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ABSTRACT BOOK
XIth International Myeloma Workshop
IVth International Workshop on Waldenström's Macroglobulinemia
25-30 June 2007 – Kos Island, Greece

Guest Editors: M.A. Dimopoulos - A. Anagnostopoulos

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S1.1

MOLECULAR PRINCIPLES UNDERLYING MYELOMA

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Multiple myeloma is a tumor of mature isotype-switched plasma cells that accumulate in the bone marrow causing anemia, hypercalcemia and bone lesions.

Classification of multiple myeloma – disease-defining genetic events

MM is a heterogeneous disease. Both a supervised[1] (TC) and an unsupervised[2] analysis of gene expression data from the University of Arkansas for Medical Sciences have yielded complementary results. These studies definitively show that the primary determinants of this heterogeneity are underlying genetic abnormalities: recurrent immunoglobulin gene translocations and hyperdiploidy. These genetic events identify five homogeneous groups of patients that can be identified today using FISH: 1) MM with t(4;14), FGFR3/MMSET 14%; 2) MM with t(14;16) and variants, c-maf 5%, mafB 2%, mafA 1%; 3) MM with t(11;14) and variants, cyclin D1 16%, cyclin D3 2%; 4) MM with hyperdiploidy, trisomies of chromosomes 3,5,7,9,11,15,19,21 40%; 5) MM not otherwise classified 20%. These are thought to be primary genetic events, with the translocations mediated by germinal center reactions (switch recombination and somatic hypermutation) that each initiate a characteristic cascade of secondary genetic, epigenetic and microenvironmental changes leading to the unique gene expression profile and clinical course. A specific genetic lesion underlying the hyperdiploidy has not been identified, although molecularly it has been associated with a low-level of ectopic, bi-allelic expression of cyclin D1 (TC D1). Both studies identified additional heterogeneity: a proliferative group (PR), a group with ectopic expression of cyclin D1 and cyclin D2 (TC D1+D2), a group with low bone disease (LB), and a group with expression of cyclin D2 (TC D2). A genetic basis for this additional heterogeneity has not been identified, and is the subject of active investigation. An important feature of this classification is that it is inherent in the genetics of the disease, and does not depend on response to treatments that will no doubt evolve. We can be confident that one hundred years from now MM will still be characterized by t(4;14), t(11;14) and hyperdiploidy. This means that as a basis for classification, these defined genetic events will not change over time. No doubt we will be able to further refine genetic subtypes however, as we identify the genetic basis for the additional heterogeneity. Importantly, although this classification does not depend on response to treatment, it is given additional clinical relevance by the fact that these groups identify patients with vastly different prognoses when treated with standard,[3] high-dose[4] or novel therapies.[5]

Disease-modifying genetic events

Interestingly, a number of genetic events that are important in the pathogenesis and prognosis of MM do not have a dominant effect on the global gene expression profile. These include activating mutations of ras, amplifications of 1q, deletions of 13q and deletions of p536. Of note, although studies in patients have yielded conflicting results as to the target of the 17p deletions, this likely represents the difficulty inherent in analyzing primary patient samples. The more thorough analysis that is possible in the myeloma cell lines clearly identifies p53 as a target in these cases, with the majority having inactivation of p53 function, including several small bi-allelic deletions, and inactivating point mutations associated with LOH. Each of these presumably secondary events is seen (with different frequency) in each of the primary genetic groups above, and represent events that modify the course, but do not define unique diseases. These events, together with gene expression risk profiles and clinical parameters (β2-microglobulin, PCLI) can be used to develop elegant prognostic models that should not be confused with a disease classification.[4,7] Recently we have identified a promiscuous array of mutations that result in constitutive activation of (primarily) the non-canonical NFKB pathway, present in up to 20% of patients.[8] The most common mutation is inactivation of TRAF3, present in 13% of patients. Preliminary analysis using gene expression as a surrogate for inactivating mutation suggests that this may have important clinical consequences. In the APEX clinical trial,[9] patients with inactivation of TRAF3 appear to have a lower response rate to dexamethasone (10%) and higher response rate to bortezomib (90%), associated with significant prolongation of PFS (83 vs 193 days). In the remaining patients there is no significant difference between dexamethasone and bortezomib in terms of response or PFS. This has important implications for the selection of patients for different treatments, and for the implied mechanism of action of both glucocorticoids and proteasome inhibitors in the treatment of MM.

The timing of oncogene activation is the critical determinant of the disease, the nature of the oncogene the critical determinant of the phenotype

Based on a novel mouse model that activates oncogene expression using somatic hypermutation (as occurs in 10% of the translocations in MM) we have developed a faithful model of myeloma. The mouse model uses the c-myc oncogene, which is critically important in human MM, translocated in 15% of newly diagnosed MM, 40% of advanced refractory MM, and over 90% of HMCL. A major clinical implication of these studies is that both the timing of oncogene activation, and the nature of the oncogene, are critical determinants of the resulting disease phenotype. Based on our studies in both mice and men, we propose that these primary genetic events form the basis for a clinically relevant classification of multiple myeloma.

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S1.2

MOLECULAR LESIONS IN MULTIPLE MYELOMA

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Primary IgH translocations (TLC) occur mainly in non-hyperdiploid tumors

There appear to be two pathways involved in the pathogenesis of MGUS and MM: hyperdiploid (HRD) and non-hyperdiploid (NHRD).[1]Nearly half of tumors are non-hyperdiploid (NHRD), and most have one of seven recurrent IgH TLC that fall into three IgH TLC groups: CYCLIN D: 11q13(CYCLIN D1), 15%; 12p13 (CYCLIN D2), <1%; 6p21 (CYCLIN D3), 2%; MAF: 16q23 (c-MAF), 5%; 20q12 (MAF B), 2%; 8q24.3 (MAFA), <1%. MMSET/FGFR3: 4p16(FGFR3 & MMSET), 15%. The recurrent TLC are thought to represent primary - perhaps initiating – events that are mediated mostly by errors in IgH switch recombination, or less often by errors in somatic hypermutation, as B cells pass through a germinal center.[2] The remaining tumors are hyperdiploid (HRD), containing 48-75 chromosomes with multiple trisomies of chromosomes 3,5,7,9,11,15,19, and 21, and only infrequently have one of the recurrent IgH TLC. Dysregulation of a CYCLIN D gene: an early, unifying event in MGUS and MM. Despite a low proliferation index, virtually all MGUS and MM tumors have dysregulated and/or increased expression of CYCLIN D1, CYCLIN D2, or CYCLIN D3.[3] The level of CYCLIN D expression in tumors is comparable to highly proliferating plasmablasts, and thus unlike normal plasma cells. About 25% of MGUS or MM tumors have an IgH TLC that directly dysregulates a CYCLIN D gene or a MAF gene that encodes a transcription factor targeting CYCLIN D2. Another 40% of MM tumors are hyperdiploid, and bi-allelically express CYCLIN D1, unlike normal lymphoid or plasma cells that express little or no CYCLIN D1. Most other tumors, including those with a t(4;14) translocation, have increased expression of CYCLIN D2. The HRD tumors that bi-allelically express CYCLIN D1 but not CYCLIN D2 are not represented among our panel of 50 human MM cell lines (HMCL), whereas the recurrent translocations are over-represented in HMCL. One possible explanation is that the HRD tumors are particularly dependent on interactions with bone marrow stromal cells, and that primary TLC might provide one step towards stromal independence.

The TC classification appears to be based on early oncogenic events

The patterns of Translocations and CYCLIN D expression led to a TC classification that includes eight groups: 11q, 6p; MAF (CYCLIN D2); 4p (CYCLIN D2); D1 (34%); D1+D2 (6%); D2 (17%) and NONE (2%).[3] Two modifications should be noted. First, apart from the dysregulated expression of different CYCLIN D genes, the 11q and 6p groups share many properties, and probably should be considered as one group. Second, many of the NONE tumors are substantially contaminated by non-tumor cells, or express virtually no RB1, which would eliminate the need for expression of a CYCLIN D gene. Despite shared progression events (below), the phenotypes of MGUS and MM tumors in the eight TC groups seems to be determined mainly by early oncogenic events. A recent molecular classification of myeloma from the Arkansas group includes seven groups that are substantially concordant with the TC groups, although there are important differences, with at least one group (PR) appearing to be defined mainly by progression events.[4] Similar to acute lymphocytic leukemia, MM seems to include several diseases (groups) that can have differences in: early or initiating events, global expression patterns, bone marrow dependence, clinical features, prognosis, and response to therapy.

Secondary translocations represent one kind of progression event

TLC involving a MYC gene (c- >> N- > L-) are absent in MGUS but occur in 15% of MM tumors, 45% of advanced MM tumors, and >90% of HMCL.[2,5] MYC TLC are a very late progression event, occurring at a time when MM is becoming more proliferative and less stromal cell dependent. They provide a paradigm for secondary (Ig) TLC, which typically are karyotypically complex, do not involve B cell specific DNA recombination mechanisms, and can occur at anytime during tumor progression, including MGUS. Secondary TLC, which include all MYC TLC, most IgL TLC λ>>κ), IgH TLC not involving the recurrent partners, and perhaps a small fraction of translocations involving the recurrent partners, have a similar prevalence in HRD and NHRD tumors. Surprisingly, two independent Ig TLC are found in 58% of HMCL, 25% of advanced MM tumors, and even, as shown by others,[6] in some MGUS tumors. Rarely, two translocations involving partners from two (all combinations) of the three primary translocation groups have been observed in the same tumor cell, suggesting that there are some unique phenotypic features associated with each of these IgH TLC groups.

Activating mutations of N-RAS, K-RAS, or FGFR3.

We have identified mutually exclusive mutations of N- or K-RAS in 40% of HMCL, and in 31% of newly diagnosed MM tumors. Surprisingly, N-RAS mutations, which are present in 14% of MM tumors, have a much higher prevalence in tumors that express CYCLIN D1 as opposed to CYCLIN D2. By contrast, K-RAS mutations are found at a similar prevalence in all tumor groups. We have also found that two of thirty-four (6%) of MGUS tumors have an N-RAS mutation, suggesting that the occurrence of RAS mutations sometimes is associated with progression of MGUS to MM. Mutations of FGFR3 in t(4;14) tumors are rare, but seem to be mutually exclusive of RAS mutations.

Inactivation of p53

We find that the function of p53 is compromised in most HMCL, including no or very low RNA expression in 14%, mutations in 63%, and increased expression of MDM2 in 19%. Others have reported that there is mono-allelic 17p deletion (including p53) in about 10% of newly diagnosed MM tumors and even a lower prevalence of p53 mutations, whereas the prevalence of deletion and mutation is much higher in advanced, extra-medullary tumors.[7,8] Moreover, 17p deletion or very low expression of p53 is associated with a very poor prognosis. Although the inactivation of p53 appears to be mostly a late progression event, there are no large studies that have comprehensively analysed the different mechanisms that inactivate the p53 pathway in primary MM tumors.

Increased proliferation and additional perturbation of the RB pathway

Increased and/or dysregulated expression of CYCLIN D RNA, which seems to occur in virtually all MGUS and MM tumors, is insufficient to cause a high level of proliferation. Methylation of the p16INK4a promoter, which occurs in about 25% of MGUS and MM tumors, is uniformly associated with lack of p16 expression, but nearly 50% of MM tumors express little or no p16 despite the absence of p16 methylation.[9] It is unclear if low or no p16 expression is an active oncogenic event. By contrast, p18INK4c, which is necessary for normal plasma cell development, is homozygously deleted in 30% of HMCL, about 2% of all MM, and nearly 10% of the most proliferative MM.[10] Although bi-allelic deletion of p18 appears to be a late progression event that contributes to increased proliferation, most HMCL and proliferative MM tumors have a paradoxical increase in p18 expression, most likely due to increased E2F that causes increased proliferation but also increased transcription of p18. Thus these tumors have become insensitive to increased expression of p18. Retroviral-mediated expression of exogenous p18 inhibits the growth of HMCL that express little or no p18, but has no effect on growth of an HMCL that already expresses a high level of p18. At present, we are unable to explain this paradox except for the rare tumors or HMCL that have inactivated RB1.

Dysregulation of the NFkB pathway in MM.

Recently, we have determined that NFkB inducing kinase (NIK), which is an activator of the alternative NFkB pathway, sometimes is constitutively over-expressed as a consequence of translocations, amplification, or enhanced protein stability. In addition, we have identified mutations that result in activation of NFKB2, which also is a mediator of the alternative NFKB pathway. Curiously, even though these abnormalities are predicted to specifically target the alternative pathway, the classical NFkB pathway also is activated, suggesting an as yet poorly defined cross-talk between the two pathways. Although we don't know the mechanisms in many cases, additional studies indicate that the NFkB pathway is constitutively active in about half of HMCL, and possibly half of primary MM tumors. We suspect that the constitutive activation of the NFkB pathway is a progression event, perhaps facilitating independence from environmental factors that extrinsically activate this pathway in primary MM tumors.

Concluding thoughts. Although we have learned much about the molecular lesions involved in the pathogenesis of MGUS and MM, many important questions remain unanswered, including the following. What are the molecular mechanisms by which hyperdiploidy contributes to tumorigenesis? What is the mechanism responsible for bi-allelic CYCLIN D1 expression in HRD tumors? What are the molecular (or microenvironmental) abnormalities that distinguish MM from MGUS?

What molecular consequences are associated with other recurrent chromosomal imbalances, such as 1q gain, 1p loss, and 13 loss?

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S1.3

TOWADS A MOLECULAR-BASED RISK STRATIFICATION OF MULTIPLE MYELOMA

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Multiple Myeloma, a malignancy of terminally differentiated antibody-secreting plasma cells, homes to and expands in the bone marrow where it causes a constellation of disease manifestations including bone marrow suppression, osteolytic bone destruction and end organ damage. In 1997 the Myeloma Institute began utilizing high throughput genomic technologies to better understand the biology and therapy of this genetically complex disease. Leveraging the large patient base available through our referral center, we have now studied more than 5,000 cases. We have compared and contrasted gene expression signatures of purified plasma cells and whole bone marrow biopsies from healthy individuals, patients with the benign precursor condition monoclonal gammopathy of undetermined significance (MGUS), newly diagnosed myeloma prior to and following 48 hour test doses of single agent chemotherapies, as well as patients in remission and at relapse. Importantly, the majority of MM cases are studied longitudinally in order to understand the molecular characteristics of disease progression.[1] Many cancers are thought to develop from well-defined pre-neoplastic or hyperplastic conditions and multiple myeloma frequently emerges from the benign condition MGUS. Comparative expression profiling has been used to distinguish 52 genes that may provide molecular clues to the transformation of MGUS to MM.[2] With the hypothesis that a stigmata of the MGUS condition might be present in newly diagnosed MM, with the possibility that such a link might point to forms of MM that are at an earlier, more manageable stage or, alternatively, represent a fundamentally different and more indolent form of the disease we showed that MM cases could be separated into those that were MGUS-like and those that were non-MGUS-like. Importantly, the MGUS-like MM had low risk clinical features and improved long-term survival relative to their non-MGUS-L counterparts. An interesting finding of this study was that a subset of MGUS cases was MM-like, suggesting that despite their clinical presentation these cases may in fact be at higher risk of conversion to MM. Thus, patients with MGUS-like MM may not require as intensive therapies as their non-MGUS-like counterparts. Likewise, the presence of molecular features of MM in MGUS may point to the need for earlier intervention, as opposed to the typical watch-and-wait approach. Molecular studies within MM have revealed that the disease can be separated into eight different molecular subtypes with tight links to known translocations, hyperdiploidy, proliferation, bone disease, and non-plasma cell hematopoietic lineage gene expression patterns.[3] Subtypes associated with *FGFR3/WHSC1* spikes and proliferation represented poor risk subtypes in patients receiving tandem transplants[3] Regardless of molecular subtypes all myeloma appear to originate through the activation of one of the three cyclin D genes.[3,4] By correlating gene expression profiles of tumor cells with MRI imaging studies we are able to show that mRNA levels of the Wnt signaling inhibitor *DKK1* in tumor cells were correlated with the presence of lytic bone disease.[5] The importance of this secreted factor lies in its potent ability to suppress Wnt signaling and osteoblast differentiation, which in turn control osteoclast development by altering RANKL and OPG levels. We have shown that *DKK1* is likely activated in myeloma plasma cells by oxidative stress via the JNK signaling cascade[6] and that a monoclonal antibody against *DKK1* can inhibit myeloma bone destruction and tumor progression in a mouse model of myeloma.[7] Long-term survival in patients with multiple myeloma can vary considerably with current laboratory and genetic tests failing to account for more than 20% of this variability. Using log rank tests of expression extremes, hypothesized to reflect gene copy number gains or losses leading to a clinically aggressive form of disease, revealed that a subset of 17 genes, 50% mapping to chromosome 1, were linked to early disease-related death. Importantly, the majority of up-regulated genes mapped to chromosome 1q and down-regulated genes mapped to chromosome 1p suggesting that abnormalities in this chromosome may be central to the development of an aggressive phenotype.[8] The high-risk signature, present in 13% of patients and found in all 8 disease subtypes experienced shorter durations of complete remission, event-free and overall survival. The high-risk score also was an independent predictor of outcome endpoints in multivariate analysis that included the International Staging System and high-risk translocations. For example, patients with the t(4;14) translocation and a low-risk score faired significantly better than patients with the t(4;14) and a high-risk score, pointing to the value of the molecular risk score in appropriate risk stratification. In longitudinal studies of paired samples taken at diagnosis and relapse we found that the risk score could increase at relapse and could predict post-relapse survival. Given that baseline and relapse samples contained the same initiating translocations these data suggests that clonal evolution can occur over time, a concept that is supported by our discovery that the percentage of cells with gains and the number of copies of chromosome band 1q21 can increase from the time of diagnosis to the time of relapse.[9] Overexpression of the gene *CKS1B*, mapping within a minimally amplified region between 153-154Mb of chromosome 1q21,[10] is overexpressed in gene in high-risk disease and a component of the 17 gene high-risk signature.[8] CKS1B is the rate-limiting component of the SCFSkp2 ubiquitin ligase that regulates the proteasomal degradation of $p27^{Kip1}$ a cyclin- dependent kinase inhibitor and tumor suppressor gene. Over-expression of CKS1B contributes to increased $p27^{Kip1}$ turnover, cell proliferation, and a poor prognosis in many tumor types and we have shown that increased CKS1B protein is inversely correlated with $p27^{Kip1}$ protein in primary myeloma.[11] Taken together these data suggests that amplification of 1q21 may target *CKS1B*. Utilizing four MM cell lines harboring *MAF, FGFR3/MMSET*, or *CCND1*-activating translocations we have shown that lentiviral delivery of shRNA directed against resulted in ablation of CKS1B mRNA and protein with concomitant stabilization of $p27^{Kip1}$, cell cycle arrest, and apoptosis. Although shRNA-mediated knockdown of SKP2 and forced expression of a non-degradable form of $p27^{Kip1}$ ($p27^{T187A}$) lead to cell cycle arrest apoptosis was modest. Importantly, while knock down of SKP2 or over expression of $p27^{T187A}$ induced cell cycle arrest in KMS28PE, a MM cell line with biallelic deletion of CDKN1B/$p27^{Kip1}$, CKS1B ablation induced strong apoptosis.[11] These data suggest that CKS1B influences myeloma cell growth and survival through SKP2- and $p27^{Kip1}$-dependent and independent mechanisms and that therapeutic strategies aimed at abolishing CKS1B function may hold promise for the treatment of high-risk disease for which effective therapies are currently lacking. In conclusion, the application of gene microarray analysis to large well-annotated and uniformly treated patients is providing unprecedented opportunities to advance therapy for multiple myeloma.

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S1.4

CLINICAL AND PATHOLOGICAL IMPLICATIONS FOR MYELOMA SUBTYPES

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It is clear that myeloma (MM) is a heterogeneous disorder and that several well defined variants exist.[1] The study of these genetic aberrations not only furthers our understanding of disease pathogenesis, but has allowed for their application in the clinic. This information improves our ability to prognosticate and predict likelihood of clinical benefit with specific therapeutics. Multiple classification schemes exist for MM, yet all describe one and the same thing; a heterogeneous condition best subclassified by genetic/cytogenetic markers, with consequent variability of phenotype.[1] However, as information has emerged the classification systems for the disease can be divide into three main categories; 1) Biology based classification: Mostly by identifying unique genetic subsets characterized by either chromosome translocations/structural aberrations, deletions, of whole chromosome changes, unlikely to change, usually with clinical utility, but not always. 2) Prognostic classification: A classification derived from understanding the natural course of the disease and independent of therapy administered, can possible change depending on therapy, but likely influential in outcome with most treatments. 3) Predictive classifications: A classification capable of providing estimates of clinical benefits associated with specific therapeutics, highly relevant but dynamic and subject to change.

Biology classification (*enduring*)

Primary genetic events. A biology classification system is one that will (should) not change with time. For instance even if MM becomes curable we will still have 15% of MM be t(11;14). This classification needs to be derived purely from biology and irrespective of clinical implications.[1,2] While biologic classifiers that attain clinical significance are more convincing, important genetic lesions do not necessarily have to discriminate outcomes. For instance MM with t(11;14) is clearly a unique biologic entity, even when it is not significantly different with regards to prognosis.[3-5] The two major subtypes include hyperdiploid MM and MM with IgH translocations involving multiple partners.

Secondary genetic events. A number of other genetic aberrations are thought to participate in disease pathogenesis acting as progression events. Some of them may act as *catalyst* factors for clonal expansion (*e.g.* chromosome 13 abnormalities; *see abstract by T Henry et al.*) while others will enhance cell survival, allow extramedullary disease and increase likelihood of therapy resistance (*e.g.* p53 deletions).[6] Lastly others are in all likelihood contributors for clonal expansion, but with specific roles still being elucidated. (*e.g.* chromosome 1 abnormalities, c-myc, non-canonical NFKB activation). Regarding to chromosome 1, it should be noted that 1q gain and 1p loss are so closely related that it is hard to provide differentiation.[7,8]

Other categories. The fundamental observation by Bergsagel and Kuehl that MM is characterized by upregulation of any one of the three cyclin D genes,[2] resulted in the observation that some patients lacking the aforementioned primary translocations will be characterized, at the gene expression level, by augmented expression of CCND2 (with or without concurrent CCND1 expression). It is possible one unifying genetic mechanism explains CCND2 elevation, but also that multiple abnormalities ultimately lead to CCND2 increased expression. Another subgroup identified by this classification is the *none* group. This group is characterized by minimal expression of *RB1* (50%) complimenting a model consistent with hyperactivity of cyclin D or down regulation of checkpoints (RB1) mediating G1/S transition.

Prognostic classification

While we believe the major prognostic variation of MM is dictated by primary genetic categories, secondary changes can also have a profound influence in outcome by providing clonal survival/proliferation advantages. Some of the basic genetic categories have not resulted (yet) in specific clinical outcome difference, yet define unique subtypes (*e.g.* t(11;14)).[9,10] The clinical consequences of secondary genetic changes tend not to be related to therapy administered. One possible way to define prognostic markers is that they associate with baseline features of aggressiveness (pathobiology) and they should exert their influence if patients are not treated (natural history). Since most patients will ultimately be treated, they usually will exert their influence in segregating outcome, even with therapy improvements (but may not). One could propose that for a prognostic marker to be considered meaningful minimal requirement could be; a) that the marker has shown effects when applied to patients treated with two different modalities and, b) that it has been reported in at least two statistically *empowered* series of patients. For instance we know for that overall patients with t(4;14) fare worse,[3-5,11] even when some selected cases might fare better. Needless to say the prognostic ability of this same markers is currently challenged by the introduction of novel therapeutics(12). The same is true for t(14;16) with at least two series showing that (ECOG and UAMS).[3,11] Minimal data is available regarding the other MAF variants.

Other models. Other prognostic markers exert effects across the major biology subtypes of MM. Some are well established, including the strongest cytogenetic prognostic marker; –17p1.[3-5,13] Another model that has been proposed and tested in at least two series is a *70 gene* signature derived from the GEP by the UAMS.[14] This last model discriminates with unprecedented ability *high-risk* disease. While not likely to be immediately in the clinic this is an excellent benchmark with which clinical trails can be compared for inclusion of high-risk individuals. Other markers could include proliferation index by GEP, centrosome index by GEP and CTA.[15] Most of these models are best considered working categories.

Table 1.

Classification	*Likely to change over time*	*Clinical utility*	*Related to therapy*	*Definitive categories*	*Need additional data or series*
1. Biologic	Not	Maybe	No		
	Primary			t(11;14) t(4;14) t(14;16) Hyperdiploidy	CCND2 D1+D2 category
	Secondary			Deletions of 13 17p13 deletions	Chromosome 1 Non-canonical NFKB ? c-myc.
2. Prognostic	Perhaps	Yes	Yes/No	t(4;14) t(14;16) -17p13 Deletions 13 by metaphase Hypodiploidy by metaphase UAMS 70 gene signature	Hyperdiploidy t(11;14) UAMS 70 gene signature Proliferation signature Cancer testis antigens Centrosomes CCND3 and other maf translocations
3. Predictive (*e.g. melphalan*)	Likely	Yes	Yes	t(4;14) t(14;16) -17p13	TRAF3 UAMS 70 gene signature Centrosome
3. Predictive (*bortezomib*)	Likely	Yes	Yes	None	t(4;14) IgH translocations Deletions 13 TRAF3 Centrosome

Predictive classification

Predictive classifications lag behind in supporting data and clinical applicability. The issue of predicting response to therapy is not as important in MM (now most patients respond), as it is to predict sustainability and quality responses. Arguably by combining these parameters (and assuming reasonable toxicity) a predictive factor will measure clinical utility. A predictive classification is more complex in that it incorporates treatment as a variable. Again, using likelihood of response alone will not be enough. For instance all preliminary data suggest that bortezomib works just as well for IgH translocated or chromosome 13 deleted cases (*not necessarily better*).[12,16] However, this has not as of yet translated into clinical benefit. The problem with t(4;14) has not been lower responses, but rather early relapse and refractory relapse.[3,4,17] The long-term outlook of t(4;14) patients treated with bortezomib is needed. Furthermore an accurate predictive model may dictate more the order (sequence) of treatments employed, since ultimately, and barring the development of curative therapy, many MM patients will be treated with all agents, just in different order. One example of prediction is our recent observation of higher likelihood of response (90%) to bortezomib amongst patients with hyperactive non-canonical NFKB pathway (using low level of expression of TRAF3 as surrogate), as opposed to those with normal level of TRAF3 expression (30%). Based on some of these considerations, despite data shortcomings, and because of the shorter benefit of autologous stem cell transplant for high-risk disease, our group has incorporated bortezomib early on in the treatment course of patients with high-risk disease (recommendations available at mSMART.org). RF is a Clinical Investigator of the Damon Runyon Cancer Research Fund.

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S1.5

PROGNOSTIC IMPLICATIONS OF FISH KARYOTYPING IN MYELOMA

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Despite recent improvements in the treatment of patients with multiple myeloma, most patients will experience relapse and ultimately will die from the disease. Although myeloma is currently not curable, even with the use of the novel drugs, some of them may enjoy a very long survival, up to 10 or 15 years. However, using similar therapeutic strategies, some patients present a highly refractory disease, and die from the disease within a few months after the diagnosis. Several prognostic classifications have been proposed in order to try to predict patient evolution. The most popular one is the International Staging System (ISS), based on 2 simple biological tests, i.e., β2-microglobulin (β2m), and albumin levels.[1] This system allows to distinguish 3 groups of patients with different outcomes. However, this classification is not very useful to identify both patients with a very poor outcome (for instance, those who will die within 24 months), and those who will be still alive more than 10 years after the diagnosis. By analogy with other hematopoietic malignancies, cytogenetics has been shown to play a dramatic role in determining the prognosis of the patients with myeloma.[2,3] However, striking differences exist between myeloma and acute leukemias for instance, that explain the gap in the use of cytogenetics in these two disease types. Basically, two major differences have been shown to prevent a widespread use of karyotyping in myeloma. The first difference is the level of tumor cell infiltrate within the bone marrow samples. In myeloma, bone marrow samples sent to the cytogenetics lab contain usually less than 10% of plasma cells, probably because of the difficulties in bone marrow harvesting in myeloma. The second difference is related to the biology of the tumor cells. Plasma cells are terminally-differentiated cells and display a very low proliferative index. Because conventional cytogenetics requires the cells to pass through the mitosis to generate metaphasic chromosomes, proliferation is crucial for cytogenetics success. If, in addition, the number of tumor cells within the specimen is very low, the generation of metaphases within the tumor clone is almost impossible. Thus, to circumvent this pitfall, the use of techniques independent of proliferation is a prerequisite. Interphase fluorescence *in situ* hybridization (FISH) presents many advantages to reach this goal. Before to describe the results of FISH analyses in predicting outcome in myeloma, it is crucial to understand the pitfalls that have to be circumvented in order to get proper interpretation of the results. The most important point is that FISH analysis has to be performed on identified plasma cells. Because of the common low percentage of plasma cells in specimens sent to the lab (median is 6% in the IFM practice), a classical direct FISH experiment would get falsely negative results. Physicians have to be aware of this major point, in order to reject results obtained on crude bone marrow cells. Plasma cells can be identified either by purification (using anti CD138-coated magnetic beads for instance, with an evaluation of the plasma cell purity after the selection), or by a concomitant immuno-labeling of the kappa or lambda light chains, or by a two-step method combining a morphological identification of the plasma cells, followed by a classical FISH experiment on these plasma cells. Many chromosomal abnormalities have been shown to impact the outcome of the patients with myeloma: del(13), t(4;14), t(11;14), ploidy changes, del(17p), t(14;16), t(14;20), 1q gains being the most commonly recognized. Abnormalities of chromosome 13 have been the first to be associated with a poor outcome.[4] Most of these abnormalities are monosomies, more rarely partial deletions. A matter of debate is still present in the myeloma community regarding the specific prognostic role of del(13). First of all, it is clear that chromosome 13 abnormalities identified on the karyotype (rather than by interphase FISH only) have a higher prognostic power, probably because it is associated by definition with a high proliferative index. However, recent data obtained by the IFM on a large number of patients did highlight the wide heterogeneity of patients with del(13).[5] They did show that even though del(13) is clearly associated with a shorter survival, this prognostic power is almost totally related to other chromosomal abnormalities frequently associated with del(13), like t(4;14), del(17p), or t(14;16). Actually, patients lacking these latter abnormalities, but presenting a del(13), have exactly the same outcome than patients lacking del(13). Thus, the debate around the method of del(13) identification will probably disappear with the disappearance of chromosome 13 expertise from the prognostic assessment of myeloma at diagnosis. Translocations involving the 14q32 region are present in about 60% of the patients with myeloma. The most frequent one is the t(11;14) (about 20% of the patients), the same than that observed in mantle cell lymphoma. This translocation has been associated with a longer survival in some studies, but more recent studies did not confirm this prognostic impact, and the t(11;14) is currently considered as neutral from a prognostic point of view.5 The second most frequent 14q32 translocation is the t(4;14). This translocation leads to the deregulation of two genes located at the 4p16 breakpoint, *FGFR3* and *MMSET*. All the authors agree to consider this abnormality as a *grave* parameter.[5,6] However, even in this small subgroup of patients (about 15% of the patients), heterogeneity has been identified. For instance, gene expression profiling has been shown to discriminate two groups of t(4;14)-positive patients, with different outcomes.[7] Similarly, using more simple methods like β2m levels, the IFM did show that β2m can discriminate two groups of t(4;14)-positive patients, with different outcomes (median survival of 22 months and 54 months, respectively). An unresolved question is the exact role of FGFR3 overexpression in oncogenesis and patient outcome. Actually, about one third of the t(4;14)-positive patients do not express FGFR3 (mostly because of a loss of the derivative chromosome 14).[8,9] However, those patients display a similar poor prognosis.[5] This finding highlights the role of *MMSET* deregulation rather than *FGFR3* in the pathogeny of t(4;14), and raises some questions on the current therapeutic strategies testing FGFR3 kinase inhibitors. Other rarer 14q32 translocations, and especially those deregulating the *MAF* genes, are also associated with a poor prognosis.[3] However, these translocations (t(14;16) and t(14;20)) are observed in less than 5% of the patients, and data on the real prognostic power have probably to be extended through larger studies. Ploidy is probably not an important prognostic factor, as recently shown by the IFM.[5] Actually, the poor prognostic impact of hypodiploidy is probably largely covered by other abnormalities like t(4;14), del(17p), or t(14;16), almost totally restrict-

ed to hypodiploid karyotypes. The last important prognostic abnormality is del(17p). This abnormality is observed in about 10% of the patients (mostly as losses of the whole short arm of chromosome 17). Several series have shown the very short survival of patients presenting this abnormality.[3,5,10] The molecular significance of this deletion is so far unknown. Most authors did focus on the TP53 gene, located at 17p13. However, so far, no clear evidence highlights the role of this gene in the prognostic impact of del(17p) in myeloma. It is rather unlikely that the sole deletion of one *TP53* allele will inactivate the gene. Thus, the inactivation of the second allele has to be present to imagine that *TP53* is the target of these deletions. To date, no convincing data have been reported to support this hypothesis. Deletions are never bi-allelic, and *TP53* mutations have been rarely reported in myeloma at diagnosis. Thus, the quest of the Graal is still open! In conclusion, FISH karyotyping has become the reference technique for the analysis of genetic abnormalities in myeloma, and should be performed at diagnosis for every patient. Actually, FISH is more informative than conventional cytogenetics, and is (so far) much more feasible than microarray techniques. However, it is also clear that genetic abnormalities have to be combined with other prognostic factors exploring other disease components (like tumor mass, or proliferation for instance) to get a strong prognostic analysis. In the IFM experience, β2m is the strongest factor after cytogenetics, allowing to split the survival curves drawn according to genetic factors.[5]

Overall survival of the 516 patients annotated for all the parameters in the IFM99 studies (patients < 66 years).

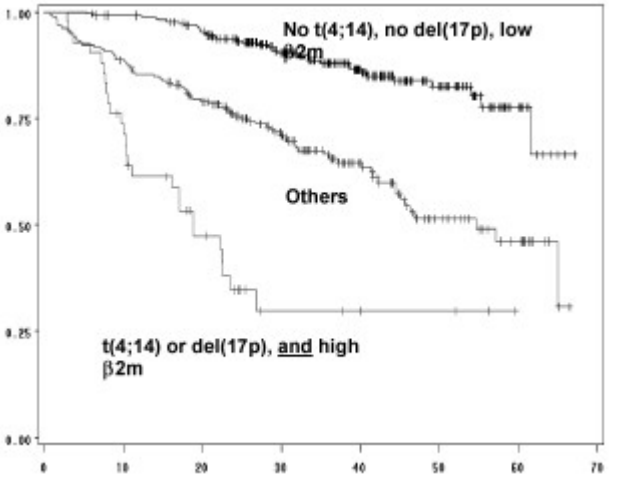

Figure.

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S1.6

SINGLE NUCLEOTIDE POLYMORPHISM MODELS IN MYELOMA – FROM THE BANK ON A CURE SNP CHIP

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Introduction and SNP Chip design

The human genome project has shown that there are large genetic variations in the human genome (polymorphisms). Unlike somatic mutations, polymorphisms are stable and heritable. Polymorphisms include single nucleotide polymorphisms (SNPs), micro- and minisatellites, and may include heritable insertions and deletions (indels). A SNP represents a single base change that may or may not cause an amino acid change in the encoded protein, potentially altering function; or a SNP may alter important regulatory elements, that affect levels of expression or RNA processing. Significantly, SNPs account for over 90% of genetic variation in the human genome. An important principle that has emerged from the consideration of genetic variation is that disease risk, and clinical outcomes can show significant variation in the population. Multiple myeloma (MM) shows significant heterogeneity with regard to morphology, disease progression, response to therapy and incidence of secondary malignancies. This heterogeneity likely is due, in part, to differences in genetic abnormalities within the malignant clone, as shown in many studies on chromosomal abnormalities, oncogenes and tumor suppressor genes in myeloma. However, it is also very likely that germline genetic polymorphisms contribute significantly to an individual's disease course and response. The growth of MM plasma cells is dependent on a complex interplay among various growth factors, adhesion molecules and other factors in the tumor microenvironment. Thus, it might be expected that genetic variations in this interplay could have a profound influence on disease initiation, progression, associated bone complications, and response. Moreover, genetic variation in immunity and inflammation is an important consideration, as are variations in genes coding for drug metabolism and transport. In order to address these issues we have engaged in an international program designated as the *Bank On A Cure* (BOAC). A cooperative program was established to bank DNA from multiple clinical and institutional trials, and develop a platform for examining the association of genetic variation with disease risk and outcomes. A directed, custom BOAC SNP chip design was developed with specific criteria from public and commercial data bases. Rather than a total genome wide screen, a plan was undertaken to develop a custom SNP chip, focusing on functionally relevant polymorphisms playing a role in normal and abnormal cellular functions, inflammation and immunity, as well as drug responses. Candidate gene lists were created and each gene in the candidate list was systematically investigated with a selection of SNP databases to harvest SNPs that may have a functional effect on gene action. Searches for genes were developed, using public and commercial software programs in PubMed, iHOP, as well as pathway data bases, such as PharmGKB Pathways, BioCarta, KEGG, Ingenuity, and Pathway Assist (Stratagene, Inc.): The Human Mutation Database contains a searchable database of polymorphisms associated with diseases cited in the literature. This database was used in conjunction with SNP500, SNPper and MutDB to obtain the SNP id (rs number) of polymorphisms in the gene lists. A systematic search for all non-synonymous SNPs (ie. resulting in coding change) with a validated, minor allele frequency greater than 2% in all of the candidate genes was completed using SNP 500, dbSNP, and Affymetrix databases. SNPs failing to meet a 2% population frequency were included if the frequency was higher than 5% in selected racial subgroups (eg. Asian, African American, Caucasian). A systematic search of the promoter regions of in all the candidate genes for SNPs present in homologous regions between human and mouse with a minor allele frequency greater than 2% were identified using Promolign Database. Many of the SNPs selected in this method were seen to lay in or adjacent to promoter and transcription binding sites. Affymetrix provided several in-house validated SNP lists, including: inflammation & immunity, drug metabolism, and cancer lists. TagSNPs in genes influencing drug metabolism and transport were added. A final custom SNP chip panel of 3,404 SNPs from 983 genes meeting the above criteria was produced for the Affymetrix/Megallele platform.

Samples and Quality Controls

A total of 861 DNA samples have been profiled by the BOAC SNP Chip, including 670 myeloma patient samples and 191 unaffected controls. Most of the patient samples were derived from cooperative group clinical trials (eg. ECOG, SWOG), institutional trials (eg. Arkansas Total Therapy II), or NCBI control cell lines (eg. Coriell). Among all samples profiled, we had an average call rate of 95.3%. The profiles of the Coriell panel allowed us to determine allelic frequencies in racial groups and unaffected populations. Of the 3,404 SNPs on the BOAC panel, 786 were contained in the SNP500 cancer data base, allowing us to determine concordance between the two data sets. We found very good agreement with an average of 97% concordance. We also duplicated the profile of a number of samples, and found better than 99.7% reproducibility between duplicate samples. This concordance and duplication rate was also equivalent when comparing the BOAC SNP panel run in the U.S. and U.K facilities, providing a cross validation between BOAC laboratory sites. We have used the SNP data base to analyze variants associated with survival, adverse effects (eg. thrombosis), and bone disease. A first analysis of genetic variation associated with survival will be presented here; other associations will be provided in separate presentations.

Analysis of SNPs associated with survival

143 samples from two clinical trials were run on the BOAC SNP chip. Because of the extensive differences in allelic frequencies among races, and the larger cohort of Caucasians in the trials, we limited our first analysis to Caucasians, but will continue to analyze samples from all races, with updates as the numbers increase. Moreover, as a first exploration of genetic variation associated with survival, we choose to compare extreme phenotypes, ie. short term survivors (less than one year, n=70) versus long term survivors (greater than 3 years, n=73), receiving combination chemotherapies in ECOG trial E9486 and intergroup trial S9321. We first determined a rank order of SNP association through a univariate analysis, using a stratified test to account for trial differences. From this list, 141 SNPs showed association with a p value <.05. Notably, there was a high content of SNPs associated with drug metabolism, transport, and DNA repair (cyp genes, ABC transporters, GSTs, XRCCs), as well as proliferation genes (PCNA) and cathepsins.When we treated the 141 SNPs as a set for classification prediction, we found a 79% correct classification rate, with confidence intervals for likely error (13.9%, 23.8%). In addition, a classification recursive partitioning tree was constructed in which 6 SNPs were found to provide an 81% classification predictor. These results are to be considered exploratory, and a number of limitations on interpretations will be presented. However, they provide some of the first attempts to model the association of genetic variations among patients with outcomes in myeloma.

S2: Pathophysiology - Microenvironment

S2.1

ANIMAL MODELS OF MULTIPLE MYELOMA: AN OVERVIEW

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Multiple myeloma (MM) cells predominantly grow in the bone marrow as a result of a complex interplay between these cells and the different players in the bone marrow stroma, resulting not only in bone marrow plasmacytosis but also in the induction of other processes involving the host microenvironment like angiogenesis and bone disease. While *in vitro* experiments mainly focus on one type of interaction in a two-dimensional network, animal models of myeloma provide a basis to study complex interactions in a three-dimensional network. Different models have been developed and used in the past decade to recapitulate the human pathophysiology in murine systems. Most established animal models however fail in one or more crucial features to resemble human MM. It is important to stress that each model has his pro and contra and investigators should use a particular model depending on the scientific question asked. In view of the important role of the bone marrow microenvironment, growth in the bone marrow is one of the critical features of MM models. Furthermore, the bone marrow engrafted MM cells should also interact with the host, inducing processes like angiogenesis and osteolytic lesions. Preclinical testing of new therapeutic strategies for the treatment of MM requires quantitative evaluation of the applied therapy, while immunological studies require an immunocompetent host. Recent developments in medical imaging allow accurate evaluation of the MM disease: follow-up of bone lesions by microCT (of whole bones or bone explants) and follow-up of tumor burden after transfection (plasmids) or transduction with viral vectors containing fluorochromes (*e.g.* eGFP), luciferases (bioluminescence) or a sodium iodide symporter (rat or human, SPECT) using specific imaging devices. Both techniques hold great promise, since animals can be repeatedly monitored throughout disease progression. The mostly widely used MM models will be discussed, including syngeneic models, different SCID xenografts models, SCID-Hu, SCID-rab and different transgenic models.

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S2.2

THE SCID-HU MODEL FOR MULTIPLE MYELOMA

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Adaptation of the severe combined immunodeficient-human (SCID-hu) mouse, originally developed for studying human hematopoiesis,[1] to the study of primary human myeloma was prompted by two factors: the need to identify the proliferative myeloma cell, (myeloma stem cell), a controversial issue stemming from the perceived lack of proliferative capacity of the recognizable myeloma plasma cell, and by the observation that when a human bone (myeloma patient biopsy core) was present, myeloma cell lines injected IV into the mice homed preferentially to the human bone and not to the mouse hematopoietic organs, their site of homing in the absence of a human bone.[2] The result of this adaptation was the first system that consistently supports growth and dissemination of primary myeloma plasma cells. Initial work established, for the first time, a model that consistently supported the growth of myeloma cells isolated to high degree of purity from fresh bone marrow aspirates.[3] This model helped prove unequivocally that given the right microenvironment, myeloma plasma cells are proliferative, capable of sustained proliferation, and that no other cell in the patient's bone marrow or blood are able to produce myeloma in this model or are required for sustained myeloma cell growth.[4] Myeloma growth was totally restricted to and dependent on the human bone marrow microenvironment, and was associated with typical myeloma manifestations, including osteolytic bone disease. The dependence of myeloma on the human bone microenvironment facilitated studies addressing biological characteristics of myeloma, especially those related to myeloma cell interactions with the bone marrow microenvironment. Pearse used the SCID-hu model to demonstrate that myeloma alters the RANKL/OPG balance in the bone marrow, causing bone destruction and promoting disease progression,[5] and Yaccoby demonstrated that myeloma depends on the osteoclastogenesis it induces for survival and growth,[6] observations also reproduced in the 5T murine myeloma model. Thus, the dependence of myeloma on osteoclast activity revealed, for the first time, that myeloma-associated bone disease is a facilitator of myeloma growth and progression, and helped demonstrate that IL-6 is an anti-apoptotic agent.[5-7] These studies also demonstrated a fundamental difference between classical and extramedullary myeloma that while still requiring a human microenvironment, does not depend on osteoclast activity for survival or growth, only for dissemination. To investigate the role of myeloma-associated osteoblast abolition in the disease process, mesenchymal progenitors of osteoblasts (MSC) were injected into the human bones of SCID-hu mice with established myeloma. In 50% of experiments MSC injection was associated with inhibition of myeloma growth and increase in bone mineral density, while in the other half no effect was discerned. Only in experiments with clear anti-myeloma activity could the injected MSC and their differentiated progeny be demonstrated histologically.[8] Whether failure of MSC to engraft in these experiments reflects characteristics of these cells or properties of the myeloma cells is being investigated. Similarly, enhancing osteoblast activity with bone anabolic agents like PTH was associated with inhibition of myeloma growth. Additional studies on the interactions between myeloma cells and the marrow microenvironment revealed that only some myelomas respond to inhibition of angiogenesis and that thalidomide's anti-myeloma activity depends on its metabolism by liver microsomes.[9,10] Ge reported that the fibroblast activation protein (FAP), a serine phosphatase was important in the myeloma interaction of myeloma cells with their microenvironment.[11] Unique characteristics of the SCID-hu model – the only one in which primary myeloma cells grow in and interact with a human bone microenvironment – render it a most attractive system for pre-clinical studies. Indeed, the model has provided a great deal of information on myeloma biology and is suitable for pre-clinical proof of principle studies. However, the human bone is the sole source of human factors that are required for myeloma survival and growth. Therefore, the effects of interference with any of the major cells in this microenvironment (osteoclasts, osteoblasts/MSCs, vascular endothelial cells) on the myeloma cells will be hugely amplified, whereas in patients some or all of the required factors can originate from other organs. The interpretability and applicability of the model-derived data to patients is therefore limited at best. Several investigators reported variants of the SCID-hu model, including the use of myeloma cell lines to investigate myeloma cell homing,[12] and the use of myeloma cells and cell lines in pre-clinical evaluation of various therapeutic agents.[13-15] Other variations on the SCID-hu model include the NOD/SCID-hu reported by Huang et.al.[16] and the SCID-rab model reported by Yata *et al.*,[17] in which a rabbit bone replaces the implanted human bone; [18] this and the SCID-hu models were used to demonstrate the effect of anti-*DKK1*, bortezomib, and bone anabolic agents on bone remodeling and myeloma growth.[19]

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S2.3

CHROMOSOMAL AND EPIGENETIC ABNORMALITIES IN MGUS AND MM POST-MGUS

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Patients with monoclonal gammopathy of undetermined significance (MGUS) may progress to multiple myeloma (MM) or a related disorder with a probability of 1% per year. However, it is at present unclear whether or not MM post-MGUS is biologically and clinically different from MM developing without a durable MGUS-phase (referred to as MM with *unknown* prior history, MM-U). We studied 41 patients with MM post-MGUS using interphase FISH to determine the cytogenetic pattern and clinical outcome. Results were compared with cytogenetic abnormalities found in a reference population of 287 patients with MM-U.

The frequency of FISH-defined chromosomal aberrations is summarized below.

	MM post MGUS	*MM-U*	*p-Value*
Any t(14q32)	25/38 (65.8%)	116/274 (42.3%)	*p*=0.11
t(11;14)	9/39 (24.1%)	39/286 (13.6)	*p*=0.19
t(4;14)	3/38 (7.9%)	24/270 (8.9%)	*p*=0.85
Del(17p13)	1/39 (2.6%)	25/157 (15.9)%	*p*=0.04
Del(13q14)	22/41 (53.7%)	115/287 (40.1%)	*p*=0.31
Del(13q14) with t(14q32)	20/22 (90.9%)	64/115 (55.6%)	*p*=0.15
Del(13q14) with t(11;14)	7/22 (31.8%)	11/115 (9.6%)	*p*=0.02
Del(13q14) only[1]	11/39 (28.2%)	29/250 (11.6%)	*p*=0.02

In MM post-MGUS, a t(11;14) was found to be more frequent than in MM-U (24% versus 14%) and it was associated with significantly shortened survival (24 months versus 70 months in MM-U; p=0.01). MM post-MGUS was further characterized by a higher frequency of 13q-deletions only (absence of all other specific abnormalities; 28% ver-

sus 12% in MM-U; p=0.02). A 13q-deletion only was an indicator of long survival in MM post-MGUS (median not yet reached) as opposed to MM-U (median survival, 29 months; p=0.001). 17p-deletions were infrequent in MM post-MGUS (3% versus 16% in MM-U; p=0.04). Survival times for patients with t(4;14) and/or 17p-deletions and other abnormalities were similar in both MM patient cohorts. Our data suggest that t(11;14) and 13q-deletions have distinct prognostic implications in the context of MM post-MGUS. DNA methylation leading to gene silencing is an important mechanism in the pathogenesis of many malignancies and has been observed in MM as well. However, compared to other malignant diseases, only a limited number of genes epigenetically inactivated in MM has been identified thus far. We therefore analyzed global changes in gene expression profiles of MM cell lines (MM1, U266, NCI-H929) after treatment with 5-aza-2'-deoxycytidine and/or trichostatin-A. A substantial number of genes as found to be up-regulated in at least one cell line in response to treatment with one or a combination of both drugs (470 genes). Our approach identified several genes which have already been reported to be epigenetically silenced in MM (*e.g. TIMP1, DDKN1A, SOCS1* or *CDH1*). However, of greater importance, we identified a large number of genes whose epigenetic regulation in MM was not known thus far (*e.g. ING1, TADA3L, BTG1, JUP, CGREF1*). Preliminary results in primary patient samples indicate that aberrant methylation of promoter regions of these genes can also be observed in MM and MGUS, and comparative results will be presented. Our results further point to an important role of epigenetic gene silencing in the pathogenesis of MM.

S2.4

MICROARRAY ANALYSIS TO PREDICT RESPONSE TO THALIDOMIDE AND THE IMIDS

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The past decade has seen incredible advances in the understanding of myeloma biology as well as in its treatment. The introduction of thalidomide and IMiDs has opened up treatment options for patients with myeloma and has improved their outcome.[1,2] However, progress does come at the price of toxicities unique to each therapy and treatment with these drugs has the potential for toxicities that can impact the quality of life. The ability to individualize therapy, to match therapies to patients that are likely to result in best efficacy with lowest potential for toxicity, has so far remained an elusive target. Better understanding of the disease biology and mechanisms of the drugs being used have brought this dream closer to reality than ever before. Identification of the underlying genetic abnormalities have been the corner stone for better understanding of the disease biology and has been true for myeloma than any other disease. Introduction of FISH overcame the limits of conventional cytogenetics in a disease characterized by low rates of proliferation and has highlighted the genetic heterogeneity of this disease.[3] Gene expression profiling using high density oligonucleotide arrays have enabled us to view a snapshot of the gene expression profile in the tumor cells and careful correlation with the clinical phenotype has allowed identification of critical changes associated with the malignant transformation. In addition to studying disease mechanisms and identifying targets for therapeutic intervention, it has also opened up the possibility of recognizing genetic signatures that can predict response to individual therapeutic approaches for various cancers. Such approaches have been employed with various tumor types including colorectal cancer, breast cancer, as well as leukemias and lymphomas.[4] Thalidomide and IMiDs along with the proteasome inhibitors has become integral part of the treatment of newly diagnosed as well as relapsed myeloma.[5,6] Randomized clinical trials have demonstrated improvement in response rates and survival with use of these agents compared to previous therapies.[7] However, use of these drugs is associated with the potential for multiple toxicities including thrombosis, neuropathy, and cytopenias among others.[8] The ability to identify patients who are most likely to respond to any of these drugs will allow us to individualize the treatment strategies and avoid potential toxicities. Given the widespread use of thalidomide and dexamethasone for initial therapy of myeloma, we evaluated the ability of gene expression profiling to identify patients who are most likely to respond to this combination, when used as initial therapy.[9] Patient samples from the Eastern Co-operative Oncology Group clinical trial (E1A00),[6] that compared thalidomide-dexamethasone combination with dexamethasone, and patient samples from the phase II Mayo Clinic study of thalidomide and dexamethasone, both for newly diagnosed MM, were used for this study. Thirty newly diagnosed patients who were evaluable for response were included in the analysis. Bone marrow mononuclear cells were separated from aspirates by Ficoll or ACK lysis and tumor cells isolated by CD138 selection. Total RNA was isolated using Qiagen RNeasy, cDNA generated and GEP performed using the Affymetrix U133A microarray platform. The Affymetrix output (CEL files) was imported into Genespring 7.2 (Agilent Technologies) microarray analysis software, normalized across chips using GCRMA followed by per gene normalization to median. For the purposes of this study, responses were defined as reduction in the serum paraprotein (or the urinary M protein in the absence of a serum M protein) of ≥50% (PR), 25 to 49% (MR), increase of ≥50% (PD) and stable disease for the remaining. Five of the 30 patients had no response to the thalidomide Dexamethasone therapy. Using the class prediction tool available in Genespring (Support Vector Machines), we identified 25 genes that reliable predicted non responders (PD, NC) from the responders (MR, PR, and CR). A representative list is provided in Table 1. We also utilized the GEP to identify patients with the common IgH translocations. None of the differentially predictor genes were associated with the five common translocations in MM (11q13 (cyclin D1), 6p21 (cyclin D3), 4p16 (FGFR3-MMSET), 16q23 (c-maf), or 20q11 (maf-B)). All 5 non-responders had one of the primary IgH translocations (11q13 -1 pt, 6p21 -1 pt, 4p16 -2 pts, 20q11 -1pt) compared to only 7 of the 25 responders with one of the translocations. Using a combination of two datasets we have identified a set of genes that can be used to reliably predict lack of response to thalidomide and dexamethasone in patients with newly diagnosed MM. We think that this represents a step towards creation of custom microarrays spotted with genes that are capable of predicting lack of response (or response) for the purpose of tailoring therapy to the patient. Terragna *et al.* presented the results of their study that examined the ability of certain GEP signatures to predict the ability to attain a complete response to therapy with thalidomide and dexamethasone treatment in patients with newly diagnosed myeloma.[10] Bone marrow plasma cells obtained at diagnosis from 32 patients enrolled in the *Bologna 2002* clinical trial were used for this study. Gene expresion profiling was performed using the Affymetrix HG133 Plus 2.0 microarray platform with the output analyzed using Genespring 7.3 (Agilent technologies) microarray analysis software. The data files were normalized across chips using GCRMA and to the 50th percentile, followed by per gene normalization to median. Genes differently expressed between the group of six patients who achieved at least a nCR after thalidomide and dexamethasone and the rest were identified an ANOVA analysis. The authors identified a gene signature of 162 genes, that was capable of distinguishing patients with nCR from those who failed to achieve a nCR. Using a nearest neighbour approach they narrowed the list down to a set of 10 genes that could reliably identify the patients achieving a nCR. These genes included those that have been incriminated in cell cycle regulation as well as those encoding anti-apoptotic proteins. Ongoing work utilizing samples from clinical trials of IMiDs will allow us to develop similar lists of genes that can potentially predict resistance or response to these agents. These studies will eventually lead to development of customized arrays that will guide optimal therapy for patients with myeloma.

Table 1.

Chromosomal Location	*Gene Name*	*Protein*
4q12	SEC3L1	Exocyst complex component 1
3q26.1	SMC4L1	Structural maintenance of chromosomes (SMC) family member
12q12-q14	TUBA6	Tubulin alpha 6
14q22	CNIH	Cornichon homolog
7q11	WBSCR5	Linker for activation of T cells family, member 2
16p12-13	NFATC2IP	Nuclear factor of activated T-cells
Xp22	CD99	MIC2, T cell activation
1p31	LEPR	Leptin receptor gene-related protein
1p32	USP1	Member of the ubiquitin-specific (UBP) family of proteases
1p35	FLJ11730	Sarcoma antigen NY-SAR-91
10q11	CSTF2T	Cleavage stimulation factor
10q23	FAM35A	Family with sequence similarity 35, member A
11p15	ILK	Integrin-linked kinase, apoptosis suppressor

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S2.5

MOSAICISM OF VESSELS IN PATIENTS WITH MM AND THERAPEUTIC APPROACHES

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General

New blood vessels form through angiogenesis and vasculogenesis.[1] Vasculogenesis prevails in the embryo, and starts from mesodermal-derived cells, the hemangioblasts, which differentiate both into a) angioblasts/endothelial cells (ECs), and b) hematopoietic stem cells (HSCs). Angiogenesis prevails in post-natal life, when develops from existing vessels. Both mechanisms occur in tumor tissues in response to growth factors, mainly vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), produced by tumor and stromal cells.[2] In addition, monocytes and macrophages can be induced to assume a number of EC properties and form capillary-like structures *in vitro* through a vasculogenic pathway.[3] Differentiation of HSCs into ECs and the vasculogenic behaviour of macrophages have not been demonstrated so far in patients with MM. Patients were grouped into active (diagnosis, relapse, leukemic phase) and nonactive (complete/objective response, off-treatment plateau-phase).

Vasculogenesis by hematopoietic stem cells

HSCs from MM patients at diagnosis were harvested from peripheral blood before conditioning therapy, using apheresis and an anti-CD133 antibody. Cells seeded on fibronectin and exposed to VEGF, bFGF, and insulin-like growth factor (IGF) were able to differentiate into cells with an MM-EC phenotype after a 3-weeks' culture. HSCs gradually lost CD133 and acquired VEGF receptor-2 (VEGFR2/KDR), factor-VIII-related antigen (FVIII-RA) and VE-cadherin, indicative of a mature MM-EC phenotype. In addition, cells adhered to fibronectin, spread and acquired a typical MM-EC shape. On day 21, differentiated cells formed a closely-knit capillary network on the Matrigel surface. At variance from nonactive MM, MGUS and benign anemia (control) patients in bone marrow biopsies of the active MM cells co-expressing FVIII-RA and CD133, VEGFR2, or VE-cadherin were involved in the formation of the microvessel wall. VEGF, bFGF, and IGF released by MM plasma cells and inflammatory cells during the active disease possibly induce the differentiation of CD133+ HSCs into MM-ECs that contribute to the development of the MM vasculature through vasculogenesis.

Vasculogenesis by macrophages

MM bone marrow macrophages exposed to VEGF and bFGF develop a number of phenotypic properties, similar to those of paired bone marrow MM-ECs, and form capillary-like structures morphologically mimicking those produced by MM-ECs. At the ultrastructural level, macrophages of active MM displayed an oblong, spindle-like shape with thin cytoplasmic extroversions, some of which were either arranged to form a microvessel-like lumen or anastomosed with each other and with those of nearby macrophages to form tubular structures. These features were lacking or minimal in macrophages of patients with nonactive MM, MGUS or controls which, however, become phenotypically and functionally similar to those of MM under the angiogenic stimulation. In bone marrow biopsies of active MM, but not of nonactive MM, MGUS or control patients, EC-like macrophages (CD68+/FVIII-RA+ and CD68-/FVIII-RA+), and apparently typical macrophages (CD68+/FVIII-RA-) were incorporated into the microvessel wall. The *in vitro* and in tissue behaviour of these macrophage types can thus be regarded as a *vasculogenic mimicry*, like that of melanoma and other tumor cells which form vascular channels to cater for their rapid proliferation and high need of vessels.[4] Data suggest that macrophages undergo a phenotypic and functional adaptation, starting to behave like MM-ECs, when stimulated by VEGF and bFGF largely secreted by plasma cells during the active disease. In nonactive MM and MGUS, they are prone to a vascular switch that marches in step with the progression toward active MM. Thus, active MM patients harbored *mosaic* vessels in their bone marrow, formed by classical MM-ECs, MM-ECs differentiated from HSCs, EC-like macrophages and macrophages themselves.

An antiangiogenic pathway

Plasma cells and MM-ECs may share symmetric receptor tyrosine kinases (RTKs), such as VEGFR and PDGF receptor beta (PDGFRβ, which have been recently reported to be expressed on $CD34^+$ $CD133^+$ $VEGFR2^+$ HSCs *switching* them toward EC differentiation.[5] A combined targeting of both RTK's signaling cascades may therefore represent a more effective MM tumor/vessel-targeted approach *in vivo*. While the activation of VEGFR1 on MM plasma cells and VEGFR2 on MM-ECs was largely documented,[6] the involvement of PDGF/PDGFRβ signaling pathway in MM pathogenesis remains elusive. We observed that a constitutive and autocrine phospho-tyrosine activation of PDGFRβ was restricted toplasma cells of newly-diagnosed MM patients, correlating with higher levels of PDGF-BB compared to plasma cells of MGUS patients or peripheral blood mononuclear cells (PBMCs) isolated from controls. Also, MM-ECs, but not MGUS-ECs or quiescent human umbilical vein ECs (HUVECs), up-regulated PDGFRβ although they failed to express PDGF-BB. Conditioned media from MM plasma cells triggered PDGFRβ phospho-activation on MM-ECs, indicating that the PDGF/PDGFRβ kinase axis could be directly involved in the MM *angiogenic switch*, hence into disease progression. A molecular dissection of VEGF/PDGF-downstream signaling mediators in MM plasma cells and ECs revealed that c-Src, a cytoplasmic tyrosine kinase, was preferentially activated in response to VEGF in both cells and sustained by a VEGF/VEGFR autocrine signaling in serum-starved cell cultures.[7-8] In particular, downregulation of c-Src expression by a small-interfering RNA (siRNA) was sufficient to suppress MM-EC growth, resulting in reduced cell migration (wound-healing assay), adhesion to fibronectin and capillarogenesis activity on a Matrigel surface. These findings suggested that a single protein, placed downstream of VEGFR2, may be crucial for MM-EC survival *in vitro*. Moreover, the inhibitory effect elicited by silenced c-Src was partially rescued by exposure of siRNA-transfected MM-ECs to PDGF-BB, which can therefore represent an important paracrine mitogen *in vivo*. To further corroborate these data, we investigated the effect of PDGF-BB on the transcription of *VEGF*, *bFGF*, *HGF*, *Ang-1* and *Ang-2* genes, that are primarily involved in the angiogenic cascade. RT-PCR and Western blotting analysis showed that MM-ECs, cultured in the presence of PDGF-BB (10 ng/mL) for 8 h, up-regulated the expression of these genes compared to serum-free control cultures, with the exception of *Ang-2* which was instead down-regulated, We also tested the pharmacological activity of TKI-204, a novel small molecule inhibitor with high binding affinity for c-Src and PDGFRβ tyrosine kinases. TKI-204 decreased proliferation and survival of both MM cell lines (RPMI-8226, MM-1R, MM.1S) and patient-derived plasma cells and ECs by blocking VEGF-induced c-Src activation and PDGF-promoted autophosphorylation of PDGFRβ. These biological effects of TKI-204 were dose-dependent and observed with an IC_{50} nanomolar value ranging between 25 and 100 nM. In contrast, growth of PBMCs derived from controls was only slightly affected at high concentrations of TKI-204, thereby suggesting a large therapeutic window. Capillary-tube formation of MM-ECs on Matrigel, as well as the angiogenic potential of con-

ditioned media from MM plasma cells and ECs *in vivo* (chick embryo chorioallantoic membrane assay) were strikingly impaired without apparent cytotoxicity or inhibition of VEGFR kinase activation (IC_{50}>2000 nM). We also found reduced expression of *VEGF, IL-8, bFGF* and *HGF* in TKI-204 treated MM-ECs. This study, therefore, validated the biological relevance of PDGF/PDGFRβ signaling activation in plasma cells and ECs isolated from MM patients, providing evidence that a combined targeting of VEGF/PDGF-signaling cascades may represent a unique and effective approach to block MM neoplastic growth and associated neovascularization. In addition, these results highlight the therapeutic potential of TKI-204, which simultaneously targets multiple angiogenic growth factor RTKs in MM.

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S2.6

THE ROLE OF T-CELLS IN MYELOMA

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There is considerable clinical and circumstantial evidence for the presence of host control of the myeloma clone during evolution of multiple myeloma. Conditions such as smouldering myeloma and long-standing plateau phase in the presence of an obvious but a hypoproliferative tumour state, suggests host control factors. Scientific evidence for the role of immuno-editing and immuno-surveillance of myeloma is also available from studies on the T-cell graft versus myeloma effect seen in allogeneic transplantation, abnormality of regulatory T-cells and the protective effects of the presence in T-cell clones both in the peripheral blood and in the bone marrow of patients with myeloma. Regulatory T-cells maintain immunological self-tolerance, control autoimmunity and modulate an immune response against infections and tumours.[1] In patients with multiple myeloma, activated regulatory T-cells as measured by upregulated expression of CD25 and FOXP3 are significantly decreased in early stages of the disease compared to healthy donors. These cells are also dysfunctional and are unable to inhibit anti CD3 mediated T-cell proliferation.[2,3] The decreased function of regulatory T-cells may be responsible for the cytotoxic T-cell clones which are present in myeloma and also the increase in hyperreactive T-cells seen in myeloma patients.[4] Activated gamma delta T-cells also express natural cytotoxicity receptors and may be stimulated by drugs such as bisphosphonates to provide anti-myeloma effects.[5] Attempts to expand Treg cells using human dendritic cells have been explored but myeloma vaccine mediated induction of $FOXP3^+$ regulatory cells may have unexpected autoimmune side effects.[6] However human dendritic cell vaccination programs for myeloma, mostly utilizing idiotype peptide as the immunogen have not shown a great clinical benefit. The functional defect in DC of patients with MM, first reported by our group, may be significant factor affecting these T-cell responses.[7,8] Dramatic responses occasionally seen in donor lymphocyte infusion programs in the context of allogeneic transplantation suggest a strong graft-versus-myeloma effect. This appears to be predominantly due to reactivity against minor histocompatibility antigens rather than myeloma specific antigens. The recent demonstrations of the differential expression of the histocompatibility antigens on normal compared to myeloma cells provides an explanation for the antitumour effect in the absence of GVHD. Also frequently occurring nucleotide polymorphism in the human ATP dependent interferon responsive gene (ADIR) was found in a patient who entered completed remission after donor lymphocyte infusion without significant graft-versus-host disease and may provide further avenues for expansion of the therapeutic graft-versus-myeloma effect.[9] We have previously reported that approximately 60% of patients with myeloma have circulating $CD8^+$ T-cell clones present. Those clones are predominantly $CD8^+$, $C57^+$, $CD28^-$ and perforin-positive T-cells in replicative senescence.[10] The presence of $CD8^+$ clones in myeloma is associated with an improved prognosis. This finding was initially documented a decade ago in our cohort of patients in Sydney. Recently it has been confirmed by the analysis of the T-cell clonal status of patients in the Australian MM6 study, which was a randomised study of Thalidomide maintenance following autologous transplantation. This study clearly demonstrates the beneficial effect of Thalidomide both in overall and progression free-survival. Blood samples from 120 patients were available for analysis of T-cell receptor V, expression by a four colour flow cytometry assay that covered approximately 70% of the TCR V, repertoire. The presence of T-cell clones associated with improvement in progression free survival from 24.1 months in patients who had never exhibited clones to 32.1 months in patients who had clones present irrespective of their randomisation arm (χ^2=4.2; p=0.04). The use of Thalidomide had a dramatic effect on those patients in whom T-cell clones were present, significantly raising the number of clones present. The number of patients with multiple clones was 49% in the Thalidomide arm compared to the control arm of 22% (χ^2=6.8; p=<0.01). Overall T-cell clones were detected in 48% of patients pre-transplant, 68% after 8 months maintenance and 57% after 12 months maintenance. 76% of patients in the Thalidomide and 60% of patients in the control arm had clones detected during maintenance therapy. In a subset analysis, progression-free survival was 40.1 months in the Thalidomide cohort who had T-cell clones present and 15.9 months in the patients who were not on Thalidomide and did not have clones (χ^2=9.5; p=0.002). These observations from a multi institutional study confirm the prognostic significance of T-cell clones and the immunomodulatory stimulatory effect of Thalidomide on these clones. Further evidence of immunomodulation and the role of T-cells comes from cross-validated differential proteomic analysis using a standardised proteomic hierarchical cluster. No single antigen can be used to discriminate between the status of myeloma and MGUS patients on Thalidomide therapy. However utilising Dotscan®, a microarray of 82 different antibodies on 160 microscopic dots, has allowed an innovative approach to the investigation of myeloma by defining a consensus signature of antigen expression on mononuclear cells from patients who have monoclonal gammopathy of undetermined significance as compared with MM patients who are or are not on Thalidomide and a normal control group. Cross-validated discriminant analysis identified normal, MGUS, MM and myeloma patients on Thalidomide disease-specific signatures. MGUS patients who were progressing towards active MM acquired the MM signature. The antigens with the highest ranking for differentiation of disease-specific signatures were T-cell antigens and included CD8 and CD57 which are elevated in myeloma, CD28 which is reduced in myeloma and CD95 in patients on Thalidomide, reinforcing the importance of immunomodulatory mechanisms in myeloma and MGUS. Traditional flow cytometry has confirmed the reduction in CD28 determined by cross-validated discriminant analysis is specific for CD8 T-cells as we have previously described. The mechanism by which these T-cell clones contribute to an improved outcome is unclear. We have no evidence that this represents anti-idiotypic or anti-cancer germline associated antigens on myeloma cells. However recent publications have found CD8 T-cells specific for cancer germ-line antigens in many patients with myeloma and correlated their frequency with disease burden. Peptide specific T-cell responses range between 0.004% and 0.1% of the total CD8 total pool and analysis has shown these immune responses are detected in individual patients at multiple time points during the course of the disease. In patients who are undergoing treatment, cancer germ-line antigen T-cell responses are correlated with the level of paraprotein.[12] However we have been unable to confirm the presence of MUC-1 or MAGE specific T-cell populations using tetramers containing the immuno-dominant peptides of MUC-1 or MAGE. In addition we have been able to expand such $CD8^+$ T-cells using the same *ex-vivo* method in which CMV-specific T-cells could be expanded. We are currently further investigating this problem by transfecting the T-cell receptor of the expanded Vβ clones into a T-cell receptor negative Jurkat line to produce a replicative line expressing the T-cell receptor which can be hybridised with a cDNA library of the tumour. We have also conducted investigations on the maturation state of the cytotoxic T-cell responsible for the protective effect. The mature $CD28^-$, $CD27^-$ T-cell is responsible for the majority of this *protection*. Gene array analysis of the CD28-

, CD27- population has shown overexpression of NKG2C (which results in activation of cytotoxic T-cells) and NKG2D, which is the immunoreceptor for the major histo-compatibility complex class 1 related A-chain molecule. The presence of soluble major histo-compatibility complex class 1 related A-chain (MICA) is an independent prognostic factor in myeloma supporting the concept of immuno-surveillance and immuno-protection, as well as possible mechanisms for escape from this process.[13] In summary, there is abundant evidence for the presence of *protection* by cytotoxic T-cells improving the prognosis in patients with myeloma. The demonstration in a randomised multicenter study has shown that clonal T-cells play a *protective role*. While cytotoxic reactions against idiotypes have been described, it is not certain whether such cytotoxic T-cells are involved in the tumour control as most of the Vβ expansions seen in myeloma are non-reactive against idiotype. Clonal T-cells are stimulated by Thalidomide and this may be one of the major actions of Thalidomide in the post autologous transplant situation. The protective T-cell appears to be a mature cytotoxic effector T-cell which is both CD28- and CD27-. Cross-validated proteomic discriminant analysis has demonstrated other possible targets involved in the immunomodulatory action of the tumour by T-cells.

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S2.7

GENETIC MODEL OF MULTIPLE MYELOMA

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Multiple myeloma (MM) a multifocal plasma cell neoplasm is characterized by serum monoclonal gammopathy and skeletal destruction. The evolution of MM is preceded by a pre-malignant condition termed Monoclonal Gammopathy of Undetermined Significance (MGUS). MM remains incurable despite conventional high dose chemotherapy, translating into a median survival of 3 years and only 10% survival at 10 years.[1] With regard to pathogenesis, MM cells show a high level of Ig VH gene somatic mutation consistent with their cellular origin as antigen-driven B cells found in post-germinal centers. Like other human malignancies, MM is regarded as a multistep process, requiring numerous genetic and epigenetic events that endow potential cancer cells with requisite malignant capabilities. The molecular characterization of several frequent translocations in MM and it precursor MGUS has revealed a juxtaposition of Ig enhancer elements with a number of cancer-relevant loci including cyclin D1, FGFR3+MMSET, and c-*maf* that are thought to be important for disease pathogenesis. Subsequent tumor progression correlates highly with deletion of chromosome 13, mutation of Ras, and inactivation of tumor suppressor genes p16INK4a and PTEN. Secondary translocations that activate c-*myc* and mutations that inactivate p53 are believed to drive progression into advanced stages of disease.[2] With the exception of XBP-1 and Blimp-1, little is known about the transcriptional factors that control the transition from activated B cell to plasma cell. XBP-1 (X-box-binding protein-1) is a basic-region leucine zipper protein in the CREB/ATF family of transcription factors, which is required for the generation of plasma cells.[3] Several mouse models of human plasma cell neoplasms (PCNs) built upon the genetic changes above have been developed. However, most of these models have the disadvantages of not having adequately recapitulated in mice the clinical characteristic of human MM or having limited preclinical use due to late onset, low incidence, and a propensity to grow in lymphoid tissues other than bone marrow. Established mouse models of PCNs include tumors that arose spontaneously in old C57BL/KaLwRij mice and resemble human MGUS and MM. Once these mice have reached 2 years of age 50% develop MGUS and 0.5% develop MM. Both tumors are localized predominantly in the bone marrow with MM developing osteolytic lesions. Virtually nothing is known about the molecular pathogenesis of these tumors except that, like early human MM, they rarely have Ig translocations involving c-*myc*.[4] The other models includes mouse plasmacytomas, which are tumors of mature end-stage B cells that can be induced in high frequency in genetically susceptible strains of mice such as BALB/cAN and NZB/BINJ by the intraperitoneal administration of plastics, paraffin oils, or pristane and further accelerated by injections of mice with transforming retroviruses.[5] Loss of Ink4a/Arf function has been shown to accelerate plasmacytomagenesis in non-permissive strains.[6] Pristane-induced plasmacytomas secrete Igs, predominantly of the IgA isotype, and greater than 95% of these neoplasms carry translocations between the myc oncogene on chromosome 15 and the Ig heavy chain locus on chromosome 12. As such, they represent a mouse model system for studying the pathogenesis of B-cell tumors, such as Burkitt's lymphoma in humans but not MM. Other models includes the transplantation of human MM cells into SCID mice that harbor preimplanted human fetal bone as a nesting ground for the tumor cells.[7] Currently emerging mouse models of human PCN are based on transgenic expression in B cells of IL-6[8] and fusion protein of nucleophosmin and anaplastic lymphoma kinase,[9] insertion of c-*Myc* into the Igh loci and targeted deregulation of both c-*Myc* and Bcl-XL in the B cell compartment.[10] More recently, the biological actions of X-box Binding Protein-1 (XBP-1), the differentiation and unfolded protein/ER stress response factor, have been examined in the lymphoid compartment of transgenic mice. On the basis of *XBP-1s* prominence in human MM and its potent transactivation potential, transgenic mice engineered to express the *xbp-1s* open reading frame under the control of the immunoglobulin V_H promoter and Eμ enhancer elements (Eμ) have been generated and characterized.[11] Through 20 weeks of age, *Eμ-xbp-1s* transgenic mice exhibited normal gross appearance, behavior, and weight curves. Histological surveys of the major organs, as well as flow cytometric profiles of spleens using the lymphoid cells markers, CD3 and B220 did not demonstrate any abnormalities. By approximately 40 weeks of age, *Eμ-xbp-1s* transgenic animals began to manifest phenotypic changes in the skin and kidneys. *Eμ-xbp-1s* transgenic mice exhibited an overall shortened lifespan resulting from severe cutaneous disease and/or myeloma. Sixty percent of *Eμ-xbp-1s* transgenic mice developed hair loss and skin thickening around axillary and neck regions, whereas control littermates were unaffected. This cutaneous presentation was observed in all independently derived *Eμ-xbp-1s* transgenic lines. Histological examination of the *Eμ-xbp-1s* skin revealed epidermal thickening and some degree of hyperkeratosis with follicular plugging as well as dermal fibrosis with mild lymphoplasmacytic infiltrates and vascular hyperplasia. By 40 weeks, *Eμ-xbp-1s* transgenic mice also showed renal pathology, including tubular cast deposition and glomerular changes characterized by mesangial widening and deposition of PAS-positive material. These renal lesions are similar to the pathologic manifestations present in human MM and other PC disorders with systemic chronic Ig overproduction and accumulation of light chains, paraproteins, and other Ig fragments. To assess whether these lesions were Ig deposits, immunofluorescence with antibodies specific for light and heavy chain Ig was performed. Non-specific trapping of light and heavy chain Ig was present and consisted of either polyclonal heavy and light chains or clonal IgM or IgG

heavy chain and kappa light chains. Histological and flow cytometric examination of the spleen and bone marrow of *Eμ-xbp-1s* transgenic mice showed alterations after 20-40 weeks of age. The spleens appeared slightly enlarged and showed increased plasma cells around periarterial sheaths. Flow cytometric analysis showed unaltered total numbers of T-cell (CD3) and B-cell (B220) populations in *Eμ-xbp-1s* transgenic spleens. The total number of mononuclear cells between wildtype and *Eμ-xbp-1s* transgenic spleens did not differ. *Eμ-xbp-1s* transgenic mice at 40 weeks of age showed a slightly enlarged population of $CD23^+$ cells and a reciprocal decrease in $CD23^-$ cells. In addition, the fraction of $B220^+$ cells in the bone marrow was significantly increased in *Eμ-xbp-1s* transgenic mice with an increase in the mature IgM^+B220^+ B cell population and a relative decrease of pro-B cells in relation to pre-B cells in *Eμ-xbp-1s* transgenic bone marrows. Consistent with the above observation of expanded B cell compartment, plasma immunoglobulin levels of both IgM and IgG types were significantly increased in *Eμ-xbp-1s* transgenic mice when assayed by ELISA. Serum protein electrophoresis revealed presence of an M spike in the majority of *Eμ-xbp-1s* transgenic mice but not in control littermates as early as 20 weeks of age, a feature that increased in frequency and magnitude with advancing age. Notably, these serum changes were associated in some cases with bone lytic lesions and increased numbers of plasma cells in the bone marrow that varied from 5-30% of the total bone marrow cellularity compared with < 5% for non-transgenic controls. The above constellation of findings prompted detailed analysis of aging *Eμ-xbp-1s* and non-transgenic controls for evidence of MGUS and/or MM. As early as 40 weeks of age a classical MGUS profile emerged only in the *Eμ-xbp-1s* transgenic mice. Between 11-20 months of age, approximately 26% of *Eμ-xbp-1s* transgenic mice showed a clonal M spike in the serum and expanded populations of clonal plasma cells in the bone marrow (<10% of the total bone marrow mononuclear cells), without bone lytic lesions and consistent with a pattern of MGUS. Between 14-24 months of age, 26% of *Eμ-xbp-1s* transgenic mice progressed to frank MM as defined by a clonal M spike in the serum, bone marrow consisting of > 10% of clonal plasma cells, and associated bone lytic lesions on radiographic examination. Neither MGUS nor MM was detected in control littermates at any age, indicating that XBP-1s overexpression promotes the development of a condition similar to MGUS and MM. In summary, spontaneous progression from MGUS to MM in the Eu-xbp-1s transgenic mice clinically mirrors disease progression in the human. Supporting a pathogenetic role for XBP-1s overexpression in the myeloma genesis in this model, several known MM genes that were dysregulated in premalignant *Eμ-xbp-1s* B cells exhibited similar patterns of alterations in XBP-1s MM cells, including Cyclin D1, MAF, CEBPA, B and D, IL6ST (upregulated), and FOS (downregulated). Conversely, some notable MM signature genes were found to be selectively dysregulated in *Eμ-xbp-1s* MM, but not in *Eμ-xbp-1s* B cells, in line with the need for additional cooperating events as reflected in the long latency of the MM phenotype. Among such genes selectively upregulated in the tumors were APRIL and BAFF, both of which have been found to be overexpressed in various B-cell malignancies, including MM. However, unlike the human counterpart, the *Eμ-xbp-1s* MM transcriptome revealed evidence of prominent activation of pro-apoptotic tumor suppression mechanisms characterized by downregulation of the anti-apoptotic genes, MCL1 and BCL2, which are typically upregulated or amplified in aggressive human MM cases. Interestingly, MCL1 and BCL2 were not downregulated in hyperproliferative premalignant transgenic B cells. Consistent with the *Eμ-xbp-1s* gene expression pattern, and in contrast to the human disease, we documented a marked increase in apoptosis and proliferation in murine MM samples relative to those in human MM samples. Such observations gain added significance in light of the observation that MCL1 maps to a region of gene amplification in aggressive human MM primary tumors and prompt speculation that MCL1 and BCL2 play critical roles in the progression of human myeloma – a theory that can now be tested in this genetic model system.

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S2.8

FROM ONCOGENOME MINING TO FUNCTIONAL VALIDATION OF MULTIPLE MYELOMA CANCER GENES

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MM is the second most common hematological malignancy and remains incurable. Unlike most hematological malignancies and more similar to solid tissue neoplasms, MM genomes are typified by numerous structural and numerical chromosomal aberrations. Extensive molecular, cytogenetic, and chromosomal CGH analyses have uncovered a number of recurrent genetic alterations in MM and its precursor MGUS, some of which have been linked to disease pathogenesis and clinical behavior, including five chromosomal translocations involving the IgH locus (11q13 (*CCND1*), 4p16 (*FGFR3/WHSC1*), 6p21 (*CCND3*), 16q23 (*MAF*) and 20q11 (*MAFB*)), resulting in deregulated expression of these target genes in neoplastic plasma cells.[1] Such translocations, present in MGUS, appear central to MM genesis, whereas progression is associated with mutational activation of *NRAS* or *KRAS*, or *FGFR3* oncogenes. Late mutational events involve inactivation of TP53 and DNA rearrangements involving *MYC*. Two oncogenic pathways have been hypothesized for MGUS/MM pathogenesis.[1] Hyperdiploid MM involves multiple trisomies of several odd chromosomes and usually presents a better prognosis, whereas non-hyperdiploid MM is associated with IgH translocations. Deletion of chromosome 13, especially band 13q14, is commonly observed and confers high risk. While these antecedent efforts have led to important insights into the pathogenesis and clinical behavior of MM, these numerous recurrent genomic alterations point to many undefined genetic elements which may prove relevant to disease initiation, progression and drug responsiveness. For example, while recurrent chromosomal gains have been mapped to 1q, 3q, 9q, 11q, 12q, 15q, 17q, and 22q and recurrent losses to 1p, 6q, 13, 16q, Xp, and Xq, the presumed cancer-relevant targets in these loci are not known. While traditional cancer treatments have not provided the hoped improvement in prognosis, the most held view in the field is that targeted therapies, directed against specific genes and pathways essential for tumor maintenance, will be the key for successful cancer therapies in the future. Encouraging successes have already been obtained with compounds, either monoclonal antibodies or small molecules, targeting proteins belonging to the kinase signaling pathways.[2] One of the technologies been used to identify such targets in MM is array comparative genomic hybridization (aCGH). Merging of aCGH data and expression data has provided powerful tools to sift through genes included in amplicons and identify bona-fide oncogenes. This smaller, more manageable list of candidate oncogenes could then be enlisted into more in-depth functional validation studies, to address their relevance in the context of carcinogenesis and most importantly on tumor maintenance.

Ouverture: gNMF classification of MM samples

We have analyzed 67 newly diagnosed MM patients prior to treatment,[3] using an Agilent high-resolution aCGH platform[4] as well as expression profiling.[5] We have modified an algorithm, based on non-negative matrix factorization,[6] to extract distinctive genomic features from aCGH profiles (gNMF).[3] Ranks K=2, 3 and 4 generated matrices showed stable cluster assignments suggesting the existence of up to 4 distinct genomic patterns among the MM samples. The rank K=2 classification divided the samples into a *kA* subgroup (n=38) characterized by odd-chromosome gains and a *kB* subgroup (n=29) characterized by loss of chromosomes 1p, 8p, 13, and 16q and amplification of ch1q. Thus, the K=2 classification yields a grouping reminiscent of the well-recognized hyperdiploid (*e.g.* odd-chromosome gain pattern) and non-hyperdiploid subclasses. With rank K=4 matrix, the samples were subdivided by gNMF into 4 distinct molecular subclasses, k1-k4. All 21 k1 and 16 k2 samples belonged to the kA subgroup, while all 13 k3 and 16 of 17 k4 samples were in the kB subgroup. These subgroups were characterized by different prognosis, with k1 having the better prognosis followed by k4 (enriched for 11;14 translocations), whereas k2 and k3 presented with the shorter survival.

Merging of expression and aCGH data

First act, the view from above: the case of chr.13 and 1q.

k1 and k2 derived from the kA, the *hyperdiploid group* and both showed odd-chromosome number gains. The only differences between the two groups were at chromosome 1q (gained/amplified in k2) and chr.13 (lost in k2). To gain an insight on the genes differentially expressed between the k1-k2 groups, a Significance Analysis of Microarrays (SAM) approach was applied to the corresponding transcriptome profiles. Significantly increased expression in k2 vs. k1 was noted for 111/2210 probes (95

genes) mapping to ch1q (FDR=15%), and decreased expression in k2 versus k1 for 48/1163 probes (46 genes) mapping to ch13 (FDR=15%). Strikingly, in both ch1q and ch13 cases, SAM-significant probes clustered in specific chromosomal bands, albeit aCGH data showed on the overall that the whole 1q and the whole chromosome 13 were affected by gains and losses respectively. We therefore tested more rigorously this apparent skewed distribution by counting significant genes in a 10 MB moving-window, and testing for significance by permutation of gene position.[7] This approach revealed an enrichment in overexpressed genes residing at 1q21-q23 (143-158MB (p<0.05)). Analogous studies of ch13 showed significant enrichment of underexpressed genes residing at 13q14 (38-50MB (p<0.05)), a region known with the highest frequency of LOH on ch13 in MM and including *RB1*. Thus, this approach of merging aCGH and expression data helped to further refine regions of gains and losses, based on the expression level of genes residing in such areas. Moreover, it provided a list of genes that might represent the targets of these genomic events in these areas.

Second act, in the trench: Gene Weight to address oncogenic overexpressed genes

Beyond whole chromosomal gains and losses, the high-definition picture of the MM genome enabled definition of recurrent CNAs with strong involvement in MM pathogenesis. Across our MM tumor collection, that includes also 43 MM cell lines, 87 prioritized Minimal Common Regions (MCRs)(47 amplifications and 40 deletions) were selected, based on the criteria of presence in primary tumors and occurrence of at least one high amplitude event (log_2 ratio >0.8).[3,7,8] These 87 MCRs had a median size of 0.89MB with an average of 12 known genes. Of interest, 14 MCRs were associated with poor survival, including an amplification on ch8 (*MYC*) and a deletion on ch17 (*TP53*) – both previously been linked to poor prognosis in MM. As copy number alterations function to alter expression of resident genes, we conducted an integrated RNA expression analyses by the Gene-Weight measure.[8,9] First, for each gene residing within an amplified MCR, we asked whether its expression showed a copy number correlated pattern by comparing the mRNA levels in tumors with and without CNAs in the region of interest. In addition, modeling after *bona fide* oncogenes, such as *MYC*, whose expressions are known to be dysregulated by mechanisms other than gene dosage alteration, we also compared expression of the gene in tumors with or without CNAs, relative to normal plasma cells, respectively. Genes showing this *oncogene-like* expression pattern – namely copy number correlated expression and significant overexpression in tumors without amplification vs. normal plasma cells – were considered high-probability candidates targeted for amplification in these MCRs during MM development. By such stringent criteria, approximately 30% of the 2151 genes residing in the high-priority MCRs were considered potential candidates. These included genes with credentialed roles in MM pathogenesis such as *MYC*, *MCL1*, *IL6R*, *HGF*, and *ABL1*, as well as many functionally diverse genes with no known link to MM development.

Third act: pathway analysis, Gene Set Enrichment Analysis (GSEA) in specific MM subgroups

The final method that we applied is Gene Set Enrichment Analysis (GSEA),[10] an analytical strategy designed to detect modest but coordinate changes in expression of functionally related genes. The expression level of normal plasma cells was compared with the expression level of plasma cells derived from patients in each gNMF group, i.e. k1-k4. On the overall, from k1 to k4 subgroups, there were a larger number of pathways showing increased levels of expression when compared with normal plasma cells. Interestingly, several oncogenic pathways were significantly altered in all MM samples, irrespective of the subgroups; prominent among them was the proteasome pathway, a finding in agreement with the proven clinical effectiveness of proteasome inhibitors in MM, and suggests that these compounds could be useful in all subgroups of the disease. Among the other pathways shared by all subgroups were the p53, KRAS and, surprisingly, mTOR pathways. Other pathways were altered in K2 and K4 but not in K1, as for example SHH, rac1 and TGF-beta. Strikingly, the k4 subgroup presented the largest number of cancer-related pathways altered, including IL3, IL6, IL8, IL2, IGF1, WNT and AKT suggesting extensive alteration and rewiring of critical cancer-related pathways with disease progression.

Finale: the future, functional genomics

The criteria for conclusively establish the causative role of a candidate oncogene are still in their infancy, and no consensus frame has been defined as yet.[2] Both gain-of-function studies, as well as loss-of-function assays, as for example siRNAs or shRNAs against specific targets, are providing useful, first-pass, albeit not conclusive information on the oncogenicity potential of a vast array of targets.[2] However, before embarking in costly drug development efforts, it is likely that *in vivo* models will be necessary to conclusively demonstrate the role of such targets, not only in the genesis and progression of cancer, but also in the maintenance of fully established cancers.

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S3: Pathophysiology - Bone disease

S3.1

INDUCTION OF MULTI-LINEAGE MARKERS AND THEIR DOWN-REGULATION BY IL-6 IN HUMAN MYELOMA CELLS

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Human primary myeloma cells lacking Pax-5[1,2] expression from multiple myeloma (MM) are well known to be heterogeneous with regard to the morphology and surface phenotype,[3,4] showing expression of multi-lineage markers such as CD33, CD7, CD56, CD4 or CD86. The expression of CD33[-5] in Liu01, a subclone from U266 cells and vitamin D3-treated ILKM3 cell lines correlated to monocytoid morphology with convoluted nuclei and increased expression of C/EBPα-[5]. The expression of CD56[-6] in myeloma cells resulted from differentiation into either neuronal cell lineage or NK cell lineage. Myeloma cell lines, NOP-2 and Liu01 cells expressed CD56, and also neuron-specific enolase (NSE) as well as primary myeloma cells. The expression of CD7 in Liu01 and forskolin-stimulated U266 cells was compatible with large granules in the cytoplasm, and showed increased expression of *perforin* mRNA and significant natural killer cell activity. Interleukin 6(IL-6)-7, a growth factor for myeloma cells, could down-regulate expression of CD33, CD7 or CD56 in primary myeloma cells as well as Liu01 cells. Therefore, these data suggest that Pax-5[-] myeloma cells, but not Pax-5[+] B cells, are capable of multi-lineage differentiation, and IL-6 can induce their dedifferentiation. *Pax-5*, a paired domain transcription factor,[1] is a master gene of B cell lineage[2] including plasma cells. In *Pax-5* knockout mice,[8] differentiation of B cell lineage is completely blocked at the pro-B cell stage, andinterestingly, Pax-5-negative(Pax-5[-]) pro-B cells can differentiate into other lineage cells within hematopoietic cell lineage such as granulocytes, macrophages, dendritic cells, NK cells or T cells with appropriate stimuli. These data suggest that any cells lacking the expression of master gene in their lineage can differentiate into other lineage cells over their own lineage (transdifferentiation) and these cells function as stem cell-like cells. Since human myeloma, a hematopoietic malignancy of plasma cells in B cell lineage, is a good example of lacking the expression of *Pax-5* gene, we examined whether myeloma cells from MM patients as well as myeloma cell lines showed expression of multi-lineage markers. By recent phenotypic analysis of plasma cells by using multi-color staining with anti-CD38 antibody, we found that there were really some not all myeloma cells expressing non-B cell markers, CD33, CD7, CD56, CD4 or CD86 in MM patients. These multi-lineage expressions were also found in human myeloma cell lines; CD33 expression on ILKM8 cells, CD7 on ILKM3, CD56 on NOP2, CD4 on AMO1 and CD86 on MSG-Y01 cells, respectively. It should be also noted that Liu01 cells, a subclone from U266 cell lines, include CD33[+] cells, CD56[+] cells, CD7[+] cells or CD56[+] cells, independently, not concurrently. As for Liu01 cells, most cells were CD33[+] with the monocytoid morphology of convoluted nuclei, compared with the morphology of parent U266 cells. Since the promoter of *CD33* gene contains three possible C/EBPα and PU.1 binding sites, profiling of expression of transcription factors concerning myeloid/monocytoid differentiation clearly showed that increased expression of *C/EBPα* gene was found in CD33[+] Liu01 cells, and also in ILKM8 and ILKM3, differentiation but still remain to be in B cell lineage. Among differentiation–inducing reagents reported in HL-60 cell system, 1α,25-dihydroxy vitamin D3 (vitamin D3) could induce ILKM3 and Liu01 cells to augment expression of CD33, while Pax-5[+] KUS or Raji cells showed no induction of CD33 or CD7 expression by vitamin D3 stimulation. CD56 expression is well known to be one of specific phenotype in Pax-5[-] primary myeloma cells from MM patients, but the

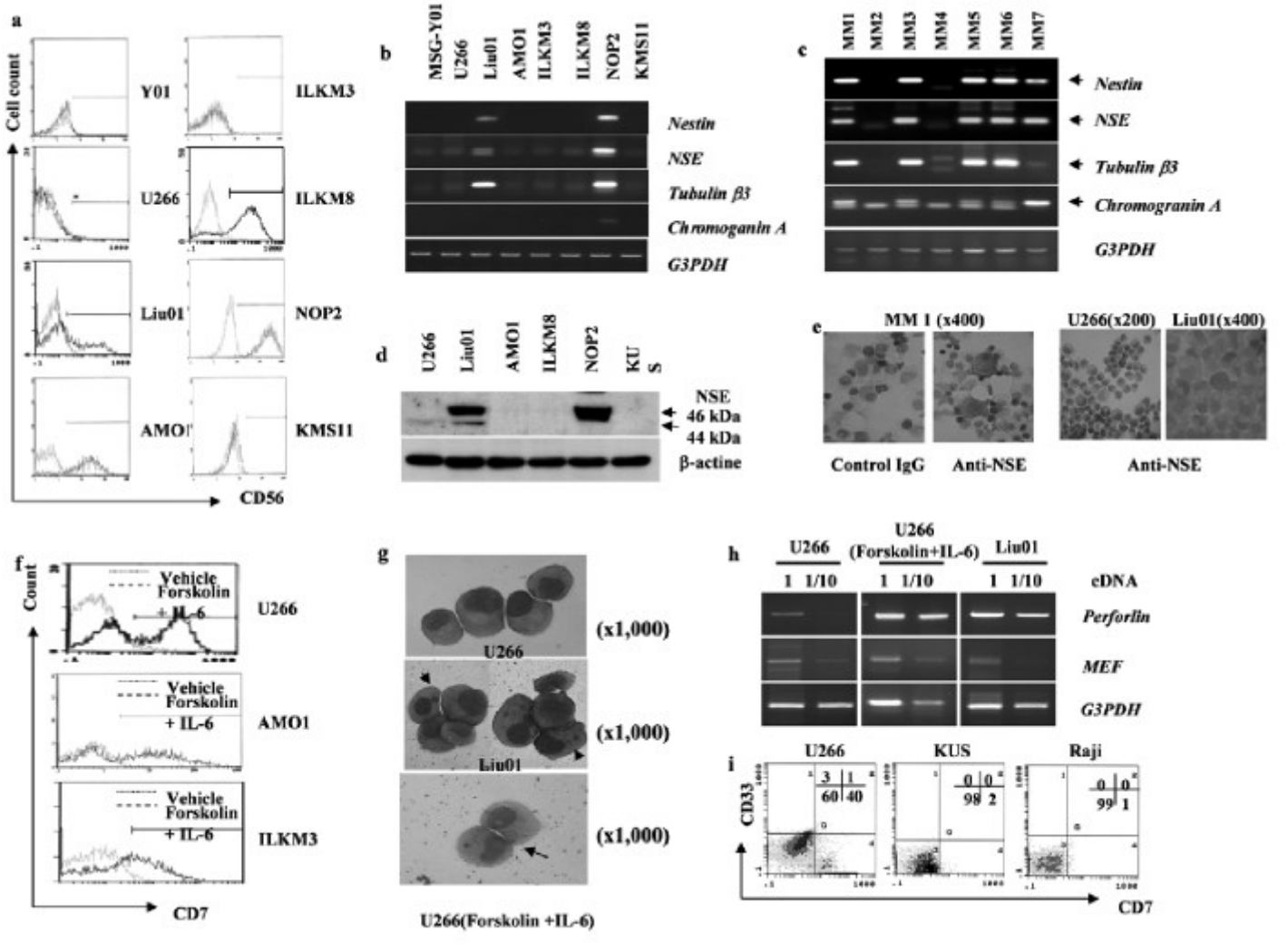

Figure 1. CD56 expression combined with the expression of neuronal cell markers or CD7 expression and NK cell activity in myeloma cells.

meaning of its expression on primary myeloma cells remained to be clarified. CD56 is also called neural cell adhesion molecule (NCAM), and is expressed on neuronal cells, natural killer cells (NK cells), a subset of T cells, neuroendocrine cells from prostate cancer, lung cancer, and pancreatic cancer, and also on myeloma cells, but not normal plasma cells. In contrast to primary myeloma cells, there existed rather a few myeloma cell lines expressing CD56 (Figure 1a). Since CD56 expression is found in either NK cell lineage or neuronal cells including neuroendocrine cells, we examined whether myeloma cell lines expressed neuron-specific genes such *as neuron-specific enolase (NSE), chromogranin A, nestin*, or *tubulinβ3*, or expressed the other NK cell marker, CD7, or not. Among CD56[+] myeloma cell lines, Liu01 and NOP2 showed expression of *NSE, tubulin β3* and *nestin gene*, while CD56[+] AMO1 and ILKM8 cells did not (Figure 1b). The expression of NSE protein in Liu01 and NOP2 was confirmed by western blotting (Figure 1d). In primary myeloma cells from MM patients, myeloma cells containing many CD56[+] cells in MM1, MM3, MM5, MM6 and MM7 did express the genes of *NSE, chromogranin A, nestin and tubulinβ3* , however, myeloma cells containing a few CD56[+] cells in MM2 and MM4 did not show expression of any these genes (Figure 1c). These data are understandable because CD56[+] primary myeloma cells are CD7[−], and so these cells are considered not to belong to NK lineage but to neuronal cell lineage. Furthermore, immunostaining with anti-NSE antibody on the cytospined slides showed expression of NSE in myeloma cells of bone marrow mononuclear cells from MM 1 patient and positive staining of NSE in some of Liu01 cells but not U266 cells (Figure 1e). Since another possibility of CD56 expression is derived from NK lineage, we examined whether myeloma cell lines could differentiate into CD7-expressing cells by forskolin stimulation. Forskolin, an activator of adenylate cyclase, is well known to induce neuroendocrine differentiation in prostate cancer and lung cancer cells.[26] CD7[−] myeloma cell lines, U266, AMO1 and ILKM3 cells, could express CD7 on their surface by stimulation of forskolin (10 μM) combined with IL-6 (5 ng/mL) for 5 days (Figure 1f). These U266 cells cultured with forskolin and IL-6 for 5 days showed large granules in the cytoplasm as shown in CD7[+] Liu01 cells (Figure 1g). The induction of CD7 expression is also compatible with increased expression of *perforin* gene in U266 cells stimulated with forskolin and IL-6, as well as Liu01 cells (Figure 1h). Also, stimulation with forskolin and IL-6 induced expression of CD7 but not combined with CD33 expression in U266 cells. In contrast to Pax-5[−] U266 cells, however, the induction of CD7 expression was never observed in Pax-5[+] KUS or Raji cells stimulated forskolin and/or IL-6 (Figure 3I). Therefore, expression of CD56 in myeloma cells resulted from differentiation into either neuronal cell lineage or NK cell lineage; CD56[+]NSE[+] means neuronal cell lineage, and CD56[+]CD7[+] means NK cell lineage. Interleukin 6(IL-6) is a growth factor for Pax-5[−] myeloma cells but not Pax-5[+] normal plasma cells; IL-6 induces proliferation of myeloma cells, which is considered to be their self-renewal by IL-6. As mentioned above, Liu01 but not U266 express CD33 on their surface, but if cultured with IL-6 (10 ng/mL) for 12 days, expression of CD33 on Liu01 cells completely disappeared as shown in Figure 2a. Also, expression of CD56 and CD7 was markedly reduced by stimulation of IL-6 in Liu01 cells (Figure 2b). By IL-6 stimulation, the morphology of Liu01 cells was also reversed to that of parent U266 cells; convoluted nuclei in Liu01 cells disappeared and returned to round nuclei as observed in U266 cells (Figure 2c). It is also confirmed that increased expression of *C/EBPα* was markedly reduced to the same level as U266 cells after 5 days culture of IL-6 (Figure 2d). Furthermore, IL-6-induced downregulation of expression of translineage markers was also observed in primary myeloma cells; as representatively shown in Figure 2e, myeloma cells from MM7 patient were cultured with or without IL-6 (50 ng/mL) for 7 days, and both CD33[+] and CD56[+] cells were significantly decreased in the presence of IL-6. Apparently, Liu01 cells are considered to be differentiated cells, and parent U266 cells are undifferentiated cells. Parent U266 cells respond to IL-6 to proliferate well, but Liu01 cells are

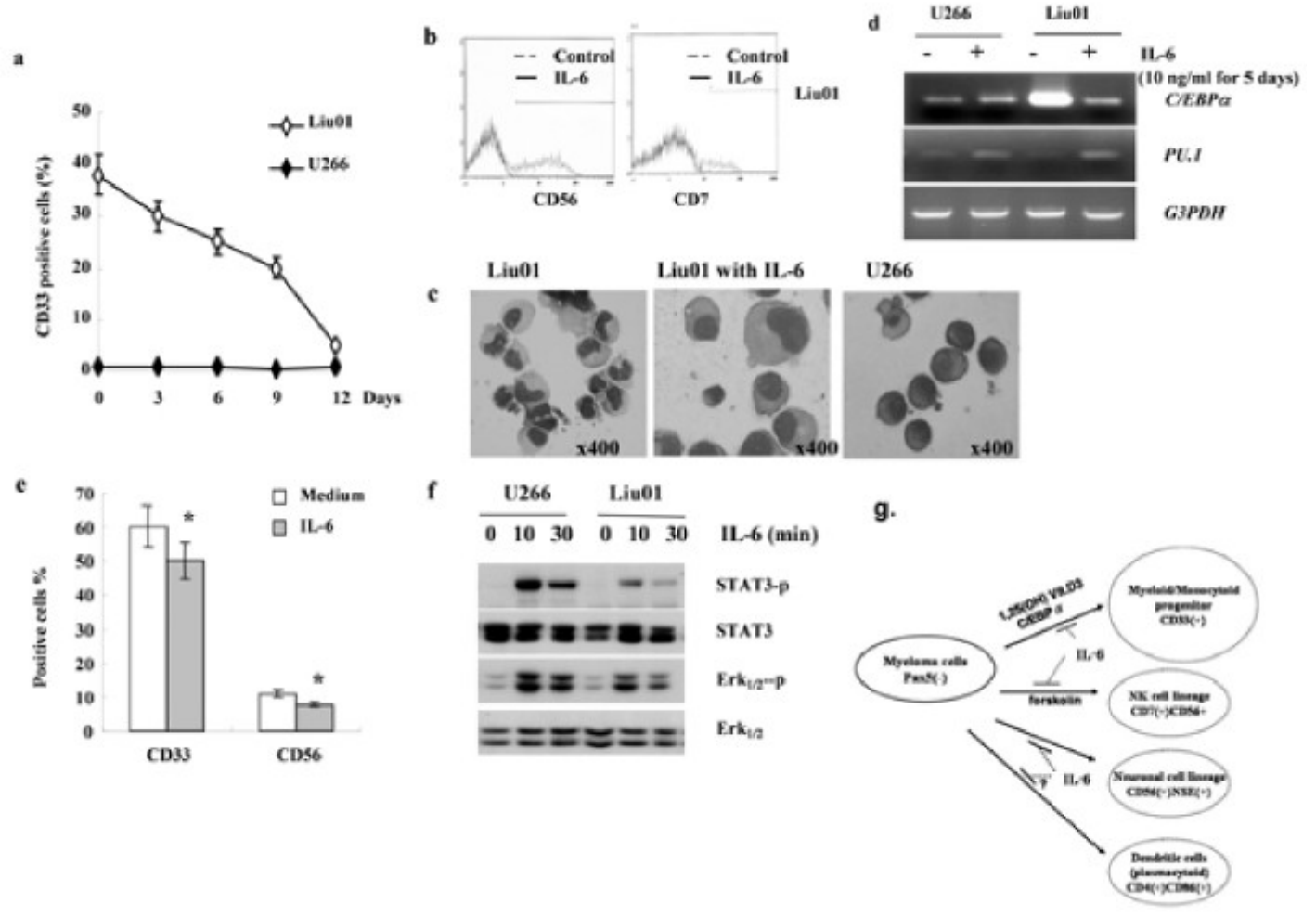

Figure 2. IL-6 reduced CD33, CD56, or CD7 expression in myeloma cells.

less responsive to IL-6 to proliferate. This is well explained by the finding that phosphorylation of STAT3 was less induced in Liu01 than U266 by IL-6 stimulation (Figure 2f). Activation of STAT3 is generally considered to be very important to maintain so-called stem cells and keep them undifferentiated state. Here, we really show direct evidence that in human multiple myeloma (MM), cancer cell lacking expression of master gene can transdifferentiate and their growth factor dedifferentiate them (Figure 2g). Now, it is believed that a small subset of cancer cells, so called cancer stem cells, can proliferate in the hematopoietic malignancies.[9] In another word, cancer cells are phenotypically heterogeneous and only a small proportion of cells are proliferative or clonogenic. Our findings provide a new aspect that in MM, myeloma cells lose the expression of *Pax-5*, a master gene in their lineage, and so these cells dropped out of their lineage (B cell lineage) can become stem cell-like cells; we called them *stone cells* (stem cell-like cells dropping out of the lineage).[10] In stead of cancer stem cells, it is one of explanations of cancer heterogeneity that some cancer cells are really stone cells that can proliferate or make self-renewal by their growth factor, and can differentiate into multi-lineage cells, presenting cancer cell heterogeneity.

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S3.2

PATHOPHYSIOLOGY OF MYELOMA BONE DISEASE

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A major clinical feature of multiple myeloma is the development of bone disease. This is characterised by the presence of osteolytic bone lesions and generalised osteoporosis, leading to pathological fracture and bone pain. The bone destruction is mediated by both an increase in osteoclast formation and resorptive activity, and a suppression of osteoblastic bone formation. Until recently the identity of molecular mechanism responsible for the development of myeloma bone disease has been unclear. However, improvements in our understanding of normal bone biology and the development of appropriate murine models of myeloma bone disease to allow functional studies, has resulted in significant new advances in our understanding. The majority of research has focused upon investigating the mechanisms responsible for the increased osteoclastic bone resorption. These studies have demonstrated that a number of molecules may play important roles. The ligand for receptor activator of NFκB (RANKL) and its decoy receptor osteoprotegerin (OPG) are abnormally expressed in myeloma. RANKL expression is increased in the cells of the bone marrow microenvironment and may also be expressed by myeloma cells themselves.[1,2] Furthermore, OPG is down-regulated in cells found in the bone marrow in response to contact with myeloma cells.[1] In patients with myeloma circulating concentrations of soluble RANKL are elevated, whereas levels of the decoy receptor are decreased. Targeting this system, with recombinant OPG or soluble RANK constructs has been shown to prevent the development of osteolytic disease.[1,2] Furthermore, OPG peptidomimetics, designed on the basis of the predicted structure of the RANKL/OPG complex, prevent the development of bone disease in the 5T2MM murine model. These observations in murine models have led to the evaluation of novel anti-RANKL strategies in clinical studies in patients with myeloma. In addition to RANKL, macrophage inflammatory 1α and β have both been implicated in the development of myeloma bone disease.[3,4,5] Myeloma cells have been shown to produce MIP1α and β and inhibiting MIP1α in murine models prevents the development of bone disease.[3,4,5] In addition to RANKL and MIP1α other molecules including hepatocyte growth factor and interleukin-3 have also been implicated in regulating osteoclastic bone resorption; however, as yet there are limited *in vivo* functional data to support a causal role. Stromal derived factor 1α is an additional factor produced by some myeloma cells. Rather than promoting osteoclast formation SDF1α promotes osteoclast activity.[6] Circulating serum concentrations of a number of these molecules are also increased in patients suggesting that may contribute to the generalised bone loss as well as reflect the development of focal osteolytic disease. Whether each of these molecules promotes bone resorpton via induction of RANKL or they do so in a RANKL independent manner also remains unclear. Equally, the relative contribution of these molecules and their role in patients remains to be established. However, their discovery has resulted in the identification of a number of potential new targets for future therapeutic exploitation. In addition to stimulatory effect on osteoclasts, myeloma cells also suppress bone formation. *in vitro* studies have shown that myeloma cells inhibit the differentiation of osteoblast precursors and can induce apoptosis in mature osteoblasts. Although, our understanding of the molecular mechanisms responsible is limited a number of pathways have recently been implicated. The Wnt signaling pathway is one system that has been shown to play a key role in normal osteoblast differentiation. Tian *et al.* have demonstrated that dickkopf-1 (*DKK1*), an inhibitor of Wnt signaling is produced by some myeloma cells and may inhibit osteoblast differentiation and activity *in vitro*.[7] Inhibiting the activity of Dkk1 with an anti-Dkk1 antibody in the SCID/ model has been shown to prevent the development of myeloma bone disease. Dkk1 is also increased in the serum of patients with myeloma. Another inhibitor of Wnt signalling, soluble frizzle-related protein-2 (sFRP-2) has been shown to be produced by myeloma cells and implicated in the suppression of bone formation.[8] Furthermore, other molecules that are likely to function independently of the Wnt signalling system, including IL-7 and IL-3 have also been reported to be increased in myeloma and inhibit osteoblast differentiation either directly or indirectly, *in vitro*.[9,10] The precise role of each of these molecules has yet to be established, and in particular their role *in vivo* is often unclear; however, the net result is the inhibition of osteoblast differentiation and reduced bone formation. Interestingly, approaches to targeting osteoblasts are effective in preventing the development of myeloma bone disease *in vivo*, suggesting that osteoblasts may play a critical role in driving the development of myeloma bone disease. Thus, recent investigations have identified some of the key signalling pathways that contribute to both the increase in osteoclastic bone resorption and the suppression of new bone formation. Clearly, this understanding will greatly facilitate the design of new approaches to treating this important aspect of multiple myeloma.

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S3.3

THE ROLE OF BISPHOSPHONATES IN THE MANAGEMENT OF MYELOMA

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Multiple myeloma (MM) is a B-cell malignancy characterized by enhanced bone loss commonly associated with diffuse osteopenia, focal lytic lesions, pathologic fractures, hypercalcemia and bony pain. Consequently, MM patients frequently require radiation therapy, surgery and analgesic medications. Even when MM patients respond to anti-MM therapies, they may still have progression of skeletal events without repair of osteolytic lesions. Bisphosphonates are specific inhibitors of osteoclastic activity and these agents have been evaluated in MM patients with bone disease during the past 15 years. Monthly intravenous infusions of either pamidronate or zoledronic acid have reduced the skeletal complications among MM patients and are now a mainstay of myeloma therapy. A large, randomized, double-blind study was conducted to determine the effect of monthly 90 mg infusions of pamidronate in MM patients. At the preplanned primary endpoint after nine cycles of therapy, the proportions of myeloma patients having any skeletal event was 41% in patients receiving placebo but only 24% in pamidronate-treated patients. The proportion of patients developing any skeletal event and the skeletal morbidity rate continued to remain significantly lower in the pamidronate group than the placebo group during the additional twelve cycles of treatment. Zoledronic acid is an imidazole-containing bisphosphonate that shows more potency in preclinical studies than any other bisphosphonate currently available. A phase III trial evaluated two doses of zoledronic acid (four and eight mg) compared with pamidronate (90 mg) infused every three to four weeks for treatment of myeloma or breast cancer patients with metastatic bone disease. The results of the study showed that the proportion of patients with any skeletal event did not differ among the three treatment arms. In addition, the time to first skeletal event and analgesic use was similar in the three groups (12 to 13 months). Moreover, after 25 months of follow-up, the overall proportions of patients developing skeletal events remained similar between the zoledronic acid (four mg) and pamidronate-treated patients. However, in an additional preplanned analysis, the multiple events analysis, zoledronic acid-treated patients showed a 16% reduction in risk of developing skeletal complications compared with those patients who received pamidronate. Importantly, during the clinical trial, rises in creatinine level were more frequently observed in the zoledronic acid arms. Because of the renal toxicity, infusion time of zoledronic acid was increased to 15 minutes and patients in the 8 mg zoledronic acid group subsequently had their dosage reduced to 4 mg. Long-term follow-up data are now available that show no difference in the renal profile between patients receiving 4 mg of zoledronic acid infused during 15 minutes and 90 mg of pamidronate infused during 120 minutes. Another complication that may result from bisphosphonate therapy is osteonecrosis of the jaw (ONJ). Over the past several years, a number of case reports in the medical and dental literature appeared suggesting that this potential complication was developing among cancer patients receiving either long-term zoledronic acid or pamidronate treatment. ONJ has also been infrequently observed in patients teated with oral alendronate and risedronate for postmenopausal osteoporosis. The frequency with which this complication occurs in cancer patients receiving intravenous bisphosphonate therapy is unknown. However, it appears that there is a higher risk of this complication among patients receiving these drugs with the incident rate of one to ten percent. Moreover, myeloma patients appear to be at increased risk compared to other cancer patients who receive intravenous bisphosphonate treatment for unknown reasons. Most cases are associated with exposed mandibular or less frequently maxillary bone with minimal symptoms, but infrequently patients may require more extensive intervention, including surgical procedures to treat this problem. Histologically, necrotic bone with associated Actinomyces colonization is consistently seen. Risk factors for ONJ may be multifactorial and include previous dental work, poor dental hygiene and tobacco or alcohol abuse. To date, no reliable, predictive pattern has been clearly identified to allow for calculations of risk for ONJ in a particular patient. It is now recommended that patients receiving bisphosphonates, including most myeloma patients, should be evaluated early in their treatment for dental problems and encouraged to maintain excellent dental hygiene. The course of this complication is variable but in many patients, the condition does not worsen and, in fact, many show improvement over time. No evidence exists that discontinued use of the bisphosphonate or replacement with other bisphosphonates changes the course of this complication. Therefore, it is critical that clinicians assess the risks and benefits of the use of bisphosphonates for individual MM patients. Several guidelines recently published have suggested less frequent dosing and possible discontinuation of these agents after a fixed period of time is warranted because of these potential complications. However, this must be weighed against the ongoing bone destruction with its devastating clinical consequences that had been the hallmark of myeloma until the era of widespread use of long term monthly intravenous bisphosphonate therapy. Alternative dosing regimens have not been evaluated for either safety or efficacy. In addition, the discontinuation of these agents among patients who are doing well after receiving them for several years may have untoward effects on their long term bone health. Indeed, it may be the group that continues to be without skeletal complications and progressive disease while on bisphosphonate treatment may be just the group that derives the most benefit from continuation of these agents. In addition, recommendations that pamidronate should be initiated rather than zoledronic acid among myeloma patients with bone loss have been made by some guidelines based on the supposed increased risk of ONJ among patients receiving the newer bisphosphonate. These studies are not prospective or randomized and their conclusions are based on small numbers of patients who have been observed during different time periods. Time-to-event (ONJ) analyses are flawed by the marked differences in the time that these different drugs have been available in the clinic. It is clear that the time period during which zoledronic acid has been available has also been accompanied by the introduction of many new agents into the clinic for myeloma patients that were not available in the pamidronate era of the 1990's. These newer agents have not only prolonged the survival of myeloma patients (increasing the time period during which the patient is at risk for ONJ) but also may serve as additional risk factors for ONJ. Some studies have supported this latter possibility. Moreover, the role of bisphosphonates for myeloma patients may go beyond simply inhibiting bone resorption and the resulting skeletal complications. Recent pre-clinical studies have established their direct and indirect anti-myeloma effects in the laboratory. Interestingly, a survival advantage was observed in the subset of patients who failed first-line therapy at the time of enrollment who received monthly pamidronate compared to the placebo-treated group. In addition, a recent retrospective analysis from the randomized trial comparing monthly zoledronic acid 4 mg to pamidronate 90 mg shows that patients with elevated bone alkaline phosphatase levels show superior survival with zoledronic acid compared to pamidronate treatment. Future clinical trials will hopefully further evaluate the potential anti-myeloma effects of these agents, and establish their role as not only important inhibitors of bone loss but as potential anti-MM drugs as well.

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S3.4

THE ROLE OF BIOCHEMICAL MARKERS OF BONE METABOLISM IN MULTIPLE MYELOMA. ADVERSE EVENTS OF BISPHOSPHONATES ADMINISTRATION

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A major feature of muliple myeloma (MM) is osteolytic bone disease, present in about 75% of patients at diagnosis. Over a median follow-up time of 21 months, more than half of all MM patients will have experienced ≥1 skeletal-related event (SRE). In addition to increased bone resorption, a significant reduction in bone formation is observed in MM, further disrupting the normal coupling of bone remodeling. As a result, bone lesions do not normally heal even if MM goes into remission. Radiographs frequently do not indicate increased bone resorption in MM progression. Thus, biochemical markers of bone metabolism have been used in an effort to better monitor the myeloma bone disease and improve assessment of disease progression.

Markers of bone resorption and myeloma bone disease

So far, bone resorption markers such as serum C-terminal cross-linking telopeptide of type-I collagen generated by metalloproteinases (ICTP) and urinary N-terminal cross-linking telopeptide of type-I collagen (NTX) have demonstrated more consistent results in determining disease state and predicting skeletal morbidity and disease progression in MM; levels are higher vs. healthy controls, and prognosis is poorer when levels are higher thereafter. ICTP produced the most accurate elevated levels among MM patients, but NTX produced better results among patients with impaired renal function. Furthermore, in histomorphometric studies of bone biopsies in MM, NTX had the strongest positive correlation with dynamic histomorphometric indices of bone resorption, while ICTP had a slightly weaker correlation. Both markers have correlated with the extent of lytic disease and advanced stage at diagnosis, markers of disease activity such as β2-microglobulin and interleukin-6, and overall survival. Serum CTX, though studied in fewer trials, was also correlated with elevated readings in patients with MM and advanced lytic disease. Finally, tartrate-resistant acid phosphatase type-5b (TRACP-5b), an enzyme produced by activated osteoclasts, was also increased in both newly diagnosed and relapsed/refractory MM patients and associated with radiographically assessed severity of bone lytic disease.

Markers of bone formation and myeloma bone disease

Markers of bone formation have shown equivocal results in monitoring bone disease in MM. The most favorable results have been with bone-specific alkaline phosphatase (bALP), which correlated significantly with bone pain, lesions, and fractures in MM patients. Serum bALP levels have been found to be either reduced or within normal limits in most MM studies. Osteocalcin (OC) has also received conflicting results in trials. OC levels were found to correlate with lytic bone disease and MM stage in some studies. In other studies, however, OC did not show any correlation with SREs or disease progression. The unique nature of OC seems to contribute to these paradoxical results. Although produced by osteoblasts, OC is released during bone matrix mineralization. Thus, inhibition of osteoblasts may not lead to suppressed matrix mineralization. Also, OC is a relatively unstable protein, rapidly metabolized in the kidney after release into the circulation. Renal dysfunction, even at an early stage of MM, may reduce the glomerular filtration rate and produce falsely increased OC levels.

Bone markers for monitoring bisphosphonate therapy

Both pamidronate and zoledronic acid are very effective in reducing bone resorption in MM. In a study of MM patients randomized to pamidronate or no treatment (n=63), both NTX and TRACP-5b were reduced from the second month of pamidronate therapy vs. patients not treated with pamidronate. In another study, comparing MM patients randomized to pamidronate or ibandronate, TRACP-5b was reduced in the second month with pamidronate and in the sixth month with ibandronate, with more reduction in the pamidronate group (p=0.014); NTX values fell for both groups from the second month, also with more reduction in the pamidronate group (p=0.002). Finally, zoledronic acid at a dose of 4mg, IV, monthly has been proven more efficacious in reducing NTX levels than 90 mg of IV pamidronate. But is this reduction of bone resorption markers clinically important First of all, Coleman *et al.* showed that NTX levels provide valuable prognostic information in MM patients with bone lesions who receive bisphosphonates. Examination of the MM subset (n=510) of a large randomized trial showed that, among patients with high NTX levels (≥100 nmol BCE/mmol creatinine) during treatment with zoledronic acid, the relative risk for developing a first SRE was 3-fold higher than for patients with low NTX (<50 nmol/mmol creatinine) during treatment (p=0.008). Likewise, for patients with moderate on-study NTX levels (50-99 nmol/mmol creatinine) vs. low levels, the risk of SREs was increased approximately 2-fold (p=0.016). These results support the hypothesis that patients who continue to have high or moderately high levels of bone metabolism during bisphosphonate therapy have an increased risk for skeletal morbidity and more rapid disease progression compared with patients who have normalization of bone metabolism with bisphosphonates. The correlation between NTX and clinical outcomes was examined in a much larger database from the randomized trial of zoledronic acid vs. pamidronate in patients with breast cancer or MM. Results from this exploratory analysis showed that among patients (n=170) who had high baseline NTX (≥64 nmol/mmol creatinine), patients with persistently elevated NTX levels after 3 months of zoledronic acid therapy 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 with patients who normalized NTX. Therefore, normalization of elevated NTX levels after 3 months of therapy appears to correlate well with a reduced risk of SREs and a delay in time to first SRE. In this study, among patients with high NTX at baseline, 15% treated with zoledronic acid and 30% treated with pamidronate did not normalize NTX after 3 months of bisphosphonate therapy.

Other anti-myeloma therapies and bone markers

Thalidomide reduces bone resorption markers (CTX, NTX and TRACP-5b), mainly through the reduction of tumor mass, in both newly diagnosed and refractory/relapsed patients. More interestingly, bortezomib seems not only to reduce bone resorption but also to increase bone formation as assessed by an increase in serum bALP, and OC, but also by an increase in osteoblast counts in bone biopsies. However, this effect on bone formation is reduced when bortezomib is combined with other anti-myeloma agents such as thalidomide, melphalan, and dexamethasone. Autologous stem cell transplantation (ASCT), the treatment of choice to-date for eligible MM patients, also suppresses bone resorption. Two months after ASCT there is a significant reduction in NTX and TRACP-5b; OC and bALP levels rise later, at 9 and 11 months, respectively.

RANKL/OPG and potential new markers

Serum markers of osteoclast stimulation, such as the soluble and total receptor activator of nuclear factor-kappaB ligand (RANKL) and osteoprotegerin (OPG) have been also measured in MM. The ratio of RANKL to OPG has correlated with bone resorption markers, osteolytic lesions, and disease activity markers in newly diagnosed MM patients and has even been prognostic for survival. It is reduced post ASCT, thalidomide/dexamethasone and bortezomib therapy. Last, molecules that inhibit osteoblast function, such as dickkopf-1 (Dkk-1), an antagonist of the *Wnt* signalling is overexpressed in the myeloma microenvironment and correlates with myeloma lytic disease. Our group has measured Dkk-1 serum levels and found them to be increased in newly diagnosed and refractory/relapsed MM patients vs. MGUS patients and controls, while they are reduced post-bortezomib therapy, possibly contributing to the increase of osteoblast function. However, more studies are needed to determine the exact role of these molecules in assessing and monitoring myeloma bone disease.

Side-effects of bisphosphonates

Bisphosphonates are, in general, well tolerated in patients with MM. Most commonly reported adverse events with pamidronate and zoledronic acid include skeletal pain, fatigue, nausea, vomiting, flu-like syndrome with fever, mild infusion site reactions, hypocalcaemia and headache; these are similar in frequency with both agents. Flu-like symptoms usually occur after the first or second dose of the drug and they do not indicate discontinuation of treatment. Uveitis and other ocular manifestations, including iritis, have also been described. A clinically insignificant decrease in hemoglobin and platelet counts has been also reported in patients receiving pamidronate or zoledronic acid beyond 24

months Renal impairment has been described in patients with prolonged administration of pamidronate or zoledronic acid. The renal dysfunction is reversible in the majority of patients and the re-treatment with the same dose, over a longer infusion time (>2 hours for pamidronate and >15 min for zoledronic acid), does not usually lead to kidney problems. In patients with pre-existing renal disease, there is no need for change in dosage or infusion time if serum creatinine levels are less than 3.0 mg/dL (265 μmol/L). In general, infusion times less than 2 hours for pamidronate or less than 15 min for zoledronic acid should be avoided.

Osteonecrosis of the jaw and bisphosphonates

Avascular osteonecrosis of the jaw (ONJ) is a recent complication that has been described in MM patients who receive potent bisphosphonates. ONJ has an incidence of approximately 5-10%, appears to be time-dependent, correlates mainly with zoledronic acid administration, older age, and dental extractions. No satisfactory therapy is currently available. Therefore, patients who receive bisphosphonates should improve their oral hygiene, while hematologists and dentists should be aware of this complication and its management. Reinitiating bisphosphonate therapy in patients suffering osteonecrosis is debated and warrants further study. Pathogenesis of ONJ remains unclear and experimental models are urgently needed to clarify its mechanisms.

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S3.5

NEW AGENTS TARGETING MYELOMA BONE DISEASE

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Osteoclastic bone destruction is responsible for many of the most distressing symptoms in patients with myeloma (MM). Release of growth factors from bone by osteoclasts (OCL) results in both tumor growth as well as increased bone destruction. In addition, osteoblast activity is also suppressed. Tumor-stromal cell interactions via VCAM-1 on stromal cells result in release of RANKL, a potent inducer of OCL formation, IL-6, MCP-1 and TNF-α, which increase adhesive interactions between MM cells and marrow stromal cells and the growth of MM cells. These adhesive interactions also increase chemoresistance and production of osteoclastogenic factors by MM cells, including macrophage inflammatory peptide-1alpha (MIP-1α), IL-3 and RANKL. Since NF-κB signaling plays an important role in these processes, targeting the NF-κB pathway should have major therapeutic benefits for patients with MM. p62 (sequestosome-1) is a recently described member of the NF-κB signaling pathway, which is activated by TNF-α, RANKL and IL-1, and is also involved in multiple other signaling pathways, which enhance MM cell survival and bone destruction. Thus inhibiting p62 expression and/or activity should profoundly diminish osteolytic bone destruction and MM cell growth by blocking the production of RANKL, TNF-α and IL-6 and upregulation of VCAM-1 by marrow stromal cells. Therefore, we used stromal cells from p62-/- mice to determine the effects of deleting p62 on the growth of MM cells and OCL formation. Cells lacking p62 showed decreased production of IL-6 and TNF-α compared to wild-type (WT) stromal cells. Furthermore, coculture of p62-/- stromal cells with MM cells resulted in decreased growth of MM cells by 70% as well as decreased production of IL-6 and TNF-α by the stromal cells. In addition, VCAM-1 expression on marrow stromal cells cocultured with or without was markedly decreased at the mRNA and protein level. Finally, p62-/- stromal cells did not support OCL formation compared to WT stromal cells. These results demonstrate that p62 plays an important role in controlling MM cell growth and bone destruction in the bone microenvironment and support p62 as the potential novel target for treating MM bone disease.

S3.6

RANDOMISED STUDY ON PROPHYLACTIC PAMIDRONATE 30 MG VS. 90 MG IN MULTIPLE MYELOMA

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Prophylactic bisphosphonates are used worldwide in multiple myeloma patients, and two major randomised studies have shown significant effect on skeletal events of i.v. pamidronate 90 mg once a month;[1] and of oral clondronate 1600 mg daily[2] compared to placebo. However, no larger clinical study has addressed the dose-efficacy question, which is increasingly interesting due to the recent knowledge of renal toxicity and osteone-crosis of the jaw (BON). In 2000 we decided to perform a randomised double blind trail on i.v. pamidronate 30 mg versus 90 mg monthly for at least 3 years. We present the data of the final analysis. Design: Patients with newly diagnosed symptomatic multiple myeloma were allocated to one of the two doses. Strati-fication according to planned high dose therapy or conventional therapy and β2-m > 2.6 mg/L or lower was performed. The patients were followed every third month with respect to skeletal event, toxicity and response, while the QLQ was mailed directly to the patients every third month. Skeletal X-rays were routinely performed before, 9 and 12 months after start of pamidronat therapy. Primary end-point was physical function at 12 months determined by EORTC QLQ30 quality of life questionnaire (QLQ), while secondary end-points were time to objective skeletal events, cost-utility analysis, response of myeloma disease, re-sponse duration and survival, fatigue and pain determined by the QLQ. Furthermore explorative subgroup analysis of patients treated with high-dose therapy and different prognostic stages was performed. *Results*. 505 patients were randomised. The median follow up was 3,7 years (1.1 to 5.7 years). The initial analysis of QLQ data shows no significant difference regarding the primary end-point. Results from the secondary endpoints will be presented together with information on the incidence of BON. *Conclusions*. The primary end-point result will be discussed and compared with the data on skeletal events and toxicity in order to make recommendation for future bisphosphonate treatment in multiple myeloma.

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S3.7

A TRANSGENIC MOUSE MODEL THAT FAITHFULLY REPRODUCES THE PATHOGENESIS, BIOLOGY AND CLINICAL FEATURES OF MULTIPLE MYELOMA

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Introduction. Multiple myeloma (MM) is an indolent tumor of immunoglobulin class switched, somatically hypermutated, post germinal center (GC), fully differentiated plasma cells (PCs) that slowly accumulate in the bone marrow (BM). No mouse model exists which faithfully reproduces human MM. Here, we report the generation of the first mouse model of MM in an immune competent mouse, with indolent PC growth confined to the BM, and in which the major biological and clinical features of the human disease are recapitulated. *Methods*. VK*MYC mice were generated by placing inactive MYC under the control of Vk regulatory elements in a way that its expression can only be activated sporadically in GC B cells that undergo somatic hypermutation. Results All Vk*MYC mice develop monoclonal PC expansion (>10% PC in BM) by 50 weeks of age, that is manifested by high levels of serum IgG (20 g/L) and major M-spikes by SPEP. The PCs in Vk*MYC mice are fully differentiated ($CD19^{-}CD138^{+}$), somatically mutated, have a very low proliferation index and are found exclusively in the BM. Vk*MYC mice also develop anemia (Hgb 8.9 vs 13.4 g/dL in wt controls) and bone disease (low bone mineral and trabecular densities), with sporadic occurrence of lytic bone lesions and hind limb paralysis. 30% of the mice progress to an extra-medullary disease with ascites or plasmablastic lymphoma (PBL), in which PCs become proliferative and express a more plasmablastic phenotype (CD45R). This suggests that the BM dependent growth of MM relies on localized survival signal(s), abrogated by the acquisition of secondary mutations. Proving this hypothesis, Vk*MYC mice crossed with mice expressing the anti apoptotic gene Bcl2 develop an aggressive disease not confined to the BM and associated with shorter survival. These features all contribute to VK*MYC mice closely mimicking the clinical behavior of human MM, and showing significant clinical responses to drugs that are known to be active against MM (melphalan, dexamethasone, bortezomib, lenalidomide) while demonstrating no response to drugs with little or no clinical activity (fludarabine, hydroxyurea, vincristine). *Conclusions*. The VK*MYC mice represent a faithful model of MM and will be useful in the study of MM disease biology as well as the development of pharmacological and immunological therapies.

S4: Novel treatment approaches

S4.1

THE TREATMENT OF RELAPSED AND REFRACTORY MYELOMA: FOCUS ON BORTEZOMIB AND BORTEZOMIB-BASED COMBINATIONS

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Relapsed and refractory multiple myeloma (MM) patients constitute a specific and unmet medical need, where median survival ranges from as little as 6 to 9 months and responses to treatment are characteristically short. Relapsed/refractory disease is defined as patients who achieve minor response or better followed by relapse and then progress on salvage therapy, or experience progression within 60 days of their last therapy. Successive treatment regimens result in progressively shorter response durations; the decrease in duration of response to consecutive regimens typically reflects emerging drug resistance. The observed decrease in response duration also reflects changes in disease biology within each patient, with tumor cells expressing a more aggressive phenotype, higher proliferative thrust and lower apoptotic rates. Whilst several prognostic factors have been identified for newly diagnosed myeloma, factors that retain prognostic value in the context of relapsed/refractory disease remain to be comprehensively defined. Nonetheless, patients with poor risk include those with t(4;14) or t(14;16), deletion 17 or deletion 13, hypodiploidy, high β2 m, and low serum albumin; clinical challenges in the relapsed and refractory population include light chain and IgA isotype, renal failure, extramedullary disease, hyposecretory myeloma, and advanced bone disease. The advent of novel therapies targeting disease biology and tumor microenvironment has dramatically improved the outlook for patients with relapsed and refractory disease. Bortezomib, thalidomide and lenalidomide now constitute *backbone* agents in this setting. Bortezomib in particular reflects a paradigm of drug development where accelerated approval emerged from studies in the relapsed/refractory patient population, followed by full approval in the relapsed setting; combinations which include bortezomib are now a priority in this patient population. As reviewed in Chauhan *et al.*[1] bortezomib reversibly inhibits proteasome function by binding to the beta-5 subunit of the complex and this, together with other effects, results in myeloma cell apoptosis, down-regulation of myeloma adhesion molecule expression, inhibition of cell adhesion-mediated resistance, and decreased cytokine transcription and secretion in the bone marrow. The effects of bortezomib are partly mediated through inhibition of the nuclear factor (NF)-κB signaling pathway, which has been shown to be especially important in the growth and survival of myeloma cells. Specifically, NF-κB remains inactivated due its binding with a specific inhibitory protein, IκB-α, which sequesters the NF-κB p50/p65 heterodimer in the cytoplasm. In response to various stimuli, including proinflammatory cytokines such as IL-1 and IL-6, I-κB-α is degraded by the proteasome, and NF-κB p50/65 then translocates to the nucleus where it activates transcription of anti-apoptotic and growth-promoting genes. The stabilization of IκB-α through proteasome inhibition with bortezomib prevents NF-κB activation, consequently overcoming the anti-apoptotic/growth response and making myeloma cells markedly more susceptible to stress. This is especially significant for the use of proteasome inhibitors in combination with other cytotoxic therapies, because proteasome inhibition may thus sensitize resistant cells to the effects of chemotherapy and other agents. Here we provide a brief review of the clinical efficacy of bortezomib-based approaches in relapsed/refractory MM patients. SUMMIT was the first open-label, Phase II trial that assessed the effects of bortezomib 1.3 mg/m^2 on days 1, 4, 8, 11 of a 21-day cycle in 202 patients with relapsed/refractory MM.[2] Dexamethasone was added for suboptimal response. Patients were heavily pretreated (with a median of six prior lines of therapy). Response rates, assessed using modified EBMT criteria, were robust; those who achieved a CR or PR after two cycles had significantly longer survival and those with CR/nCR had longer survival than those with PR. Results indicated that global QOL, pain, fatigue, and other disease symptoms improved with bortezomib treatment. Grade 3 peripheral neuropathy was an important but reversible toxicity and occurred in 25 (12%) of the patients, resulting in discontinuation in 4%.[2] With a median of follow-up of 23 months, median OS was 17.0 months and median duration of response was 12.7 months; in all patients, median TTP was 7 months while in responding patients it was 15 months.[3] CREST was an open-label, randomized trial investigating the effect of bortezomib 1.0 or 1.3 mg/m^2 on the same schedule.[4] Again, dexamethasone was added for suboptimal response.

Both doses showed activity with and without dexamethasone. The activity of the 1.0 mg/m^2 dose suggested that if reduction of the 1.3 mg/m^2 dose is required, response may still be achieved. Notably, grade ≥ 3 treatment-emergent peripheral neuropathy was lower in the 1.0 mg/m^2 dose group (7% vs 15% for the higher dose group). In APEX, an international, randomized phase 3 trial of 669 patients with relapsed and relapsed/refractory MM (1-3 prior therapies), the efficacy and safety of bortezomib 1.3 mg/m^2 was compared with that of pulsed high dose dexamethasone. The trial was stopped when a pre-planned interim analysis demonstrated superiority of bortezomib in terms of response rates, median TTP and survival. Final results confirmed these findings, as well as superior response rates (43% including 15% CR/nCR) with bortezomib. A recent analysis with extended follow-up confirmed that bortezomib retains a 6-month survival advantage, despite a >62% crossover to bortezomib from the high-dose dexamethasone arm.[5] Combinations with conventional agents include bortezomib, oral cyclophosphamide, and prednisone used to treat patients with relapsed/refractory MM. In one representative study, 13 assessable patients achieved 38% CR/nCR and 46% PR.[6] The combination of bortezomib, low-dose cyclophosphamide, and high-dose dexamethasone also appears to be active with a response rate in 50 evaluable patients of 82% and a median event-free survival (EFS) of 12 months. A phase 1/2 dose-escalation study evaluated bortezomib 0.7-1.0 mg/m^2 twice weekly plus melphalan 0.025-0.25 mg/kg on days 1-4 of a 4-week cycle.[8] Responses in 34 evaluable patients included 15% CR/nCR and 32% PR, with manageable toxicities. In patients who had previously been treated with either drug, response rate was 67% (including MR). Grade 3 events were mainly associated with myelosuppression. The maximum tolerated dose (MTD) was bortezomib 1.0 mg/m^2 and melphalan 0.1 mg/kg. Another phase 1/2 study assessing bortezomib and low-dose melphalan recently reported an ORR (CR+PR+MR) of 76%, increasing to 80% with the addition of dexamethasone. MTD was bortezomib 1.3 mg/m^2 and melphalan 7.5 mg/m^2.[9] Most notably, in a recent, large phase 3 study, the combination of bortezomib plus pegylated liposomal doxorubicin (PLD) was compared to bortezomib alone (N=646)[10] Planned interim analyses showed that the combination resulted in significantly longer duration of response and time to progression (9 months vs. 6 months): median OS had not been reached in either arm. Combinations with other novel agents include trials of bortezomib, PLD, and thalidomide (VDT), bortezomib, melphalan, prednisone, and thalidomide (VMPT), and others. In one study, overall response rate among 18 heavily pre-treated patients treated with the VDT combination was 56%, included 22% CR, and of no significant grade 3/4 non-hematological toxicities were noted.[11] In an important phase I/II study of the VMPT regimen, MTD was determined to be bortezomib 1.3 mg/m^2 on days 1, 4, 15, 22 of a 35-day cycle, with melphalan 6 mg/m^2 and prednisone 60 mg/m^2 days 1-5, and thalidomide 50 mg daily. The response rate in all patients (N = 30) was 67%, including 43% VGPR or better. The regimen appeared more effective in patients in first relapse than in patients in subsequent relapse.12 Minimal neuropathy was seen, and no DVT was reported. Lenalidomide has been investigated in combination with bortezomib in a phase I trial in patients with relapsed/refractory MM. The combination was well tolerated, with responses in 39% of patients, including 6% CR. No significant treatment-emergent peripheral neuropathy was reported.[13] The high response rate observed is especially encouraging, as all the patients had received prior thalidomide, most had received bortezomib, and some had received lenalidomide. Phase II studies with this regimen are underway. Bortezomib in combination with the HSP-90 inhibitor tanespimycin has also shown promise. Responses were seen in 35% of patients, including 14% CR/nCR. Importantly, responses were seen in patients who were bortezomib naïve, as well as those who had been previously treated with bortezomib or were refractory to bortezomib.[14] Studies of bortezomib and numerous other novel agents are ongoing or planned, including other small molecules, such as perifosine and histone deacetylase inhibitors, including SAHA and LBH, as well as monoclonal antibody-based approaches. Given that patients with relapsed/refractory MM typically are more symptomatic, with potential co-morbidities and have characteristically resistant disease, relapsed/refractory disease remains especially challenging to treat. Bortezomib-based treatments constitute a paradigm of therapy for such patients, especially given its activity in older patients and patients with elevated β2 microglobulin and low serum albumin[15] patients with renal disease,[16] and patients with adverse cytogenetics, including chromosome 13 deletion.[17] Studies are on-going to evaluate the role of bortezomib in patients with light chain myeloma, advanced bone disease, and extra-medullary spread. With the introduction of novel, targeted therapies and combinations with these and other agents, the potential to further improve responses in this patient population has increased. Our growing understanding of gene expression profiling, multiple cellular-signaling pathways, and microenvironmental events will aid in the design of additional novel strategies to improve patient outcome.[18] Moreover, the prospect of less toxicity is especially appealing given data on combinations, such as bortezomib and lenalidomide, showing less neuropathy and lower rates of thrombo-embolic complications.

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S4.2

LENALIDOMIDE FOR THE TREATMENT OF RELAPSED OR REFRACTORY MULTIPLE MYELOMA

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For decades, chemotherapy for multiple myeloma consisted of standard combinations of alkylating agents, anthracyclines and steroids with or without hematopoietic stem cell rescue. While these therapies can provide rapid responses and result in modest gains for patients, the disease eventually relapses in all patients and becomes resistant to treatment. In 1998 a new era of discovery of novel agents for treatment of myeloma began after thalidomide was noted to be clinically active for the treatment of relapsed (rel) or refractory (ref) multiple myeloma. This was soon followed by the introduction of the proteosome inhibitor

bortezomib which also has significant activity for this disorder. Although these drugs have provided exciting new directions for treatment for myeloma, side effects including fatigue, neuropathy, constipation, and thromboembolic events remain problematic and continued development of new agents for treatment of patients with resistant disease remains necessary. Subsequently, in an effort to improve the toxicity profile of thalidomide, while maintaining or surpassing the efficacy of the drug, the immunomodulatory derivative (IMiD) lenalidomide was developed, and has demonstrated significant clinical activity in previously treated patients with multiple myeloma. Lenalidomide is a structural analogue of thalidomide that *in vitro* is more effective in inhibiting tumor necrosis factor-α secretion, down regulates interleukin-6 and nuclear factor kappa-B, activates caspase 8, may promote natural killer cell-mediated myeloma cell death and directly induces apoptosis of myeloma cells.[1] Such *in vitro* activity was initially confirmed by the 20% and 29% partial response rates (>PR) in 2 phase I trials among patients with relapsing or refractory myeloma.[2, 3] These studies identified myelosuppression, particularly neutropenia, as the dose limiting toxicity of lenalidomide and indicated a daily dose of 25mg for 21 days (courses repeated q28 days) was the maximum tolerated dose of the drug. In the trial by Richardson *et al.*, grade 3-4 myelosuppression was noted in 12 of 13 pts treated with a dose of 50 mg/d, but marrow cellularity was reduced in only 2 of these patients suggesting causality other than marrow hypoplasia, although the precise etiology remains unclear. A follow-up phase II trial comparing different schedules of lenalidomide in patients with relapsed/refractory MM demonstrated that a daily dose of 30 mg was well tolerated and produced at least a partial response (PR) in 18% of patients with relapsed and refractory MM.[4] Although a twice daily dose of 15 mg of lenalidomide produced response in 14% of patients, grade 3-4 myelosuppression was significantly higher in this group (41% vs. 13% with 30 mg/d, p.03) therefore accrual to the 15 mg twice daily arm was stopped and an additional 32 patients received the 30 mg/d dose. The overall response rate (OR) of the study was 17% and an additional 9% of patients achieved a minor response (MR); median survival was 28 months for patients receiving the 30 mg/d dose. An additional 22% of patients that had not responded to lenalidomide as a single agent achieved partial remission with the addition of intermittent pulses of dexamethasone. Significant peripheral neuropathy and thromboembolic events occurred in only 3% of patients. A phase II trial of single agent lenalidomide 30mg/d x 21d (28d cycles) reported by the same authors revealed similar results including an overall response of 25% and a median time to progression (TTP) of 22.4 weeks.[5] Subsequently based on these phase I and II trials a 25 mg/d dose of lenalidomide given for 21 days of a 28 day cycle is considered standard for treatment of patients with rel/ref MM. Recently the results of 2 double-blind, randomized phase III trials (MM-009, North American, 353 pts; MM-010, Europe, Australia, and Israel, 351 pts) of lenalidomide-dexamethasone (L-D) versus dexamethasone-placebo (D-P) were updated.[6,7] Patients received dexamethasone 40 mg daily on days 1-4, 9-12, 17-20 (dose reduced to d1-4 beginning with cycle 5) every 28 days and were randomized to receive either lenalidomide 25 mg orally on days 1-21 every 28 days or identical placebo. L-D was superior to D-P with respect to overall response, median TTP and median overall survival (OS). OR was 61% and 59.1% for L-D and 20.5% and 24% for D-P (p,.001) for each study respectively. At a median follow-up of approximately 17 months from randomization, both studies show significant improvement in median TTP (11.1 and 113.3 months vs. 4.7 months, p<.001, respectively) and median overall survival (MM-009:29.6m, MM-010: NR vs MM-009: 20.5 m, MM-010: 20.6 m, p<.001) for L-D compared with D-P, respectively. The combination of L-D was generally well tolerated but grade 3-4 neutropenia occurred more commonly in patients treated with L-D (24 and 16.5% vs. 3.5 and 1.2%). Unlike thalidomide, treatment with lenalidomide resulted in grade 3-4 neuropathy in less than 5% of patients. Thrombosis occurred more frequently in the L-D arm than with D-P (15 and 8.5% vs. 4.5 and 3.5%, for MM-009 and MM-010, respectively). Concurrent use of erythropoietin or darbepoetin in these trials was associated with a higher incidence of thromboembolic events. Aspirin may, however, have some role in preventing these thrombotic events as demonstrated in a study of 34 previously untreated patients given aspirin for prophylaxis during treatment with L-D with a resultant thrombosis rate of only 3%.[8] Similar results were noted with aspirin prophylaxis in a retrospective analysis of melphalan-prednisone-lenalidomide (MPR).[9] In contrast, in a Southwest Oncology Group (SWOG) study that randomized patients to receive either L-D or D-P, 9/12 patients (75%) treated with L-D verses no patients treated with dexamethasone had thromboembolic events.[10] Subsequently aspirin prophylaxis (325 mg) reduced the incidence of thromboembolic events, but these still remained increased at 26%. The lack of consistency among these studies suggests the need to further clarify the optimal agent for thromboembolic prophylaxis for patients treated with L-D. Based on these encouraging results of lenalidomide for refractory myeloma, many new combinations are beginning to be evaluated. Knop *et al.* have reported a phase I dose escalation study of doxorubicin-lenalidomide-dexamethasone (RAd) in previously treated patients with MM.[11] The MTD of the combination was lenalidomide 25 mg/d x 21d, doxorubicin 9mg/m^2/d on days 1-4 and dexamethasone 40 mg/d on days 1-4, 9-12, and 17-20. Among 31 evaluable patients, the partial response (PR) rate was 84%, with a complete response (CR) rate of 3%. Grade 3-4 toxicities included one patient with each of the following: neutropenia, renal failure, catheter related-infection and pneumocystis pneumonia. Similarly, Baz *et al.* evaluated a regimen of lenalidomide, liposomal doxorubicin, vincristine, and dexamethasone (DVd-R: MTD: liposomal doxorubicin 40 mg/m^2 and vincristine 2 mg on day 1, dexamethasone 40 mg/d days 1-4 and lenalidomide 10 mg/d po x 21d in 28-day cycles).[12] Although all patients received prophylaxis with amoxicillin 250 mg bid, and acyclovir 400 mg bid, the dose limiting toxicity was non-neutropenic sepsis. Overall response was 75% (PR 60%, CR 15%) with a median progression free survival (PFS) of 12 months; median overall suvival had not been reached at the time of publication. Grade 3 or 4 neutropenia occurred in 32% of patients, with febrile neutropenia in 7%. Thromboembolic events occurred in 9% of patients despite daily prophylaxis with aspirin (81 mg). Cyclophosphamide, has also been combined with L-D (CRD) (500 mg/d po days 1, 8, 15, and 21; lenalidomide 25 mg/d x 21d, and dexamethasone 40 mg/d on days 1-4 and 12-15 q28d x maximum 6 cycles).[13] Among 17 evaluable patients, 65% achieved PR and 6% CR. G-CSF was given to 67% of patients to maintain their neutrophil count and neutropenic fever was seen in 22% of patients (prophylaxis with acyclovir and trimethoprim sulfate was given to all patients). Deep vein thrombosis occurred in 11% (aspirin prophylaxis given to 1 patient) of patients, an incidence similar to that noted by Baz *et al.* (*see above*) despite low dose aspirin prophylaxis. Richardson *et al.* have reported preliminary results of a phase I/II study of 36 patients treated with lenalidomide and bortezomib.[14] The MTD was reached at a dose of lenalidomide 15 mg/d on days 1-14 and bortezomib 1.0 mg/m^2/d d1,4,8,11 given on a 21 day cycle. In patients developing progressive disease, dexamethasone 40mg was added on the day of and the day after each bortezomib dose. Overall response was 39% (CR 3%) and among 14 patients with progressive disease 70% achieved at least MR after the addition of dexamethasone. Significant toxicities included transient hyponatremia, herpes zoster reactivation, grade 4 neutropenia, and deep vein thrombosis. No grade 3-4 fatigue or peripheral neuropathy was observed. These studies illustrate the tremendous clinical activity and potential of the immunomodulatory derivative lenalidomide for treatment of resistant and relapsing myeloma, particularly given the generally tolerable side effect profile that is notable for an absence of significant neuropathy. While this drug has single agent activity, response rates are higher when lenalidomide is given in combination with other agents with known antimyeloma activity (steroids, anthracyclines, alkylating agents, and other novel agents). In combination therapy some form of prophylaxis for thromboembolism appears justified, although the ideal agent and schedule remains unclear. Lenalidomide in combination with known agents, and in the future, with promising new drugs, contributes positively and significantly to the growing list of treatment options that impact survival for myeloma patients with resistant or relapsing disease.

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S4.3

THE IMPACT OF NOVEL AGENTS IN PATIENTS WITH POOR PROGNOSIS MYELOMA DEFINED BY UNFAVORABLE CYTOGENETICS, HIGH AGE, AND RENAL FAILURE

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Poor prognosis patients with multiple myeloma is conferred by unfavorable cytogenetics, such as del13, and t(4;14), by laboratory (*e.g.* high β2-microglobulin, low albumin, hypercalcemia) and by clinical parameters (*e.g.* high age, renal impairment, primary resistant disease).

Unfavorable cytogenetics

In previously untreated patients with poor prognosis multiple myeloma, standard chemotherapy usually results in low response rates and short survival, and prognosis is even worse for relapsed patients with high-risk features. Unfavorable cytogenetics also predict for short survival in patients treated with high-dose therapy and autologous transplantation[1] and dose reduced allogeneic transplantation.[2] This unsatisfactory scenario has partly changed with the introduction of thalidomide but more so with the advent of bortezomib and lenalidomide. Thalidomide and thalidomide-based combinations render remarkable response rates in patients receiving first line treatment and also in those refractory to primary treatment or relapsing from previous treatment lines[3] Unfavorable cytogenetics have been shown to influence treatment outcome with thalidomide. Shingal *et al.* [4] reported an association between del13 and poor response, at least in univariate analysis. This finding was confirmed in a later analysis on 169 relapsed or refractory patients receiving thalidomide, identifying del13, high labeling index and high β2-microglobulin as predictors for poor response[5]. Interestingly, dose escalation of thalidomide seemed to benefit patients with high-risk profile, but the doses used are unlikely to be tolerated in clinical practice. A recent Italian phase II trial employing upfront thalidomide-dexamethasone (Cavo *et al.* ASH 2006, abstract 3081) revealed low rates of VGPR in patients with both del13 and t(4;14) compared to patients with del13 only (12% vs. 41%, p=0.012) and to those with t(4;14) only (12% vs. 50%, p=0.006). Bortezomib and lenalidomide have shown high response rates in relapsed and refractory myeloma patients and data indicate that these novel agents have the capacity to overcome the impact of unfavorable cytogenetics on the course of disease. In the SUMMIT trial 202 patients with relapsed or refractory myeloma were treated with single agent bortezomib or, after 2 cycles without response, with bortezomib plus dexamethasone. Although patients with del13 appeared to have a poorer prognosis, response rates (24% vs. 33%, ns) and overall survival (10 vs. 15 months, p=0.135) did not differ significantly between the 25 patients with del13 compared to 120 patients without this abnormality.[6] These patients also had a higher median level of β2-m and a higher proportion of patients were older (>65 years). In the APEX trial, 246 of the 669 patients randomized either to dexamethasone or bortezomib treatment were evaluated for del13 either by FISH or metaphase analysis. Del13 was detected in 24 of 168 patients by metaphase cytogenetics. Median survival was shorter in del13 patients with the difference being more pronounced in the dexamethasone compared to the bortezomib arm (8.3 months vs. NR, p<0.0073, 12.5 months vs. NR, 0.0379, respectively). In a matched pair analysis 25% of patients with del13 responded to bortezomib and 9% to dexamethasone. The respective response rates in del13 negative patients were 35% and 26%, respectively. Overall survival did not differ between del13 positive and negative patients with bortezomib therapy (12.5 months vs. NR, p<0.7919) but in those treated with dexamethasone (3.3 months vs. NR, p<0.002). We treated 62 patients with relapsed and refractory myeloma with bortezomib and found an overall response rate of 45% and 55% (p=0.66) in patients with and without del13 as defined by FISH.[7] There was also no statistical significant difference in duration of response (9.3 months in patients without del13 and 12.3 months in patients with del13, p=0.25). Patients with del13 who did not respond to bortezomib had a very short survival, which resulted in a trend towards shortened overall survival of all del13 positive patients (9.9 months vs. NR, p<0.057). Bortezomib was also effective in the small number of patients (n=3) with presence of a t(4;14), which is otherwise associated with extremely poor prognosis. Importantly, we noted that patients relapsing after bortezomib treatment could successfully be treated with bortezomib combination therapy, even in the presence of unfavorable cytogenetics. In a small study of patients with advanced myeloma, bortezomib plus dexamethasone produced responses that were independent of del13 defined either by FISH or metaphase cytogenetics. Overall, 11 of 15 patients responded (CR or PR), including 8 of 10 patients with del13.[8] Early data are available for efficacy of lenalidomide in high-risk myeloma. In the MM016 trial, relapsed and refractory myeloma patients were treated with a combination of lenalidomide and dexamethasone. Bahlis *et al.* (ASH 2006, abstract 3557) reported on 36 patients enrolled at their site, for all of whom cytogenetic analysis was available. A del13 was detected in 16 patients (44.5%) and a t(4;14) in 7 patients (19.4%). The overall response rate was 90% in the group without del13 and 75% in the group with del13. Event free survival estimates at 6 months were also similar in the 2 groups (no del13: 73% vs. 81% with del13; p=0.61). Lenalidomide also seemed to be effective in the small group with a t(4;14). Importantly, lenalidomide exerts activity in thalidomide pretreated patients (Wang *et al.*, ASH 2006 abstract 3553), but response rates are lower (54% vs. 61%, p<0.01) and time to progression shorter (36.9 weeks vs. 61.3 weeks, p<0.001) compared to thalidomide naïve patients

Elderly patients

Thalidomide-based combinations seem to achieve a better outcome compared to standard therapy in elderly patients. In a trial comparing Thal-Dex to MP, higher response rates and a shorter time to response were seen with the thalidomide combination in a patient population with a median age of 72 years (Ludwig *et al.*, EHA 2007). Similarly, Palumbo *et al.*[9] observed a better outcome with MP combined with thalidomide compared to MP alone in patients >60 years. In a 3-arm French trial, a higher progression-free survival and overall survival were observed with MP-T compared to MP, but, notably, also compared to double autologous transplantation with MEL 100 in patients 65-75 years old (Facon *et al.*, ASCO 2006, abstract 1). Bortezomib exerts significant activity in elderly patients, but response rates were lower in patients aged ≥ 65 years compared to younger patients (19% vs. 32%, p=0.06) in the SUMMIT trial. Remarkably high response rates, however, were obtained in a recent phase II study[10] employing bortezomib plus melphalan/prednisone therapy as frontline treatment in elderly patients (age ≥ 65 years). The overall response rate was 89%, with 32% of patients achieving immunofixation negative CR. This regimen also overcame the poor prognosis conferred by retinoblastoma gene deletion and IgH translocations. All patients with del13 responded and response rates, EFS and PFS were similar between patients with and without the deletion. Lenalidomide or lenalidomide-dexamethasone (or prednisone) induced similar response rates in 24 patients aged 65 years or older compared to 49 younger patients (58% vs. 56%, p=0.15) with relapsed/refractory myeloma. Similarly, no difference was found in the actuarial progression free survival (43% vs. 43%), overall survival (74% vs. 76%) and toxicity between older and younger patients (Reece *et al.*, ASH 2006, abstract 3550).

Renal failure

Renal failure is another important risk factor for shortened survival. In light chain -induced acute renal impairment, renal function can only recover if the level of pathogenic myeloma proteins can rapidly and substantially be reduced. Thalidomide-dexamethasone results in higher remission rates than MP and time to response is significantly shorter than with MP (Ludwig *et al.*, EHA 2007). Hence, this regimen is an attractive choice for patients with renal failure. Dose reductions of thalidomide are not required, since it is mainly metabolized by the liver. Bortezomib is rapidly distributed into tissues with an initial plasma distribution half-life of less than 10 minutes, followed by a terminal elimination half-life of more than 40 hours.[11] In renal failure dose reduction is not required. This, together with its high activity, makes it an ideal substance for treating patients with acute myeloma induced renal failure. We treated 8 patients with light chain induced renal insufficiency with bortezomib or bortezomib-based combinations. The median creatinine level decreased from a median of 9.05 mg/dL at start of therapy to a median of 2.1mg/dL (range: 0.8-2.3) after median of 48 (range 41-71) days. Five of the 8 patients achieved creatinine levels below 2.3 mg/dL. All patients with improvement of renal function achieved an objective tumor response (CR or nCR: 3, VGPR: 1, PR:1) while in the non-responders only 1 achieved a transient PR after cycle 2, but relapsed before cycle 5. Two other studies have documented a significant anti-myeloma effect in patients with renal failure. In the Apex trial, 3 of 10 patients with renal impairment responded to bortezomib, and in another study by Chanan-Khan *et al.*[12] an overall response rate of 75% was obtained with bortezomib or bortezomib-based regimens in patients on hemodialysis. In all three studies bortezomib-based treatment was well tolerated. Lenalidomide-Dex has been shown to be equally effective in patients with creatinine clearance > or < 50 mL/min, but for 16 pts with creatinine clearance <30 mL/min, median TTP and OS was shorter than for those with better renal function, but still significantly higher than for patients treated with dexamethasone only. Grade 3-4 thrombocytopenia was significantly higher in patients with impaired renal function (<50 mL/min, 13.8%; >50 mL/min 4.6%, $p<.01$; <30 mL/min, 18.8%, >30 ml/min, 5.5%, $p<.05$), but there was no difference for G3-4 neutropenia at either cut-off. In patients with renal impairment dose reductions are required because lenalidomide is mainly excreted by the kidney (Niesvizky *et al.*, ASH 2006 abstract 3549).

Conclusion

These data indicate substantial activity of bortezomib and lenalidomide in patiens with cytogenetically defined high-risk disease as well as in those with relapsed or refractory myeloma. Both drugs and combinations thereof are effective in elderly patients with myeloma, as well as in those with renal impairment. The short time to remission with bortezomib-based, and possibly also with lenalidomide-based regimens, renders these drugs an optimal choice for patients with acute renal failure, while their potential to overcome unfavorable cytogenetics offers for the first time effective treatment in patients who otherwise respond poorly to traditional therapies.

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S4.4

NOVEL PROTEASOME INHIBITORS AS THERAPY IN MULTIPLE MYELOMA

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Proteasomes are large multi-subunit protease complexes that selectively degrade intracellular proteins that are tagged for destruction by ubiquitination.[1] They regulate normal cellular processes, such as cell cycle, inflammation, transcription, DNA replication, apoptosis via proteolysis of key enzymes and regulatory proteins. In addition, proteasomes are also involved in stress response, by eliminating abnormal/redundant protein, and in the immune response through generation of antigenic peptides. The proteolytic activity is due to a multi-enzyme complex 26S protease that degrades ubiquititnated protein into small fragments in an ATP-dependent manner.[1,2] Deregulations in the Ubiquitin-Proteasome signaling (UPS) pathway are linked to the pathogenesis of various human diseases.[1] Because the UPS pathway regulates many essential cellular processes, proteasome inhibitors offer a great promise as therapeutic agents. Blockade of proteasome activity stabilizes cell cycle and proapoptotic proteins, thereby abrogating growth/survival and ultimately inducing apoptosis.[1,3] Bortezomib (Velcade™), the first in class of proteasome inhibitors, has become a standard therapy for treatment of refractory multiple myeloma (MM).[3] Even though bortezomib therapy is a major advance (35% overall response rate), it can be associated with toxicity and the development of drug-resistance. Nonetheless, the success of bortezomib as an anti-cancer therapy has generated interest in discovery and development of other novel proteasome inhibitors. Our recent study characterized a novel proteasome inhibitor NPI-0052, a small molecule derived from fermentation of Salinospora, a new marine gram-positive actinomycete.[4] Importantly, NPI-0052 is distinct from bortezomib in its chemical structure, effects on proteasome activities, mechanisms of action, toxicity profile against normal cells, and is orally bioactive. NPI-0052 induces apoptosis in MM cells resistant to conventional and bortezomib therapies, without significantly affecting normal lymphocyte viability. Biochemical and genetic studies showed that NPI-0052, in contrast to bortezomib, relies more on FADD-caspase-8-mediated cell death signaling. Combinations of low doses of NPI-0052 and bortezomib trigger synergistic anti-MM activity, which is likely due to their differential effects (additive inhibition) on proteasome activities[5] and distinct signaling mechanisms. *In vivo* studies using human MM-xenografts shows that NPI-0052 is well tolerated, prolongs survival and reduces tumor recurrence.[4] These preclinical studies provided the basis for ongoing Phase-I clinical trial of NPI-0052 in relapsed/refractory MM patients. NPI-0052 has also been evaluated in other cell systems. For example, a recent study using CLL cells showed that NPI-0052 is a more effective proapoptotic agent than bortezomib.[6] Another study demonstrated that NPI-0052 is a potent, well-tolerated proteasome inhibitor with distinct pharmacodynamic properties than bortezomib since it achieves significantly higher and more sustained levels of proteasome inhibition.[7] Combination of NPI-0052 with chemotherapy (5-fluorouracil, CPT-11, Avastin/benacizumab, leucovorin, and oxaliplatin) improves tumoricidal response in colon cancer xenograft model. As noted above, the higher anti-tumor activity of NPI-0052 than bortezomib may be due to its ability to block all three proteolytic activities (chymotrypsin-like,

trypsin-like and caspase-like proteasomal activities) and distinct signaling mechanisms; however, further studies are needed to unveil the precise mechanisms mediating combined anti-tumor activity of NPI-0052 and chemotherapeutic agents. Our ongoing studies are delineating the molecular mechanisms mediating proteasome-induced cytotoxicity, defining targets of sensitivity *versus* resistance, allowing for the development of more specific novel proteasome inhibitors, and providing the rationale for combination therapies.

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S4.5

INHIBITION OF HEAT SHOCK PROTEINS: THERAPEUTIC PERSPECTIVES

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Heat shock protein-90 (hsp90) is a molecular chaperone which interacts intracellularly with a broad range of client proteins and functions to preserve their 3-dimensional conformation to a functionally competent state, as well as facilitate their intracellular trafficking. The interaction of hsp90 with its client proteins involves formation of a multiprotein complex whereby binding of ATP to the ATP-binding domain of hsp90 allows it to facilitate the proper folding and conformational stabilization of a target protein. In the absence of that ATP-hsp90 interaction, client proteins are more likely to remain unfolded/misfolded and become ubiquitinated, thus leading to their proteasomal degradation. Compared to many other heat shock proteins, hsp90 presents the intriguing feature that it interacts with a set of client proteins which prominently includes cell surface receptors for diverse cytokines and growth factors; intracellular kinases and kinase targets; as well as other effectors of signal transduction cascades. Although many of these hsp90 client proteins share limited if any structural similarities, their respective functions tend to promote cell proliferation, survival and resistance to pro-apoptotic stimuli. Neoplastic cells, in particular, typically require a high degree of hsp90 function, not only because many of these hsp90-depenent molecular cascades play critical roles in the biological behavior of tumor cells, but also because the 3-dimensional conformation of many mutated oncoproteins (including mutant versions of src, raf or p53), as well as chimeric oncogenic kinases (including bcr/abl), which drive the malignant phenotype, is more dependent on hsp90 function compared to their respective wild-type counterparts. Therefore, from a therapeutic standpoint, inhibition of hsp90 function would present the rare advantage of being directed against a singular molecular target, which in turn facilitates concomitantly the biological activities of a multitude of pathways (some of which can be targeted at potentially multiple molecular levels) that contribute to the establishment, and progression of neoplasias. Work from diverse centers, including our group, has shown that indeed small molecule inhibitors which competitively inhibit the ATP-binding domain of hsp90, such as the ansamycin geldanamycin and its analogs *e.g.* 17-allylamino-17-demethoxygeldanamycin (17-AAG) or 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin (DMAG) can suppress the chaperoning function if hsp90 and therefore perturb the stability and function of its client proteins, leading to anti-proliferative pro-apoptotic effects in various solid tumor models and hematologic neoplasias. In particular, our work in MM models has shown that MM cells are responsive to hsp90 inhibitors *in vitro* (at pharmacologically achievable concentrations) and in clinically relevant orthotopic *in vivo* xenograft models,[1] and can also function to sensitize MM cells to other pro-apoptotic agents.[2] These original pre-clinical observations have since been confirmed in multiple MM experimental settings, both *in vitro*[3-7] and *in vivo*,[3,5,8] using diverse members of the ansamycin class, as well as different drug formulations.[3,5,8] The aggregate pre-clinical experience with hsp90 inhibitors indicates that the mechanistic basis of their anti-MM effects is related to the pleiotropic nature of their molecular sequelae.[1] Indeed, hsp90 inhibitors abrogate cytokine (*e.g.* IGF- and IL-6)-induced signaling cascades at multiple molecular levels, including suppression of cell surface expression of IGF-1R and IL-6R and inhibition of downstream signaling (via PI-3K/Akt/mTOR, Ras/Raf/MAPK, IKK/NF-κB), via molecular events which include the suppression of expression and/or function of Akt, Raf, IKK-α, and p70.[S6K] These events lead to multiple downstream pro-apoptotic sequelae, including increased nuclear translocation of pro-apoptotic members of the Forkhead family of transcription factors; suppressed expression of intracellular inhibitors of apoptosis (*e.g.*, FLIP, XIAP, cIAP-2); as well as decreased constitutive and IGF-induced activity of NF-κB, telomerase, HIF-1a and 20S proteasome. These molecular events not only contribute to decreased MM proliferation, but can also increase their chemo-/Dex-sensitivity (*e.g.* through NF-kB inhibition); suppress the long-term replicative potential of MM cells (*e.g.* through inhibition of telomerase function); or blunt pro-angiogenic effects (*e.g.* via suppression of HIF-1a transcriptional activity). These pleiotropic anti-proliferative/pro-apoptotic events allow hsp90 inhibitors to abrogate bone marrow stromal cell-derived protection on MM tumor cells, and sensitize them to other anticancer agents, including cytotoxic chemotherapy. Furthermore, because MM cells exposed to the proteasome inhibitor bortezomib upregulate the expression of heat shock proteins, including hsp90, as a stress response to counteract the intracellular accumulation of misfolded proteins, hsp90 inhibitors can sensitize MM cells to the anti-MM effects of bortezomib. These studies provided the rationale for ongoing clinical trials of tanespimycin (17-AAG in the KOS-953 cremophor-based formulation) either as a single agent[9] or in combination with bortezomib[10] in patients with relapsed or refractory MM. In these trials, tanespimycin has shown a manageable profile of side effects (without significant cardiotoxicity, peripheral neuropathy or deep vein thrombosis), durable disease stabilization and minor responses with single-agent treatment in relapased and refractory MM patients, as well as encouraging anti-MM activity by the combination of tanespimycin with bortezomib. This experience, coupled with the lack of additive toxicity or pharmacokinetic interactions in the tanespimycin + bortezomib combination, provides a platform for future phase III trials of this regimen. Hsp90 inhibitors therefore represent an emerging class of anti-tumor agents with a specific target, but pleiotropic and versatile anti-proliferative/pro-apoptotic properties, which can allow them to function as possible sensitizers that can enhance the response to existing anti-MM therapies or investigational agents. The multitude of molecular pathways modulated by hsp90 inhibitors is particularly important for MM, given its marked genetic complexity, at diagnosis and, even more so, as the disease progresses through successive lines of treatment. Ongoing preclinical and translational studies are focusing on identifying biomarkers of sensitivity vs. resistance of MM cells to hsp90 inhibitors and/or their combinations with other anti-MM agents. Such efforts, coupled with as the accumulating experience on the pharmacodynamics and pharmacokinetics of hsp90 inhibitors in clinical trials, will hopefully improve our understanding on how to best develop this drug class for incorporation in the therapeutic management of MM.

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S4.6

TARGETING KINASES AND IGH TRANSLOCATIONS IN MYELOMA

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The t[4;14] translocation, which simultaneously dysregulates both fibroblast growth factor receptor 3 (FGFR3) and Multiple Myeloma SET (MMSET), is detectable in 15% of patients with Multiple Myeloma (MM). Although FGFR3 has been shown to be oncogenic in a number of assays, expression of FGFR3 is lost in approximately 30% of patients with the translocation as analyzed by gene expression profiling while expression of MMSET is always retained. The presence of the t[4;14] is highly associated with poor outcome following both conventional chemotherapy and high-dose Melphalan-based regimens thus detection of patients bearing the t[4;14] is critical in any MM molecular work up.[1,2] Recent clinical observation demonstrates however that even within this poor prognosis group patients with low beta 2 microglobulin and normal hemoglobin fare better. Treatment may be altered on the basis of this high risk genetic feature since t[4;14] patients have a short time to progression following stem cell transplant and since bortezomib may have some ameliorative effect on the poor prognosis.[3] Finally, inhibitors of the FGFR3 tyrosine kinase are now in clinical trials. Overall it is apparent that detection of t[4;14] patients is increasingly important in patient management. In MM, no other frequent recurrent kinase translocation or mutation has yet been defined. To better define kinase targets in MM, a systematic assessment of the entire MM kinome is required. With this goal in mind kinase sequencing and kinome wide RNAi silencing(4) has been performed by our laboratory, These experiments permit identification of (a) kinases in Myeloma that are essential for tumor survival and (b) kinases in Myeloma that most effectively synergize with bortezomib or lenalidomide and will allow the identification of currently available inhibitors that specifically target the kinases identified. We have now screened in 384-well format a 1800-oligo (639 gene) kinome siRNA in the absence and presence of titrated low dose bortezomib (IC_{10}-IC_{50}). Of 41 putative Myeloma-lethal kinase targets derived from initial screening, 15 have been fully validated and are cytotoxic with three or more independent siRNA. We have also identified 25 kinases that putatively induce synergistic cytotoxicity with bortezomib. In a second cell line, JJN3, 50 RNAi silencing 25 validated lethal kinase targets, 44 (88%) were lethal in both Kms11 and JJN3, 4 (8%) were lethal in Kms11 only and 2 (4%) were lethal in JJN3 alone. This suggests that our screening strategy is capable of identifying kinase targets that may have a broad spectrum of activity for MM tumors, despite the genetic heterogeneity that typically exists. However, it will be important to substantiate this conclusion by testing other MM variants. We have also been seeking more global inhibitors of myeloma survival by employing high throughput screens of small molecule libraries. Specifically, to identify novel pharmaceutical inhibitors of maf and its ability to promote cyclin D2 (*CCND2*) transactivation we developed an assay employing NIH 3T3 cells stably co-expressing the *CCND2* transactivator c-Maf and the cyclin D2 promoter driving firefly luciferase (luc) and screened the Lopac (n=1280), Prestwick (n=1120) and Spectrum (n=2000) libraries of drugs and natural compounds. In a parallel MTS assay, the effect of each compound on 3T3 viability was determined, allowing exclusion of compounds that caused secondary suppression of *CCND2* due to non-specific cytotoxicity. Thirty-one of the 40 hits from the screen were members of the glucocorticoid family and findings were consistent with a c-Maf-dependent inhibition of *CCND2* transactivation. In further analysis glucocorticoids repress c-Maf dependent transactivation of *CCND2* through ubiquitination and subsequent down regulation of Maf. These findings demonstrate a new mechanism for glucocorticoid-induced apoptosis and help explain their activity in multiple myeloma. Of the remaining *CCND2* promoter inhibitors studies in patient marrow samples demonstrated selective activity for the triterpenoid pristimerin and kinetin riboside against $CD138^+$ myeloma cells. Together this chemical biology screen has identified a number of compounds or classes of compounds that may be further explored for preclinical and ultimately clinical anti-myeloma activity.

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S5: Frontline treatment of myeloma

S5.1

INTERNATIONAL STAGING SYSTEM FOR MM

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S5.2

THE ROLE OF THALIDOMIDE IN MYELOMA PATIENTS ELIGIBLE FOR HIGH-DOSE THERAPY

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Over the last decade new insights into the biology of multiple myeloma (MM) have provided the framework for the development of novel therapies to reverse drug resistance and improve patient prognosis. In particular, recognition of the pivotal role of bone marrow microenvironment in promoting myeloma cell growth, survival and drug resistance has allowed for identification of specific therapeutic strategies targeting myeloma-stromal cells interactions, cytokine secretion and their sequelae in the bone marrow milieu. Examples of novel targeted drugs which have quickly translated from the bench to the bedside include thalidomide, its immunomodulatory analog lenalidomide and the first-in-class proteasome inhibitor bortezomib. Thalidomide, an old drug with an infamous past due to its teratogenicity, has redeemed itself as a new treatment paradigm for myeloma patients refractory to multiple prior therapies and actually represents a standard of care also for patients with newly diagnosed disease. Three phase II clinical studies for previously untreated patients with symptomatic MM[1-3] provided demonstration that thalidomide at the daily dose of 200 mg combined with pulsed high-dose dexamethasone, both administered for a maximum of 4 months, effected an overall response rate [partial response (PR) or higher] in the range of 64% to 72%, including complete response (CR) or near complete response (nCR) between 8% and 12%. Following these studies, the use of thalidomide and dexamethasone has increased significantly in clinical practice. Moreover, impressive results of phase II trials[1-3] prompted the inititiation of phase III studies aimed at comparing thalidomide-dexamethasone with standard regimens as up-front treatment for candidates to subsequent autologous stem-cell transplantation. We performed a retrospective matched case-control analysis of 200 patients who were treated with thalidomide-dexamethasone (100 patients) or vincristine-doxorubicin-dexamethasone (VAD) (100 patients) for 4 months in preparation for single or double autologous transplantation.[4] In comparison with VAD, thalidomide-dexamethasone resulted in a significantly higher overall response rate (52% with VAD vs. 76% with thalidomide-dexamethasone, including 13% CR or nCR; p=0.0004), greater reduction in serum M protein concentrations and comparable peripheral blood stem cell (PBSC) harvests. These results were recently confirmed by a prospective randomized study showing that among patients who received thalidomide-dexamethasone for 3-4 months the probability to attain at least a very good partial response was 25%, as opposed to 7% for patients treated with VAD (p=0.027); the percentage of patients who had successful PBSC yields and underwent autologous transplantation was almost superimposable in both treatment arms.[5] A superior rate of at least PR to thalidomide-dexamethasone (73%, including 16% CR) in comparison with VAD (53%) was also found in an observational study of 60 patients.[6] Rajkumar *et al.* conducted a phase III clinical trial of thalidomide-dexamethasone compared with dexamethasone alone in 207 previously untreated MM patients.[7] Results showed that the best response within 4 cycles of therapy was 63% with thalidomide-dexamethasone versus 41% with dexamethasone alone (p=0.0017). However, superior rates of response in the thalidomide-dexamethasone arm were at the expense of a significantly higher frequency of grade 3-4 nonhematologic toxicities (67% vs. 43% with dexamethasone alone), highlighting the need to balance toxicity with response. As in our study,[4,8] deep vein thrombosis (DVT) emerged as the single most serious complication of primary thalidomide-dexamethasone therapy, occurring in 17% of patients.[7] Thus, effective antithrombotic prophylaxis using oral warfarin or low molecular weight heparin or aspirin should be recommended in all patients with newly diagnosed MM who start thalidomide therapy combined with pulsed high-dose dexamethasone. The emergent treatment paradigm of targeting the *soil* bone marrow microenvironment as a means of interfering with the growth of the myeloma *seed* has provided the rationale for investigational clinical trials of thalidomide combined with conventional chemotherapy in an attempt to enhance cytotoxicity and reverse multiple mechanisms of drug resistance.[9] This concept was pioneered by Barlogie's group at the University of Arkansas and was tested in a randomized fashion in the context of a multiphase treatment program, the so called *Total Therapy 2*, which included intensified chemotherapy in preparation for and after melphalan-based double autologous PBSC transplantation.[10] Intensified induction therapy in preparation for high-dose melphalan included one cycle of VAD followed by DCEP (dexamethasone, cyclophosphamide, etoposide and cis-platin), CAD (cyclophosphamide, doxorubicin and dexamethasone) with PBSC collection and further DCEP. According to study design, patients were randomized to receive or not thalidomide at daily doses of 400 mg during primary remission therapy, of 100 mg between the two transplants, of 200 mg during D-PACE (dexamethasone, cis-platin, doxorubicin, cyclophosphamide, etoposide) as consolidation therapy and of 100 or 50 mg during interferon maintenance. Results of the entire treatment program showed that with a median follow-up of 42 months the thalidomide group was superior to the control arm in terms of significantly higher CR rate (62% vs. 43%; respectively; p<0.001) and longer 5-year event-free survival (56% vs. 44%, respectively; p=0.01). Five-year overall survival rates in the range of approximately 65% were noted in both treatment groups and were attributed both to the routine use of thalidomide as salvage therapy for patients relapsing on the no-thalidomide arm and to the shorter post-relapse survival of patients assigned to receive thalidomide, reflecting a higher frequency of chromosome 1q21 amplification. With an extended median follow-up of 53 months, post-relapse survival was 5.3 months among controls vs. 4.3 months among thalidomide-treated patients. Alternative regimens including thalidomide combined with cytotoxic agents in preparation for subsequent autologous transplantation were explored by other groups. In a randomized study of the Greek Myeloma Study Group addition of thalidomide to DVD (*e.g.* a VAD-like regimen in which doxorubicin was replaced by pegylated liposomal doxorubicin) resulted in a higher rate of at least PR in comparison with the same regimen without added thalidomide (81% vs. 66%, respectively; p=0.048).[11] However, the increased rate of response to thalidomide combined with DVD needed to be balanced against the increased toxicity, particularly neurological; at the opposite, the incidence of DVT was similar in both treatment arms. Consistently with the results of this study, in a limited series of patients reported by Chanan-Khan the combination of thalidomide with VAD effected an overall response rate of 91%, including 27% CR.[12] In a large phase III study conducted in Netherlands and Germany, response to 3 monthly courses of VAD or thalidomide-doxorubicin-dexamethasone (TAD) was evaluated in 406 patients who subsequently underwent either single or double autologous transplantation.[13] Comparison between the two treatments arms showed the superiority of TAD over VAD in terms of at least PR (80% vs 63%, respectively; p=0.001), but not of CR rate (7% vs 3%, respectively). On the basis of clinical experiences so far reported, thalidomide combined with dexamethasone appears to be an effective and relatively well tolerated oral alternative to conventional, and often more cumbersome, induction regimens used so far in preparation for autologous stem-cell transplantation in MM (including VAD or VAD-like regimens or dexamethasone alone). In most studies, both thalidomide-dexamethasone and the combination of thalidomide with cytotoxic agents provided superior rates of response in comparison with control treatments not including thalidomide. Whether more marked pre-transplant reduction in tumor cell mass effected by thalidomide-including regimens would favorably affect long-term outcome of autologous stem-cell transplantation still remains an unresolved issue. In a retrospective study of 164 patients who were followed for a median of 29 months after autologous transplantation, it was reported that patients who received thalidomide-dexamethasone as primary therapy had a significantly longer 2-year progression-free survival than those initially treated with anthracyclines-containing regimens (93% vs. 58%, respectively; p=0.039).[14] At the opposite, in a study conducted at the Mayo Clinic, Rochester, and involving 340 patients (of whom 105 initially treated with VAD, 140 with dexamethasone alone and 95 with thalidomide-dexamethasone), the rate of CR after subsequent autologous transplantation and the length of survival, both overall and progression-free, were comparable between the three treatment arms.[15] Consistently with these latter results, in the HOVON 50/GMM-HD3-Trial comparing TAD vs VAD, post-transplantation rates of response (PR+CR) were 91% vs. 88%, respectively, including 19% CR in the TAD arm and 13% following VAD plus autologous transplantation.[13] Different conclusions were offered by the final analysis of *Total Therapy II*, in which integration of thalidomide in the context of double autologous transplantation signif-

icantly increased the final CR rate and extended event-free survival in comparison with the no-thalidomide arm of the study.[10] In conclusion, thalidomide combined with either dexamethasone or cytotoxic agents constitutes an important new addition to currently available options for MM patients who are candidates to subsequently receive autologous stem-cell transplantation. Nevertheless, more effective and safer treatments aimed at maximizing the rate and speedy of response without interfering with patients' capability to undergo PBSC-supported high-dose therapy are needed. In this setting, the opportunities afforded by lenalidomide or bortezomib are considerable. In addition, synergistic activity of some of these agents holds promise to furtherly improve therapeutic results in the near future. Bases on these premises, a large prospective, randomized, phase III clinical study is currently ongoing in Italy in an attempt to compare the role of bortezomib-thalidomide-dexamethasone (VTD) with thalidomide-dexamethasone as induction therapy and consolidation therapy before and after double autologous stem-cell transplantation for patients with newly diagnosed MM and less than 65 years of age.

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S5.3

THE ROLE OF BORTEZOMIB IN MYELOMA PATIENTS ELIGIBLE FOR HIGH-DOSE THERAPY

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High-dose therapy (HDT) plus autologous stem-cell transplantation (ASCT) is considered the standard of care for frontline treatment of multiple myeloma patients aged ″70 years. HDT-ASCT has demonstrated greater benefit for patients in terms of improved overall response and complete response (CR) rates and, in some studies, improved overall survival (OS) compared with conventional chemotherapy. Double transplantation provides higher CR rates and longer OS versus single transplantation in patients with less than a very good partial response (VGPR) after first transplant. CR/VGPR following HDT-ASCT is associated with longer event-free survival (EFS) and OS. Before HDT-ASCT, patients receive induction therapy to reduce tumor burden, followed by stem-cell mobilization and collection. Conventional induction regimens such as vincristine/ doxorubicin/dexamethasone (VAD) result in low (<10%) CR rates. Improving post-induction overall response and CR rates may result in improved CR/VGPR rates post-transplantation, which could translate into prolonged OS. With this aim, the proteasome inhibitor bortezomib (VELCADE®, Millennium Pharmaceuticals, Inc., and Johnson & Johnson Pharmaceutical Research & Development, L.L.C.) has been investigated as a single agent and in various combinations as induction therapy prior to HDT-ASCT. Single-agent bortezomib has shown encouraging activity; combination regimens have demonstrated enhanced efficacy, providing substantial clinical benefit compared with conventional treatments in terms of consistently high overall response and CR rates, both following induction and post-transplantation. Consequently, these bortezomib-based treatments are playing an increasingly important role in frontline treatment of patients eligible for HDT-ASCT. My colleagues and I reported notable overall response, CR, and survival rates with bortezomib ± dexamethasone.[1] In our phase 2 study, 49 newly diagnosed patients received bortezomib for up to six 3-week cycles. Dexamethasone was added for patients achieving less than a partial response (PR) by the end of cycle 2 or a CR by the end of cycle 4. Response rate was 88%, including 18% CR/near CR (nCR) and 20% VGPR.[1] Toxicities were predictable and manageable; grade ≥2 events included sensory neuropathy (36%), fatigue (20%), and constipation (16%). With median follow-up of 26.7 months, estimated 2-year survival rate was 85%. Among 25 patients who went on to receive HDT-ASCT, estimated 2-year post-transplant survival rate was 91%.[1] Other bortezomib and dexamethasone regimens have been investigated by the IFM[2,3] and PETHEMA[4] study groups. In an IFM phase 2 study in 48 patients administered four cycles of bortezomib plus dexamethasone, response rate was 67%, including 21% CR and 10% VGPR. Most toxicities were mild or moderate; grade ≥3 toxicities included infection (10%), and peripheral neuropathy (6%). Forty two patients proceeded to HDT-ASCT; post-transplantation response rate was 90%, including 33% CR and 21% VGPR.[2] The IFM is now comparing this regimen with VAD prior to HDT-ASCT in an ongoing, randomized phase 3 study.[3] Data from a preliminary analysis of the first 165 patients demonstrate that induction with bortezomib plus dexamethasone results in a higher overall response rate (82% vs 67%), CR/nCR rate (20% vs 9%), and VGPR rate (23% vs 17%) compared with VAD. Overall toxicity profiles were similar for the two regimens, with grade ≥3 adverse events reported in 36% of patients treated with VAD versus 30% for bortezomib plus dexamethasone. Patients received a second transplant if they did not achieve VGPR or better after their first transplant. Based on preliminary analysis of available data from those who had undergone their first transplant, more patients treated with bortezomib plus dexamethasone than with VAD achieved a VGPR or better (78% vs 55%, respectively), and therefore fewer required a second transplant.[3] The PETHEMA group studied a different regimen of bortezomib and dexamethasone in a phase 2 study of 40 newly diagnosed patients.[4] The agents were administered in alternating cycles, starting with bortezomib, for six cycles. This novel approach yielded a best response rate of 67%, including 13% CR and 10% VGPR. Toxicities were mostly mild or moderate, although 15% of patients experienced grade 3 neutropenia. To date, half the patients have received HDT-ASCT; response rate 3 months post-transplantation was 80%, including 40% CR and 20% VGPR.[4] Due to the substantial activity demonstrated, bortezomib plus dexamethasone has been added as a first-line therapeutic option for patients proceeding to HDT-ASCT in the recently updated National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology for Multiple Myeloma. Also added in this setting was the combination of bortezomib, doxorubicin, and dexamethasone (PAD), which has demonstrated impressive results in two phase 2 studies in which the dose of bortezomib differed.[5,6] In one study, 21 patients were treated with four 3-week cycles of PAD with the standard 1.3 mg/m^2 bortezomib dose. Response rate was 95%, including 29% CR/nCR and 33% VGPR.[5] All hematologic toxicities were grade 1/2. Grade 3/4 events included infection, herpes zoster reactivation, line infection (each 14%), peripheral neuropathy, febrile neutropenia, nausea and vomiting, postural hypotension, and atrial fibrillation (each 5%). Following HDT-ASCT in 18 patients, overall CR/nCR rate increased to 57%, and VGPR rate was 24%.[5] In a second study of PAD in 20 patients,

with bortezomib 1.0 mg/m^2, overall response and CR/nCR rates post-induction appeared lower with this reduced-dose PAD regimen, at 89% and 16%, respectively.[6] The overall incidence of neuropathy was also lower compared with the higher-dose PAD study (9% vs 48%), with no grade 3 neuropathy reported. Following HDT-ASCT, the efficacy of the reduced-dose regimen appeared similar to higher-dose PAD. Among 13 patients who proceeded to HDT-ASCT, post-transplantation response rate was 100%, including 54% CR/nCR and 8% VGPR.[6] PAD with standard-dose bortezomib is currently being compared with VAD prior to HDT-ASCT in an ongoing HOVON phase 3 study. A similar bortezomib-based regimen, using liposomal doxorubicin instead of doxorubicin, has been investigated in a phase 2 study.[7] The VDD regimen was administered to 28 patients for up to six 3-week cycles, resulting in an overall response rate of 89%. As with PAD, the CR/nCR and VGPR rates were very high, at 32% and 21%, respectively. The combination was well tolerated; common grade ≥3 toxicities included fatigue (14%), pneumonia (11%), thrombocytopenia (7%), and deep-vein thrombosis/pulmonary embolism (DVT/PE, 7%). Among 18 patients who underwent HDT-ASCT, post-transplantation response rate was 96%, including 54% CR/nCR and 25% VGPR.[7] Another phase 2 study, by the CALGB, investigated eight 3-week cycles of the steroid-sparing regimen of bortezomib plus liposomal doxorubicin in 63 newly diagnosed patients.[8] Response rate among 29 patients who had completed therapy was 79%, including 28% CR. The regimen was generally well tolerated, with key grade ≥3 toxicities including neutropenia (18%), fatigue (16%), thrombocytopenia (14%), sensory neuropathy (13%), and lymphopenia (13%). To date, six patients have proceeded to HDT-ASCT.[8] Bortezomib has also been investigated in combination with thalidomide and dexamethasone (VTD).[9] In a study of 38 patients given up to three 4-week cycles of VTD prior to HDT-SCT, overall response rate was 92%, with 18% CR/nCR.[9] Grade 3/4 toxicities included myelosuppression, neuropathy, infection (each 8%), and DVT/PE (5%). Following induction, 26 patients proceeded to HDT-ASCT, increasing overall response and CR rates among all 38 patients to 100% and 34%, respectively.[9] Bortezomib, thalidomide, and dexamethasone have been combined with cisplatin, doxorubicin, cyclophosphamide, and etoposide (VDT-PACE) in a phase 1 study of 12 patients.[10] Following two 5-week cycles, overall response rate was 83%, including 17% CR/nCR. Toxicities were predictable. Following HDT-ASCT and subsequent maintenance therapy with low-dose thalidomide and dexamethasone, overall response rate was 100%, including 75% CR/nCR.[10] VDT-PACE is currently being used as induction therapy in the Total Therapy 3 approach at the University of Arkansas. An important finding in these studies was that use of bortezomib in induction therapy prior to HDT-ASCT neither hampered successful harvesting of stem cells for subsequent transplantation nor affected the time to engraftment following HDT-ASCT. Median yields of CD34$^+$ stem cells ranged from 3.75 to 21×10^6 cells/kg; the numbers harvested were generally sufficient for single or, where planned, double transplantation.[1-8,10] These data confirm the activity of bortezomib-based combinations as induction therapy prior to HDT-ASCT. Substantial benefit to patients has been demonstrated, including notably high rates of CR/nCR and VGPR both following induction and post-transplantation. As achievement of these responses is associated with prolonged EFS and OS, it may be expected that use of bortezomib-based regimens will improve patient outcomes compared with conventional therapies; prolonged follow-up data will be required to confirm survival benefits. Importantly, bortezomib-based therapies appear effective in patients with poor prognostic factors, providing suitable treatment options for a broad range of patients. In the IFM phase 3 study, CR/nCR rate following bortezomib plus dexamethasone was similar to that in the overall population among patients with chromosome 13 deletion by FISH or β2-microglobulin >3 mg/L.[3] In the relapsed setting, bortezomib alone or in combination has been shown to be effective in patients with chromosome 13 deletion,[11] advanced renal failure,[12] or advanced stage disease,[13] and those aged ≥65 years.[13] Notably, the established safety profile of bortezomib was not markedly different among elderly patients.[13] Bortezomib-based therapies are becoming increasingly widely used as highly effective frontline treatment options for multiple myeloma patients eligible for HDT-ASCT; final results from ongoing phase 3 studies will help further define the optimal role of these regimens in the myeloma treatment paradigm.

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S5.4

THE ROLE OF LENALIDOMIDE IN MYELOMA PATIENTS ELIGIBLE FOR HIGH DOSE THERAPY

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Initial therapy in newly diagnosed myeloma is dependent on eligibility for autologous stem cell transplantation (ASCT).[1] It is important to avoid protracted melphalan-based therapy in patients with newly diagnosed myeloma who are considered eligible for ASCT, since it can interfere with adequate stem cell mobilization, regardless of whether an early or delayed transplant is contemplated. Typically patients eligible for ASCT are treated with approximately 4 cycles of non-alkylator-based induction therapy prior to stem cell harvest. Thalidomide-dexamethasone (Thal/Dex) has emerged as one of the most commonly used induction regimen for the treatment of newly diagnosed myeloma in the United States with response rates of approximately 65-70%.[2] Efforts are underway to identify regimens that are more effective. Lenalidomide (CC-5013) belongs to a class of thalidomide analogues termed immunomodulatory drugs (ImiDs) that appears safer and more effective than thalidomide.[3] A phase II trial conducted at the Mayo Clinic demonstrated remarkably high activity with lenalidomide plus dexamethasone

(Rev/Dex) as initial therapy for newly diagnosed myeloma without significant impact on stem cell mobilization or transplantation.[4] Lenalidomide was given orally 25 mg daily on days 1-21 of a 28-day cycle. Dexamethasone was given orally 40 mg daily on days 1-4, 9-12, 17-20 of each cycle. Thirty-one of 34 patients (91%) achieved an objective response, including 56% of patients who achieved very good partial response (VGPR) or better.[5] In the subset of 21 patients receiving Rev/Dex as primary therapy without ASCT, 67% achieved VGPR or better. Approximately fifty percent of patients experienced grade 3 or higher non-hematologic toxicity, similar to rates seen with dexamethasone alone. ECOG recently reported preliminary findings of a randomized trial testing Rev/Dex versus Rev/low-dose Dex (40 mg dexamethasone once weekly).[6] Results so far show that toxicity rates are significantly higher with Rev/standard-dose Dex compared to Rev/low-dose Dex. Early (first 4 month) mortality rates were low in both arms, 5% and 0.5% respectively. Based on these studies, Rev/low-dose dexamethasone is currently the regimen of choice in the Mayo Clinic SMART protocol for the treatment of standard-risk myeloma in patients who are candidates for ASCT outside the setting of a clinical trial. The incidence of DVT is low with single-agent lenalidomide or lenalidomide plus low-dose dexamethasone, but rises markedly when the agent is combined with high-dose dexamethasone. Aspirin alone is probably sufficient for patients receiving lenalidomide plus low-dose dexamethasone. Warfarin (dose adjusted to therapeutic INR of 1 to 2) or low molecular weight heparin (equivalent of enoxaparin 40 mg once daily subcutaneous) are recommended for patients receiving lenalidomide plus standard dose dexamethasone. A randomized trial is currently underway in the United States testing aspirin versus warfarin in patients receiving lenalidomide plus standard dose dexamethasone for newly diagnosed myeloma.

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S5.5

FRONTLINE TREATMENT IN PATIENTS NOT ELIGIBLE FOR STEM CELL TRANSPLANTATION

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Combination chemotherapy with melphalan and prednisone (MP) has been used in the treatment of multiple myeloma (MM) since the 1960s, and remained, as little as two years ago, the most widely accepted treatment option for elderly patients ineligible for high-dose therapy.[1,2] More complex combinations with alkylating agents have been substituted but often with added toxicity and inconvenience and no survival advantage over standard MP.[3] Dexamethasone-based regimens have also shown no treatment advantages compared with MP in elderly patients and were associated with higher toxicity.[4] Standard MP is associated with a response rate of 30%-50%, with very few complete responses (CR) and a median survival of approximately 3 years, thus providing ample room for improvement. Over the last several years, innovative drug combinations have been used to achieve this goal.

Initial treatment with MP-based regimens (MPT, MPV, MPR)

In two large randomized phase III studies, one from the Italian Multiple Myeloma Network (GIMEMA) and the other from the IFM (IFM99-06),[5,6] MPT was found to be significantly superior to MP. The superiority was demonstrated based on response, including CR rate and progression-free survival (PFS) in both studies. In the IFM study, the PFS advantage observed with MPT translated into a significant overall survival (OS) advantage. The addition of thalidomide to MP in the IFM study increased median OS by almost 2 years compared with MP alone. And even though OS prolongation with MPT versus MP in the GIMEMA study did not reach statistical significance, MPT results in both studies looked very similar. In the IFM study, MPT was also superior to a reduced-intensity autologous stem cell transplantation regimen using melphalan 100 mg/m^2, in terms of PFS and OS. MPT has also been found to be superior to MP in patients >75 years of age in the IFM 01-01 study to be presented at the 2007 ASCO meeting.

Table 1.

	MPT (GIMEMA)	*MPT (IFM)*	*MP (GIMEMA/IFM)*
CR (%)	16	13	2/2
VGPR (%)	37	47	12/7
PR (%)	76	76	48/35
PFS	54% EFS at 24 mo.	median 28 mo.	27% EFS at 24 mo. / median 17 mo.
OS	80% OS at 36 mo.	median 54 mo.	64% OS at 36 mo. / median 32 mo.
≥grade 3 adverse events (%)	48	42	25 / 16

Bortezomib (Velcade, Vel) has also been incorporated into the standard MP regimen (MPV)[7] in the treatment of elderly untreated patients with myeloma. The overall response rate was 89%, including 32% of patients with a CR and an additional 11% with a near-CR. EFS and OS at 16 months of follow-up were 83% and 90%, respectively. These promising results form the basis for the international phase III randomized VISTA study comparing MP with MPV. Based on the success of the phase III MPT versus MP trial, the GIMEMA group initiated a phase I/II study of MP + lenalidomide (Revlimid) (MPR). Higher response rates were achieved in the cohort of patients receiving melphalan 0.18 mg/kg and lenalidomide 10 mg/d for CR (24%), VGPR (24%), PR (33%), and MR (19%). None of the patients receiving this regimen have progressed. This study formed the basis for the ongoing European Myeloma Network phase III study comparing MP with MPR (with or without lenalidomide maintenance).

Initial treatment with Dexamethasone-based regimens (Thal/Dex, Vel/Dex, Len/Dex)

Thal/Dex and Len/Dex have been mainly used as induction regimens before autologous stem cell transplantation (ASCT). Their development was driven by the disadvantages of existing regimens such as intravenous VAD. In elderly patients, interim results of a phase III study comparing Thal/Dex with MP have reported higher response rates (67% vs 48%) for patients receiving Thal/Dex. However, increases in toxicity were also reported.[8] The combination of thalidomide, pegylated liposomal doxorubicin, and dexamethasone (ThaDD) has also been studied in a small series of patients > 65 years of age. An overall response rate of 84% was reported with 38% of patients achieving CR or near-CR. Grade 3 and 4 adverse events observed in at least 10% of patients included infections, thromboembolic events, neutropenia, and constipation.[9] Preliminary results with Len/Dex indicated that this regimen is highly active with a CR+VGPR rate of 67% and a PFS rate of 59% at 2 years in patients receiving this regimen as primary treatment. Toxicity was modest with grade 3 and 4 adverse events observed in at least 10% of patients, including fatigue and neutropenia.[10] Data with Vel/Dex are limited in newly diagnosed patients not eligible for ASCT. Long-term follow-up of patients treated with Vel with or without dexamethasone as front-line treatment reported that the median time to alternative treatment was 22 months in patients who did not proceed to ASCT.[11] Importantly, Dex given at high pulsed doses (40 mg on days 1-4, 9-12, 17-20 every 4 weeks) is associated with a high incidence of serious side-effects.[4,12] However, recent research suggests that high doses of Dex may not be necessary when used in combination with newer agents. The ECOG group has conducted a large phase III study of lenalidomide plus high-dose Dex versus lenalidomide plus low-dose Dex in newly diagnosed patients with MM.[13] Lenalidomide plus high-dose dexamethasone was associated with

greater toxicity, including increased thrombotic events compared with lenalidomide plus low-dose dexamethasone. This result could be of major importance for all Dex-based regimens. Clearly, it would be of major benefit if the same degree of efficacy could be obtained with less toxic doses of Dex.

Conclusions

At present, given the results of the IFM and GIMEMA studies, MPT appears to be the treatment of choice for a large proportion of elderly patients ineligible for ASCT. It seems certain that in the near future, MPV and MPR will also be proved superior to MP, thus providing three therapeutic options, MPT, MPV and MPR, in this patient group with MM. These therapeutic options could lead to more personalized treatment approaches, based on patient comorbidities, as the three novel agents have somewhat different toxicity profiles. MP would only be appropriate for a minority of patients with poor perfomance status and/or significant comorbidities, such as severe neuropathy or a contraindication to anticoagulants. Questions regarding the relative efficacy of melphalan-based regimens versus dexamethasone-based regimens (preferably with low-dose dexamethasone) will require randomized phase III trials. More intensive approaches with new drug combinations or with the incorporation of pegylated liposomal doxorubicin will also require additional studies. Additionally, the important issue of maintenance treatment in elderly patients with MM needs to be investigated.

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S5.6

MANAGEMENT OF TOXICITIES FROM NOVEL AGENTS

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Proteasome inhibitors and immune modulators are the two main classes from which agents are approved for multiple myeloma therapeutics. Experience with thalidomide and Bortezomib has underscored the need for diligent monitoring and management of side effects to achieve optimal therapeutic benefit. Initial phase II and III results with lenalidomide, a thalidomide analogue, have confirmed impressive response rates with a significantly more favorable toxicity profile. All these compounds have not only contributed to improved response rates as single agent but in combination with chemotherapy appear to be positively influencing the overall survival for myeloma patients.[1-4] Two different types of bisphosphonates have also been approved over the past decade for the management of skeletal destruction. Those agents have resulted in a clinically significant decrease in skeletal related events and are well tolerated over the short term.[5;6]

General side effects associated with novel therapy

Peripheral neuropathy which is an issue with first generation proteasome inhibitors as well as immune modulators appear to have been minimize with the development of the second generation immune modulator (lenalidomide) and will probably be the case with the second generation proteasome inhibitors. Hypercoagulable events continue to be a significant issue in the management of patients receiving immune modulator based therapy but has always been noted in plasma cell dyscrasia in general however at lesser extent.[7;8] The new immune modulator lenalidomide to have leucopenia and mild thrombocytopenia as part of its side effect profile.[9] There is early indirect evidence to suggest that the dose and schedule of lenalidomide influences the outcome of therapy in patients with multiple myeloma and thus it is critical to understand the true clinical implications of this abnormality. On the supportive care end, management of side effects is critical as the main role of these agents is to prevent further end organ damage and the expectation is end organ damage related to their use should be minimal if not non-existing. Bisphosphonates have been felt to be linked to the occurrence of osteonecrosis of the jaw.[10;11] In this section the management and deep venous thrombosis (DVT), neutropenia and its impact on therapy as well as osteonecrosis of the jaw will be discussed.

Neutropenia

Neutropenia as grade 3/4 toxicity was noted in 15% of the multiple myeloma patients receiving lenalidomide as a single agent. However, this was associated with a less than 5% incidence of neutropenic fever. In the DVd-R regimen (Pegylated doxorubicin-Doxil, Vincristine, reduced dose dexamethasone and Revlimid, even though therapy was given to neutropenic patients, the incidence of neutropenic fever in the phase II portion of the trial was less than 6%.[3] Notably, neutropenic fever was uncommon, but caution must be exercised as multiple myeloma patients have a compromised humoral immune system and late in the disease both the cellular system and the immune system become less effective. Management of neutropenia should be individualized depending on the patient's overall clinical status such as intact gastrointestinal linings and skin as well as the severity of neutropenia. A short course of growth factors should be considered in patients with compromised gastrointestinal linings, but this was rarely required with the use of single-agent lenalidomide except as a protocol-specified intervention. Dose reduction should be considered if growth factors are not effective.[12] We do not generally recommend lenalidomide treatment delays because of risk of disease progression during the interim. From a clinical standpoint, grade 3 neutropenia occurring with the use of lenalidomide as a single agent or in combination therapy did not result in a major clinical challenge as long as the patients were well educated to recognize early evidence of active infection and were monitored carefully.

VTE and Immunomodulatory drug combination regimens

Thalidomide and lenalidomide as a single agents do not appear to increase the incidence of VTE.[13;14] However, in another Lenalidomide phase 1 study, 2 of 6 patients (33%) who initiated treatment with lenalidomide 50 mg/day experienced VTE suggesting possible correlation with dose intensity.[15] When thalidomide was combined with chemotherapy, including thalidomide-doxorubicin[4] or melphalan-pred-

nisone-thalidomide (MPT),[16] a higher incidence of VTE was reported. Regimens combining thalidomide with anthracyclines resulted in VTE rates ranging from 7% to 58%.[17-24] The combination of thalidomide and dexamethasone elevated the incidence of VTE to 15%-26% in patients with newly diagnosed MM.[25-29] Overall, the median time to onset of a thrombotic event appears to be around 3 months. The same trend was noted when lenalidomide was combined with steroids or other chemotherapeutic agents especially n newly diagnosed patients where the incidence could be as high as what is noted with the use of anthracyclines n combination with thalidomide.[24;30] Risk factors for developing a hypercoagulable event was studied by Zangari *et al.* in 535 patients treated either with thalidomide in combination with cytotoxic chemotherapy regimens or with dexamethasone only.[31] By multivariate analysis, the combination of thalidomide with chemotherapy regimens containing doxorubicin was associated with the highest odds ratio (OR) for DVT (4.3; *p*" 0.001). Newly diagnosed disease (OR, 2.5; *p*=0.001) and chromosome 11 abnormality (OR, 1.8; *p*=0.048) were also independent predictors for DVT. The development of VTE did not adversely affect survival when examined as a time-dependent variable and adjusted for standard risk features (hazard ratio, 0.8; *p*=0.162). The absence of a negative impact on survival was also noted by Baz *et al*.[24] Preventing the occurrence of hypercoagulable events has been the focus of several groups. As expected several prophylactic regimens have been evaluated but to date there has been no randomized trials to favor a regimen vs. another. Strategies for use of prophylactic anticoagulants in conjunction with thalidomide or lenalidomide combinations have included prophylactic warfarin, low molecular weight heparin, or low-dose aspirin.[26;32-44]

Why low dose aspirin?

Out of all he regimens low dose aspirin seems the most attractive as it is the least likely to result in high risk of bleeding and appears to be as effective as other more complex regimens. Aspirin (acetylsalicylic acid) has antiplatelet effects; by acetylating the active site of cyclo-oxygenase enzymes 1 and 2 (COX-1 and COX-2), it prevents the binding of arachidonic acid and the production of thromboxane A_2. Thromboxane A_2 causes activation and aggregation of platelets, which is an early step in thrombosis. The low dose is preferred over the full dose as lower aspirin doses are known to inhibit thromboxane generation; higher doses inhibit both thromboxane and prostacyclin. The improved ratio of prostaglandins obtained with lower doses is thought to be less thrombogenic, and thus lower doses are preferred for prophylaxis.[45] Promising evidence has also been reported for use of low-dose aspirin as antithrombotic prophylaxis with the lenalidomide/dexamethasone-containing regimens DVd-R (pegylated doxorubicin, vincristine, reduced-frequency dexamethasone, and Revlimid® [lenalidomide]),[3] BiRD (Biaxin [clarithromycin], Revlimid® [lenalidomide], and dexamethasone),[46] and MPR (melphalan, prednisone, and Revlimid® [lenalidomide]).[47]

Osteonecrosis of the Jaw (ONJ)

ONJ is a process without a clear etiologic factor in multiple myeloma. There has been reports of the event occurring in different disease processes including diabetes mellitus [48] some risk factors have been defined in multiple myeloma. The process appears to be time-dependent with higher risk after long-term use of bisphosphonates, older multiple myeloma patients and often after often after dental extractions.[10] This latter characteristic does not necessarily has to be a predisposing event as our group did not note any dental manipulations in seventeen multiple myeloma cases.[11] In this report by Thacker *et al.* all patients were being treated with bisphosphonates for a median of 5 month prior to the onset of jaw symptoms. None of the patients had been irradiated in the jaw nor had obvious osseous manifestation of multiple myeloma in the jaw. Microorganisms were isolated in 7/17 patients and all improved with anti-microbial therapy.[11] Since this publication we have encountered 3 cases of severe jaw pain requiring intravenous pain therapy; non of those 3 patients demonstrated radiologic evidence of ONJ however all three patients recovered with intravenous anti-biotic therapy and 2/3 relapsed with the discontinuation of therapy and remain on chronic suppressive therapy suggesting an infectious etiology. We have initiated the following guidelines in an effort to ameliorate the incidence of this complication. Patients should have a full dental examination at the time of diagnosis of the plasma cell dyscrasia especially if' bisphosphonates are to be considered as part of the therapy. In addition, bisphosphonates are held for a period of 3 month prior to invasive dental procedures to allow for the osteoclastic recovery, therefore enhanced debris removal and lessening the chance of creating a fertile bacterial medium. Following the dental proce¬dure we would reintroduce bisphosphonates only after the healing process is complete. We also recommend that multiple myeloma patients diagnosed with jaw osteonecrosis probably have a concurrent infection and should be aggressively treated with antibiotics.

In summary

Immunomodulatory drugs, proteasome inhibitors and bisphosphonates have revolutionized therapy for multiple myeloma. However, end organ damage that could result from these agents could compromise the ultimate outcome of therapy.

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S5.7

PROGNOSTIC FACTORS FOR MULTIPLE MYELOMA IN THE ERA OF NOVEL THERAPIES

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With the use of conventional chemotherapy the median survival of patients with multiple myeloma (MM) has been of about 3 years. However, there is a wide variability in survival that is due to differences related to both the host and the tumor. Since the first report, forty years ago, by Carbone *et al.*,[1] many studies dealing with prognostic factors have resulted in the recognition of a large number of factors with prognostic value. In this review we will briefly foccus on: 1) factors associated with host characteristics, tumor mass and disease complications, 2) staging systems, 3) factors related to the malignant clone, particularly the molecular genetic status, 4) prognostic impact of response to therapy with emphasis on the newer treatment approaches and 5) possible impact of new drugs on mechanism of disease control/progression.

1. Host Factors, Tumor Mass and Complications

It is well established that age and performance status are crucial prognostic features. Concerning plasma cell mass and disease complications the most important prognostic parameters are the beta2-microglobulin serum levels, hemoglobin level and the renal function status.

2. Development of Staging Systems

Based on prognostic features a number of staging systems, usually derived from multivariate regression models, have been developed. However, none of them has been entirely satisfactory. In the search for a useful and reproducible prognostic classification, the International Myeloma Working Group has recently developed the so-called International Staging System (ISS) derived from a large number of patients included in both cooperative studies and individual institutions. The ISS is based on easily available parameters such as beta2-microglobulin and albumin.[2] The ISS will be largely discussed in this session by Doctor Philip Greipp.

3. Factors Related to the Malignant Plasma Cell Clone

The cytogenetic status has emerged as the most important prognostic feature in MM. Patients with hyperdiploidy have a good outcome, in contrast with those with hypodiploidy, while patients with t(11;14) have an *average* survival. The cytogenetic poor prognostic factors are retinoblastoma (Rb) and p53 deletions as well as the immunoglobulin heavy-chain (IgH) translocations t(4;14) and t(14;16). The coexistence of Rb deletions with IgH translocations has raised the question of whether the prognostic impact of each abnormality may be influenced by the other. In this regard, the Spanish Myeloma Group has just reported that the only factors independently affecting survival were: t(4;14), Rb deletions associated with other cytogenetic abnormality, age > 60 years, high proportion of cells in S-phase and advanced disease stage by the ISS.[3] On the other hand, up-regulation of 1q genes, such as high expression of the gene CKS1B, located in 1q21, is becoming a signature of very poor survival.[4] Gene expression profiling (GEP) provides information on the expression level of an extensive number of genes related to important disease features such as proliferation, apoptosis, DNA repair and drug resistance. Expression profiling has been used to define molecular subgroups of MM with clinical correlations and a *translocation / cyclin D* classification for MM has been proposed.[5] In addition, based on gene expression signatures driven by recurrent translocations and hyperdiploidy, seven distinct molecular entities of MM with both different clinical features and long-term outcome have been recognized.[6] Very recently, high resolution array comparative genomic hybridization, mRNA microarray, FISH analysis and novel bioinformatics approaches

defined different clinicopathologic subgroups of MM based on recurrent DNA copy number changes.[7] Thus, two forms of hyperdiploid myeloma have been identified: one with chromosome 11 gains (good prognosis) and other with chromosome 1q gains and chromosome 13 losses (poor outcome).[7] The features from these and future molecular genomic studies should be the framework for a better understanding of the disease pathogenesis, facilitating the discovery of drugs targeting the molecular pathways of specific myeloma genomic subgroups.[6]

4. Response to Therapy as Prognostic Factor

Before the introduction of novel drugs, few patients treated with conventional chemotherapy achieved CR and the correlation between the degree of response and survival was questioned.[8] In fact, in many studies the stabilization of the disease was a more powerful prognostic indicator that the degree of tumor reduction. In contrast, 35 to 50% of patients undergoing high-dose therapy/stem cell transplantation (HDT/SCT) enter CR and patients attaining CR post-transplant have a significantly longer EFS and OS than those achieving only a PR. This would suggest that there is a difference in the quality of CR after conventional chemotherapy and after HDT/SCT. However, in the MD Anderson experience patients achieving CR with primary therapy did as well as those achieving CR post-transplant.[9] With the incorporation of new agents with novel mechanisms of action an increased number of patients achieve CR with primary therapy. In addition, new drugs such as bortezomib are highly effective in patients with unfavourably cytogenetics.[10,11] Further follow-up is needed to establish the impact of these CR in non-transplant population on PFS and OS. On the other hand, with the incorporation of new drugs in the induction pre-transplant regimens a higher tumor reduction is currently achieved, this will hopefully result in a higher CR rate postransplant. However, the impact of new drugs on the post-transplant outcome remains to be determined.

5. Possible Impact of Influencing on Mechanisms of Disease Progression

The main efforts in the treatment of MM have been foccused on plasma cell killing, being the main goal of myeloma therapy the achievement of the lowest tumor mass. However, this is a fight against a resistant population that will ultimately re-growth. Ideally, the therapy impacting the final outcome should be designed to avoid disease progression once a low tumor mass has been achieved. This will only be possible by advancing in the understanding of the physiopathology of the monoclonal gammopathies. In this regard, the model is monoclonal gammopathy of undetermined significance (MGUS), a situation in which a plasma cell clone, which shares the phenotypic and cytogenetic features of malignant plasma cells, expands to a certain limit and remains stable usually for years. However, in the authors experience about 10% of patients with MGUS have an slowly rising M-protein from the time of diagnosis and virtually all of them will develop MM.[12] These patients likely have *slowly evolving myelomas*, scaping the still unknown growth-restraining influences which maintain MGUS in an stable state. The physiopathologic mechanisms limiting plasma cell expansion in individulas with MGUS should be deeply investigated since they can be crucial in the development of drugs preventing disease progression. This would represent a major step forward for the long-term outcome of patients with MM. Importantly, the era of a much better understanding of the disease in both the molecular genetics and mechanisms of disease progression is also the era of new drug development. This *coincidence* will hopefully result in a real impact in prolonging the survival of patients with MM. At this point, the so-called *prognostic factors* will be progressively lossing their importance due to the much better control of the disease.

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S6: Debate I

S6.1

SHOULD CR ACHIEVEMENT BE A MAJOR TREATMENT OBJECTIVE?

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In Multiple Myeloma (MM), it was not possible to assess the role of complete remission (CR) achievement before the introduction of high dose therapy, since CR was a rare event with conventional dose chemotherapy. In the very first studies on Autologous Stem Cell Transplantation, it became evident that high response rates could be achieved including CR and that CR achievement was correlated to longer survival. In the IFM 90 trial which was the first randomized trial showing the superiority of high dose therapy compared to conventional chemotherapy, 5-year overall survival (OS) rate was significantly longer in patients achieving CR or very good partial remission (VGPR) with ≥ 90% reduction of the M-component than in patients achieving only partial remission (PR) (72% versus 39%).[1] The prognostic value of CR achievement in the context of high dose therapy has been confirmed by other investigators,[2] which induced two consequences: 1) CR achievement became one of the objectives of clinical trials and is even the primary objective in a number of recent studies. 2) Criteria for response assessment have been changed. In the EBMT criteria which are currently the most frequently used CR has been defined by a negative immunofixation with less than 5% plasma cells in the marrow.[3]

I) In newly diagnosed patients, survival benefit related to CR achievement is likely due to a longer duration of first response

In almost all randomized studies when one arm induces more CR than the other it also yields longer progression free survival (PFS) which is usually associated to a longer OS. However in some studies higher CR rate and longer PFS were not converted into younger OS. This is particularly the case when the best treatment is offered as salvage therapy in the majority of relapsed patients explaining in part why survival after relapse is shorter in patients having received the best treatment upfront.

High-Dose Therapy

Usually, when PFS and OS are better in the high dose therapy arm of randomized studies, the CR rate is also higher. Therefore the survival benefit from high dose therapy compared to conventional chemotherapy can be explained at least in part by a higher CR rate. On the opposite, in the US Intergroup study the CR rate is not different between high dose therapy and conventional chemotherapy and no survival benefit from high dose therapy is observed.[4]

Conventional Dose Therapy

This impact is not only evident with high dose therapy but also with conventional chemotherapy when the number of patients is large enough. In a randomized ECOG study on 613 previously untreated patients, patients who achieved CR had longer survival (5.1 years) than those achieving only PR (3.3 years) ($p < 0.0001$).[5] With regimen including novel agents upfront, the CR or CR+VGPR is much higher than with conventional chemotherapy. The combination of Melphalan-prednisone with either Thalidomide or Bortezomib yield CR or CR+VGPR rates that are comparable to those achieved with high dose therapy and are associated with significantly longer PFS and possibly OS.[6-8]

II) Relapsed MM

The impact of CR achievement is not limited to frontline treatment. But it is also shown in the context of relapsed MM especially with the introduction of novel agents that are able to induce CR even in heavily pretreated patients. For instance in the Summit Phase II trial on Bortezomib, a better quality of response was associated to a longer time to progression (TTP): TTP was 16.4 months for patients achieving CR or near CR (negative electrophoresis with positive immunofixation) versus 9.2 months in patients achieving only PR. In the APEX randomized trial TTP 12.2 months in CR/near CR patients versus 8.3 months in PR patients.[9] In the large randomized studies performed in relapsed patients the arms with Bortezomib and Lenalidomide were superior to the control arms in terms of CR rate, PFS and OS. Again, like for newly diagnosed patients, the best arms induced more CR.[10-12]

III) However several questions should be addressed

Which level of CR is clinically relevant?

A number of investigators have shown that true CR with negative immunofixation is superior to near CR. This can be better demonstrated when a large number of patients is evaluated while when the number of true CR is too small, the difference may not be significant. More generally, like in other haematological malignancies, there is probably a relationship between quality of response and outcome. In the IFM 99 trials on 849 evaluable patients we have shown that CR+near CR was associated with a better outcome than VGPR and PR.[13]

Table.

	N (%)	*Median EFS*	*5-year OS*
CR	274 (32)	42 m	77%
VGPR	191 (22,5%)	38 m	63%
PR	311 (37%)	30 m	55%

The prognostic value of stringent CR or of molecular CR is not yet known, although molecular CR obtained with allogeneic stem cell transplantation can be associated with very long remissions and possibly cure. However, from a practical point of view, the IFM has always found that CR+VGPR is a relevant and single prognostic factor. In the recent analysis of IFM 99 trials again survival in patients achieving CR+VGPR was dramatically superior to survival in patients with only PR.[13]

Is consolidation or maintenance after CR achievement useful?

Unfortunately, until now, no treatment has been successful in increasing survival once CR is achieved. In the context of single ASCT, there is no evidence that achieving CR with induction treatment prior to ASCT is converted into a higher trial CR rate and improved survival. In the IFM studies, CR after VAD induction was not associated with a significantly better outcome but that could be explained by a low CR rate. Accordingly, in randomised trials comparing Thalidomide + Dexamethasone with conventional chemotherapy with VAD, the better response rate prior to ASCT was not converted by a higher CR rate after ASCT.[14,15] In a recent retrospective analysis on 721 patients the median survival was comparable in patients achieving CR after induction treatment and in those achieving CR only after intensive therapy.[16] In the context of double ASCT, in the IFM 94 trial patients achieving CR+VGPR after one transplant did not benefit from the second transplant.[17] Again in the IFM 9902 trial, maintenance therapy with Thalidomide was beneficial only in patients with PR after double ASCT while for patients in CR/VGPR after double ASCT, thalidomide did not improve outcome.[18] Therefore we still need a maintenance therapy that would be able to prolong CR.

Are there subgroups of patients for whom CR achievement is not associated with longer PFS and OS?

Patients with poor-risk cytogenetics like t(4;14) can achieve CR but usually the responses are short. On the opposite, some patients who have long remission may have a small residual M-component (like in a MGUS status).

Conclusion

Achieving CR should be the objective of the treatment of MM in any situation. However we still have to define the optimal CR level and to find the treatment able to maintain CR.

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S6.2

SHOULD CR ACHIEVEMENT BE A MAJOR TREATMENT OBJECTIVE?

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The discussant play a role against the value of the CR in the treatment of Myeloma. The following presentation does not necessary corresponds to the Authors opinions. Multiple myeloma (MM) is an incurable malignancy of terminally differentiated B-cells accounting for approximately 1 to 2% of all human cancers and 10% of hematologic malignancy.[1] Chemotherapy is the preferred initial treatment for overt symptomatic MM. Oral administration of melphalan and prednisone to patients with MM produces an objective response in 50% to 60%, and rarely patients achieve complete remission (CR).[2] Complete remission has been increased to 20 – 40% with autologous transplantation,[3] and the the median survival has been extended beyond 6 years.[3-5] Achieving CR has been considered key to long-term disease control in MM with high-dose therapies (HDT). Some patients, however, survive beyond 5 and even 10 years after HDT without ever having achieved CR.[6] The relation between overall survival time (OS) and response level was analysed in 432 MM patients from 4 prospective Finnish Leukaemia group trials, treated with conventional chemotherapy (CCT). No survival advantage was evidenced for those achieving CR.[7] In another study on 243 MM patients treated with CCT the median survivals of patients with objective and partial response were 43.4 and 42.8 months, respectively, versus 19 months for non responders.[8] However, median survival of 14 patients who achieved a CR was 42 months. A significant correlation between response and survival was observed ($p<0.0001$). In this study neither the degree of response nor the response kinetics had a significant influence on survival. However, the response to therapy was associated with a significantly longer survival in MM patients.[8] Four Southwest Oncology Group (SWOG) standard-dose chemotherapy protocols for MM including 1,555 previously untreated patients with MM were also evaluated for this purpose. Six-month and 12-month landmark analyses were performed to evaluate the outcome for patients in each response category. The overall and event-free survivals for the four protocols combined were 33 months and 18 months, respectively. Using 6- and 12-month landmarks, the median survivals of 30 to 35 months were not different for responders (50% and 75% regression) versus non responders in patients without disease progression before the landmarks. Conversely, at the 6- and 12-month landmarks, the median survivals for patients who had experienced disease progression were 13 and 15 months, respectively, versus a 34-month median for patients who did not experience progression. The magnitude of response, as a single variable, was not predictive of survival duration. Patients with response and stable disease had equivalent outcome. Only patients with progressive disease had a poorer outcome. The best indicator of survival was time to first progression.[9] The significance of obtaining CR in chemotherapy versus transplant populations remains unclear. In a French study 190 patients between 55 and 65 years old who had newly diagnosed MM were randomly assigned to receive either CCT or HDT and autologous blood stem-cell transplantation. Complete remission rates were 36% in the HDT arm and 20% in the CCT arm. The response was not predictive of survival in the HDT group. Median OS time was 59 months (from random assignment) for the 34 patients who achieved a CR and 40.5 months for the other patients. The OS (and EFS) curves of the two groups were not significantly different ($p=0.22$).[10] Blade' *et al.* evaluated the impact of giving HDT intensification compared to continued standard chemotherapy in MM patients responding to the initial chemotherapy. The HDT intensification significantly increased the CR rates (30% vs 11%; $p=0.002$), but had no significant impact on survival, median OS was 61 months for HDT vs 66 months for chemotherapy ($p=0.89$).[11] In another study of Rajkumar *et al.* a CR rate of 33% in 126 patients with MM who underwent HDT with stem cell transplantation for MM was reported, with progression-free duration of survival of 15 months for patients who achieved CR and 11 months for those who did not ($p=0.2$), whereas OS was the same. The authors found that overall duration of survival was less than 30 months in high-risk patients regardless of CR status and 57 months in the low-risk patients. Overall duration of survival was not different in patients who obtained CR (25 months) compared with those who did not (24 months).[12] The new drugs thalidomide, lenalidomide and bortezomib effect CR rates[13] especially when used in combination with each other and with standard agents, little is known, however, about the durability of the responses induced by these treatments, especially after the discontinuation of the drugs. Furthermore data translating these responses into survival prolongation are lacking. When thalidomide was incorporated into the high-dose therapy followed by autologous transplantation, a higher CR rate (62% vs 43%) and improved 5-year event-free survival (56% vs 44%) was observed compared with high-dose therapy without thalidomide. Unfortunately, the 5-year overall survival was similar in both groups ($p=0.9$).[14] In the same study it has been reported that in MM evolving from a documented monoclonal gammopathy of uncertain significance (MGUS), or a smoldering phase, a CR was infrequent, but when one did occur, it had no effect on the likelihood of survival[6]. Recently, the International Myeloma Working Group has developed new criteria for assessing response in MM trials[15] and it has been suggested that duration of response may be an important major treatment objective, since it can predict ultimate overall survival.[8] This reflects the understanding that clinical outcomes for multiple myeloma (MM) are highly heterogeneous: despite high frequency of response, early relapse are seen also in patients achieving CR. Thus, CR cannot be included as a major unique objective for clinical trials.

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S7A: Oral presentations I

S7a.1

ESTABLISHMENT OF AN AMYLOID-FORMING HUMAN CELL LINE

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Introduction. Primary amyloidosis (AL) is a rare monoclonal plasma cell disorder in which insoluble immunoglobulin light chains are produced. These proteins undergo conformational changes because of protein misfolding and become deposited in vital organs throughout the body. Because of the typically small numbers and low proliferative capability of plasma cells associated with AL, advances in the field have been limited by a lack of cell line model systems. To our knowledge, there is no human cell line in existence that has been established from a patient diagnosed with AL. In this study, we have established 2 novel AL cell lines from the same patient that have been shown to secrete free lambda light chain which exhibits the ability to form amyloid fibrils. *Methods*. The ALMC-1 and ALMC-2 cell lines were established from a patient diagnosed with amyloidosis before and after a peripheral blood stem cell transplant and were extensively characterized for phenotypic markers, cytokine responsiveness, and genetic abnormalities. To determine whether these cell lines could be used as tools to study the amyloidogenic LC, LC was purified from cell supernatants using size exclusion chromatography. Secondary structure was measured by circular dichroism spectroscopy (CD) and thermal denaturation was calculated to determine melting temperatures. Fibril formation was verified using Thioflavin T (ThT) to measure plaques composed of beta sheets. Electron microscopy (EM) was used to visually identify the presence of amyloid fibrils. *Results*. CD spectroscopy determined that the LC purified from ALMC-1 and ALMC-2 cell lines contained a beta structure, expected for an immunoglobulin molecule. Fibril formation was achieved by incubating these proteins at their melting temperature and was confirmed using ThT. In addition, EM visibly proved that production of amyloid fibrils was feasible. Long, straight, unbranching fibrils consistent with the size and shape of amyloid fibrils were observed. *Conclusion*. The formation of amyloid fibrils from these naturally secreting human LC cell lines is unprecedented and will clearly prove to be an invaluable resource to better understand AL, from the combined perspectives of amyloidogenic protein structure and amyloid formation, genetics and cell biology.

S7a.2

RNAI SCREENING OF THE KINOME FOR LETHAL TARGETS AND BORTEZOMIB SENSITIZORS IN MYELOMA

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Introduction. The paucity of validated kinase targets in Multiple Myeloma has delayed the establishment of kinase inhibitors in the treatment of this malignancy. We have conducted a high throughput, kinome wide, RNAi lethal screen in Myeloma cells to identify kinases essential to the survival of human Myeloma tumors. In addition, we have identified kinase targets that sensitize tumor cells to bortezomib therapy by RNAi screening in the presence of this drug. Methods We optimized siRNA transfection conditions for a number of human Myeloma cell lines (HMCL), achieving 90% efficiency. The HMCL, KMS11, was screened in duplicate with an 1800-oligo (639 gene) siRNA library targeting the kinome both in the absence and presence of titrated bortezomib (IC10-IC50). siRNA were used at low concentration (13 nM) to minimise off-target cellular effects. To provide confidence in the specificity and adequacy of gene silencing, each gene was screened initially with at least 2 validated siRNA and scrambled-sequence siRNA were tested in parallel. The specificity of lethal RNAi was established by repeat targeting of short-listed kinases using two additional custom-designed siRNA per gene. Myeloma-lethal and bortezomib-sensitizing kinase targets were thus selected by multiple (up to 4) independent siRNAs. The activities

of KMS11-derived kinome targets have been assessed in other HMCL and are currently being tested in non-myeloma lines to develop a catalog of kinase targets with specific cytotoxicity in Myeloma. *Results*. Approximately 5% of kinome siRNA caused greater than four standard deviation reductions in HMCL viability. A shortlist of 80 kinases either putatively essential for myeloma proliferation or synergistic with bortezomib (on inhibition) was derived from initial screening. RNAi specificity was subsequently confirmed for 60% of candidate kinase targets. At least 75% of validated myeloma-lethal RNAi were active against more than one myeloma cell line. *Conclusion*. We have identified 24 kinases essential for proliferation and survival in Myeloma tumors and have additionally defined kinase targets that are synergistic with bortezomib either with or without independent cytotoxicity on silencing. Small molecule inhibitors of identified kinase targets are now being studied with and without bortezomib.

S7a.3

5-AZACYTIDINE INDUCES DNA DAMAGE RESPONSES AND APOPTOSIS IN MM CELLS

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Introduction. Aberrant DNA methylation and related aberrant gene expression contributes to the development of hematologic malignancies. Specifically, methylation of CpG islands in promoter regions are associated with transcriptional silencing of tumor suppressor genes, suggesting DNA methylation as a target for novel therapeutics. 5-Azacytidine is an inhibitor of DNA methylation and demonstrates clinical efficacy in MDS and AML. However, despite the widely accepted demethylating activity of 5-azacytidine, the exact basis of its cytotoxic mechanism still remain unclear. The activity of 5-azacytidine requires its incorporation into cellular DNA/RNA, with subsequent sequestration of DNA methyltransferases (DNMTs) via irreversible bond formation. Based on this, several non-mutually exclusive mechanisms were proposed for 5-azacytidine-induced cytotoxicity including (i) demethylation of cellular DNA via sequestration of DNMTs; and (ii) induction of DNA damage due to the irreversible formation of enzyme-DNA adducts. Although the former has been studied extensively, the DNA damage related sequeale of 5-azacytidine in MM have yet not been delineated. Here, we investigated the cytotoxicity of 5-azacytidine against MM cells, and characterized DNA damage-related mechanisms of 5-azacytidine-induced cell death. *Materials/Methods*. Cytotoxicity was detected by MTT; flow-cytometry, immunocytochemistry and western-blotting were used for mechanistic studies. *Results*. 5-Azacytidine showed significant cytotoxicity against both conventional therapy-sensitive and -resistant MM cell lines, as well as multidrug-resistant patient-derived MM cells (IC50 ~0.8-3 uM). Conversely, 5-azacytidine was not cytotoxic to peripheral blood mononuclear cells at these doses. Importantly, 5-azacytidine overcame the survival advantages conferred by exogenous IL-6, IGF-1, or by adherence of MM cells to bone marrow stromal cells. 5-Azacytidine induced DNA double strand break (DSB) responses, as evidenced by H2AX, Chk2 and p53 phosphorylations, and apoptosis in MM cells. 5-azacytidine-induced apoptosis was both caspase-dependent and -independent, with caspase 8 and caspase 9 cleavage; Mcl-1 cleavage; Bax, Puma and Noxa upregulation; as well as release of AIF and EndoG from mitochondria. Finally, we show that 5-azacytidine-induced DNA DSB responses were mediated predominantly by ATR, and that doxorubicin, as well as bortezomib, synergistically enhanced 5-azacytidine-induced MM cell death. *Conclusion*. These data provide the preclinical rationale for the clinical evaluation of 5-azacytidine in combination with doxorubicin and bortezomib, to improve patient outcome in MM.

S7a.4

NOVEL CDK4/6-BASED COMBINATION THERAPIES IN MYELOMA

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Deregulation of Cdk4 or Cdk6 is central to the loss of cell cycle control in cancers, in particular myeloma because overexpression of either Cdk4-cyclin D1 or Cdk6-cyclin D2 predisposes bone marrow (BM) myeloma cells to proliferation *in vivo*. Conversely, silencing Cdk4/6 by its physiologic inhibitor p18INK4c is required for G1 cell cycle arrest in the generation of normal plasma cells. PD 0332991 is a novel orally bioactive small molecule that potently and specifically inhibits Cdk4/6. We have demonstrated that PD 0332991 rapidly inhibits Cdk4/6 (IC50 ~ 60 nM) and induces complete G1 cell cycle arrest in primary human myeloma cells in the presence of BM stromal cells and in cycling myeloma cell lines. Non-invasive whole body imaging further reveals that PD 0332991 nearly completely prevents tumor growth in a rapidly disseminated NOD/SCID xenograft human myeloma model without overt toxicity. On this basis and the favorable outcome of a PD 0332991 Phase I clinical trial, a randomized PD 0332991 Phase I/II combination therapy clinical trial for myeloma will soon be initiated. Because PD 0332991 does not induce apoptosis when used as a specific Cdk4/6 inhibitor as intended, we have further developed two strategies for PD 0332991-based combination therapy for myeloma. In the first, we show by BrdU pulse-labeling that the release of PD-0332991 induced G1 arrest leads to synchronous S phase reentry, which in turn markedly enhances the killing of myeloma cells by Bortezomib. In the second, we show that prolonging PD 0332991-induced G1 arrest does not induce apoptosis, but greatly heightens the susceptibility of myeloma cells to Bortezomib or dexamethasone killing largely through synergistic induction of mitochondria depolarization. Analyses of Kaplan-Meier curves and tumor growth by imaging in the xenograft model have validated that the anti-myeloma effect of the combination therapy is superior to that of either PD 0332991 or Bortezomib (dexamethasone) alone. Our *in vitro* and *in vivo* studies shed lights on the mechanism that underlies the coupling between cell cycle and apoptotic controls in myeloma. Targeting Cdk4/6 by PD 0332991 in combination with a cytotoxic compound, therefore, represents the first promising cell cycle-based therapy in myeloma.

S7a.5

TARGETING BETA 1 INTEGRIN MEDIATED SURVIVAL SIGNALS IN MULTIPLE MYELOMA

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Introduction. Multiple myeloma (MM) is a disease that will initially respond to chemotherapy. However, the disease is not curable with standard chemotherapy, indicating that standard therapy fails to eliminate minimal residual disease. Myeloma homes to the bone marrow an environment that is considered a rich source of extracellular matrixes. We propose that MM-matrix interactions, contributes to failure of chemotherapy to eliminate minimal residual disease. In support of the importance of MM-matrix interactions in evaluating drug response our laboratory previously demonstrated that adhesion of MM cell lines and primary patients specimens via beta 1 integrins is sufficient to confer a multi-drug resistant phenotype. We have referred to this phenotype as cell adhesion mediated drug resistance or CAM-DR. Beta 1 integrin signaling induces a network of signaling pathways that could contribute to drug resistance which include activation of Src family kinases, AKT and a reduction in the levels of the pro-apoptotic Bcl-2 family member Bim. Because of the multiplicity of integrin signaling we proposed that targeting the integrin receptor may be an effective strategy to block integrin mediated survival signals. The goal of this study was to determine whether inhibition of beta 1 integrin mediated adhesion with an inhibitory peptide referred to HYD1 is a viable strategy for increasing the efficacy of standard chemotherapy. *Material and Methods.* Peptide: The D-amino acid peptide referred to as HYD1 was discovered using combinatorial chemistry. HYD1 was identified as the lead peptide based on the potency of blocking beta 1 integrin mediated adhesion. Co-culture model: The bone marrow stroma cell line HS-5 was used for co-culture studies. Myeloma cells were treated with 50 ug/mL HYD1 for 45 minutes prior to co-incubating the target myeloma cell line with HS-5 cells. Following 24 hrs of drug treatment apoptotic cells were measured by Annexin V positivity by FACS analysis. SCID-Hu *in vivo* model: SCID mice that were 4 to 6 weeks old received two fetal human bones subcutaneously (humerus, femur or tibia). Six weeks after implantation of human bone, 5X104 RMPI8226 myeloma cells in PBS were injected directly into the bone. Following four weeks of tumor engraftment, mice were randomized into drug treatment groups and tumor burden was measured by circulating lambda levels as detected by ELISA. On day 28 appropriate mice, were treated with either, vehicle control, HYD1 peptide or the scrambled peptide HYD1S which was administered I.P. daily for 14 days. For mice randomized to receive melphalan treatment, 1.5 mg/Kg melphalan was administered I.P. on day 29 and day 32. *Results*. In this report we demonstrate that the beta 1 integrin D-amino acid

inhibitory peptide HYD1, induces apoptosis as single agent in 8226 and H929 multiple myeloma cells lines. In addition, we show that HYD1 treatment significantly enhanced melphalan induced cell death in suspension cultures, as well as in the bone marrow stroma HS-5 co-culture model of drug resistance ($p<0.05$ students t-test). Finally in this report we show that HYD1 has activity as a single agent, and enhances melphalan activity in the SCID-Hu *in vivo* model. HYD1 was injected I.P. indicating that the peptide is likely to have good bioavailability. Furthermore, our finding that HYD1 has activity as a single agent *in vitro* and *in vivo* indicates that multiple myeloma cells are dependent on beta 1 integrin mediated signaling for cell survival. Further studies are warranted to determine whether MM cells are more dependent on beta 1 integrin signaling for cell survival compared to non-malignant plasma cells. *Conclusions*. Together these data provide essential proof of principle that beta 1 integrins represent a novel target for drug development in the treatment of multiple myeloma. In addition, our data demonstrate that HYD1 represents a potential novel therapeutic agent for enhancing the efficacy of standard chemotherapy in the treatment of multiple myeloma. These data provide the rationale for further pre-clinical studies of HYD1, which may ultimately provide support for designing clinical trials with HYD1 for the treatment of multiple myeloma.

S7a.6

RESPONSE BY SFLC AND MARROW FLOW CYTOMETRY IN MRC MYELOMA IX

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Introduction. In the intensive pathway of the Medical Research Council Myeloma IX trial younger patients (those suitable for high dose therapy) are randomized between two induction schedules namely CVAD and CTD. All responding patients then receive high-dose melphalan (200mg/m^2) with stem cell support and are subsequently randomized to no further therapy or maintenance with thalidomide (50-100 mg daily). The purpose of this preliminary evaluation was to determine the applicability of the serum free light chain (SFLC) assay and bone marrow flow cytometry in assessing response to therapy and determine their merits in comparison to conventional response assessment using serum and/or urine paraprotein estimations. *Materials and methods*. To date (19/01/07) 1007 patients have been enrolled in the intensive pathway of Myeloma IX and 349 have undergone the second randomization to thalidomide maintenance or observation. SFLC as well as standard serum and urine paraprotein assessments were performed in a central reference laboratory at the following time points: presentation, following 3 cycles of induction, end of induction, 6 weeks and 3 months post high-dose therapy and 3 monthly thereafter until relapse. Similarly multiparameter flow cytometric evaluation of plasma cells was evaluated (again in a central laboratory) at presentation, at the end of induction and day 100 following high-dose therapy and annually thereafter until relapse. *Results*. Preliminary assessment has confirmed the general applicability of the SFLC assay including virtually all patients with light chain disease and approximately 65% of patients with non-secretory disease. Similarly aberrant plasma cell phenotypes (based on expression of CD19, CD56 and CD45) were demonstrable in >95% of patients with assessable bone marrows. The SFLC provided for a more rapid assessment of response to induction therapy compared to standard paraprotein estimations and provided an early identification of patients with refractory disease. Flow cytometry was more valuable in determining complete responses to both induction and high-dose therapy as conventional assessment was influenced by the long half life of the paraprotein in many patients. *Conclusions*. The SFLC assay and multiparameter flow cytomeric assessment of plasma cell numbers allow for a more rapid and detailed assessment of response in patients receiving intensive sequential therapy.

S7B: Oral presentations II

S7b.1

FINAL RESULTS OF A PHASE II PETHEMA TRIAL OF ALTERNATING BORTEZOMIB AND DEXAMETHASONE AS INDUCTION REGIMEN PRIOR AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) IN YOUNGER PATIENTS WITH MULTIPLE MYELOMA (MM): EFFICACY AND CLINICAL IMPLICATIONS OF TUMOR RESPONSE KINETICS

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Background. Dexamethasone-based combinations are the standard induction regimens for younger patients with MM prior ASCT. This is the first study in which bortezomib and dexamethasone were administered on an alternating basis. *Aims.* efficacy and kinetics of response. Patiens and *Methods.* patients with newly diagnosed MM under the age of 66 years were treated with bortezomib at 1.3 mg/m^2 on days 1, 4, 8 and 11 (cycles 1, 3, 5) and dexamethasone 40 mg p.o. on days 1-4, 9-12 and 17-20 (cycles 2, 4 and 6), followed by ASCT with melphalan-200. Responses were evaluated by the EBMT criteria but a VGPR was included. Random effects models were utilized to analyze the tumor response kinetics to bortezomib and dexamethasone with the absolute value of M-protein overtime and decrease by cycle. Because the nonlinearity in the change of M-protein overtime, a piecewise linear model was employed. *Results*. between August, 2005 and March, 2006, 40 patients (18 M, 22F, median age 57) were enrolled. The overall response rate was 82% (12% CR, 10% VGPR). The response was quick with 82% M-protein reduction achieved with the first 2 cycles. There was no further decrease of the mean M-protein in cycles 5 and 6. The M-protein decrease was not significantly different with dexamethasone and with bortezomib (p=0.48). Chromosome 13 deletion, t(4;14) and t(14;16) did not had a negative impact on response. Toxicity was low: ten (25%) patients developed mild peripheral neuropathy (grade 1:9 cases, grade 2:1 case) and 11 grade 1 thrombocytopenia. Grade 3 toxicity was observed in 7 patients (neutropenia 6, skin/liver 1 case). No patient developed grade 4 toxicity. In all patients stem cells could be adequately collected (median of CD34$^+$ 5×10^6/Kg). The overall response rate after ASCT was 90% with 40% CR plus 20% VGPR. *Conclusions*. Bortezomib alternating with dexamethasone is highly effective as up-front therapy in patients with MM, and is associated with a low toxicity. The results of the tumor response kinetics analysis support a short program of alternating bortezomib and dexamethasone (i.e., maximum of 4 cycles) as an effective and safe therapy for younger myeloma patients prior ASCT.

S7b.2

LONG-TERM AND UPDATED RESULTS OF THE IFM9903 AND IFM9904 PROTOCOLS COMPARING AUTOLOGOUS FOLLOWED BY RIC-ALLOGENEIC TRANSPLANTATION AND DOUBLE TRANSPLANT IN HIGH-RISK *DE NOVO* MULTIPLE MYELOMA

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The IFM99-03 and IFM99-04 trials were conducted from 04/2000 to 08/2004. Pts younger than 66 years with high-risk (b2mic > 3 and chromosome 13 deletion at diagnosis) *de novo* multiple myeloma (MM) were prospectively treated according to HLA-identical sibling availability. In both protocols, induction regimen consisted of VAD followed by melphalan 200 mg/m^2 plus autologous SCT. When a HLA-sibling donor was available, ASCT was followed by RIC-allogeneicSCT (fludarabine, ATG and low dose busulfan): IFM9903 protocol (Garban, Blood 2006;107:3474). When no donor was available, pts were randomised to receive a second ASCT with HDM220 ± anti-IL6 moAb: IFM99-04 protocol (Moreau, Blood 2006;107:397). 284 pts met eligibility criteria and received at least one course of VAD. 65 had an available HLA-identical sibling donor and were included in the IFM99-03 trial, and 219 were

included in the IFM 99-04 trial. At the reference date of November 1, 2006, on an intent-to-treat basis, considering the entire population of 284 pts, with a median follow-up of 38 months for living pts, EFS did not differ significantly between studies (median EFS 22 months in the IFM99-04 trial vs 18 in the IFM99-03 protocol; p=0.14). Conversely, OS was significantly superior in the tandem ASCT trial (median OS 56 months in the IFM99-04 trial vs 34 in the IFM99-03 protocol; p=0.03). OS and EFS were similar in the 2 treatment arms of the IFM99-04 trial (second ASCT with HDM220 + anti-IL6 moAb, n = 81, or second ASCT with HDM220 without anti-IL6 moAb, n = 85); thus, the results of pts in both arms were pooled for comparison with those of 46 pts who underwent the entire ASCT plus RIC-allogeneic transplantation program. EFS of the 166 pts randomly assigned in the tandem ASCT protocol was similar to the EFS of the 46 pts who underwent the entire IFM99-03 program (median, 25 months vs 21; p=0.38). Conversely, OS was significantly superior for the randomly assigned pts in the tandem ASCT trial than for patients treated with the combination of ASCT followed by RIC-allogeneic transplantation (median OS, 59 months vs 35; p=0.016), due to a longer survival after relapse in the tandem ASCT arm. These long-term results indicate that, in a subgroup of high-risk pts with *de novo* MM, a tandem ASCT procedure is superior to a combination of ASCT followed by RIC-allogeneic SCT. The better OS described in the tandem ASCT trial is related to a better feasibility of salvage regimens after relapse.

S7b.3

NONMYELOABLATIVE ALLOGRAFTING OR AUTOGRAFTING FOR NEWLY DIAGNOSED MULTIPLE MYELOMA

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Introduction. Allografting is the only potential cure for multiple myeloma. We compared clinical outcomes after combined autologous-nonmyeloablative allogeneic hematopoietic cell transplantation to double autologous transplantation in newly diagnosed patients. *Materials and Methods*. From September 1998 to July 2004, 162 consecutive patients aged < 65 with siblings were assigned treatment according to the presence or absence of an HLA-identical sibling donor. Initial treatment consisted of 2-3 courses of vincristine, adriamycin, and dexamethasone, followed by peripheral hematopoietic cell mobilisation. Patients with an HLA-identical sibling then received a melphalan-based autograft of hematopoietic cells followed, two to four months later, by low dose total body irradiation (200 cGy) and an allograft from an HLA-identical sibling (Tandem Autograft-Allograft). Patients without an HLA-identical sibling received a second treatment with melphalan and rescue by an autograft (Double-Autografts). Primary endpoints were overall (OS) and event-free (EFS) survivals by intention-to-treat analysis. *Results*. After a median follow up of 45 months (range 21-90), by intention-to-treat analysis, median OS and EFS were significantly longer in patients with HLA-identical sibling donors (n=80) than those without (n=82): 80 months versus 54 months (p=0.01) and 35 months versus 29 months (p=0.02), respectively. By multivariate analysis, after adjusting for age, sex, disease stage, β-2 microglobulin, albumin, lactate dehydrogenase, creatinine, platelet count at diagnosis, and myeloma protein isotype, the presence of an HLA-identical sibling was significantly correlated with longer OS and EFS (HR 0.35, CI 95% 0.19-0.64, p=0.001 and HR 0.54, CI 95% 0.35-0.81, p=0.003). Median OS was not reached in the 58 patients who completed Tandem-Autograft-Allograft and was 58 months in the 46 who completed Double-Autografts (HR 0.33, CI 95% 0.14-0.80, p=0.01). EFS was 43 and 33 months (HR 0.47, CI 95% 0.27-0.83, p=0.009). Complete remission rate was significantly higher in Tandem Autograft-Allograft (55%) than in the Double-Autografts group (26%), (p=0.004). Transplant-related mortality did not significantly differ between the two groups (p=0.09), but disease-related mortality was significantly higher in the Double-Autografts group (43%) than in Tandem Autograft-Allograft (7%), (p<0.001). *Conclusions*. Tandem Autograft-Allograft should be considered for newly diagnosed myeloma patients with an HLA-identical sibling donor.

S7b.4

DOUBLE VERSUS SINGLE AUTOLOGOUS STEM-CELL TRANSPLANTATION FOR MULTIPLE MYELOMA: A REGION BASED STUDY IN 485 PATIENTS FROM THE NORDIC AREA

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Background. Autologous stem cell transplantation is now considered the standard of care in young patients with multiple myeloma (MM). This disease is the most common indication for high dose therapy supported by haematopoietic stem-cell transplantation, and more data support the benefit of this procedure in MM than in any other disease. The available results of randomized studies are in favor of tandem autologous transplantation; however the effect on long term survival is unclear. During 1994-2000 we have conducted sequential registration trials in the Nordic area, evaluating the treatment of multiple myeloma with high-dose chemotherapy. This has included a regional phase II registration study of double autologous stem-cell transplantations. *Methods*. During 1994-2000 we have registered a total of 485 previously untreated patients under the age of 60 years at diagnosis who on a regional basis were treated with single (Trial NMSG #5/94 and #7/98 (N=384)) or double (Trial HKTH (N=101)) high dose melphalan (200 mg/m^2) therapy supported by autologous stem cell transplantation. *Results*. A complete or a very good partial response was achieved by 40 percent of patients in the single-transplant group and 60 percent of patients in the double-transplant group (p=0.0006). The probability of surviving event-free for 5 years after the diagnosis was 25 (18-32) percent in the single-transplant group and 44 (33-55) percent in the double-transplant group (p=0.0014). The estimated overall 5-year survival rate was 50 percent in the single-transplant group and 50 percent in the double-transplant group (p=0.9). In a multivariate analysis of variables including single versus double transplantation, beta-2 microglobulin level, age, sex and disease stage only beta-2 microglobulin came out significantly (p<0.0001) and (p=0.001) for overall and event free survival respectively. In accordance with these results a 1:1 case-control matched comparison between double and single transplantation did not identify significant differences in overall and event free survival. *Conclusions*. As compared with single autologous stem-cell transplantation double transplantation did not seem to improve the final outcome among patients with multiple myeloma in the Nordic area.

S7b.5

THALIDOMIDE IMPROVES SURVIVAL WHEN USED FOLLOWING ASCT

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Since 2002 the Australasian Leukaemia and Lymphoma Group has been conducting a randomized trial of limited duration Thalidomide (T) combined with maintenance alternate day prednisolone (AP) (ARM 1) vs AP (ARM 2) following a single melphalan 200 mg/m^2 conditioned ASCT for multiple myeloma (MM). Patients underwent a first ASCT following no more than 12 months of prior anti-MM therapy and at 6 weeks post-ASCT those with no evidence of disease progression were randomised to AP 50mg with or without T 200mg daily. T was limited to a maximum daily dose of 200 mg and for a maximum duration of 12 months because of concerns related to toxicity, development of drug resistance and cost. AP was scheduled to continue until disease progression. The primary and secondary end-points of the trial were progression-free (PFS) and overall survival (OS). Analyses were on an intention-to-treat basis. Between January 2002 and March 2005 243 patients from 29 centers were randomised - ARM 1 114 and ARM 2 129. The arms were well matched for patient and disease characteristics. Median follow-up for both arms is approximately 2.5 years. At the time of randomisation 9% vs 11% of patients in ARM 1 and 2, respectively, were in CR (immunofixation negative). 64% of patients were able to complete 12 months of T at a median dose of 100 mg. At both 8 months and 12 months post-randomisation ARM 1 patients demonstrated a greater likelihood of maintaining a PR or better, 89% vs 67% and 83% vs 52%,

respectively (both $p<0.01$). Post-ASCT ARM 1 demonstrated superior PFS ($p=0.0003$). Estimates of PFS at 1, 2 and 3 years were 91% vs 69%, 63% vs 36% and 35% vs 25% for ARM 1 and 2, respectively. Likewise, ARM 1 demonstrated superior OS ($p=0.02$) with estimates of OS at 1, 2 and 3 years of 97% vs 95%, 91% vs 80% and 86% vs 75% for ARM 1 and 2, respectively. We conclude that consolidation with thalidomide in combination with alternate day prednisolone maintenance is an effective approach that prolongs the duration of disease response and survival following ASCT for MM.

S7b.6

PROGNOSTIC IMPACT OF POSTRANSPLANTATION COMPLETE REMISSION (CR) IN MULTIPLE MYELOMA(MM). FINAL RESULTS OF A PROSPECTIVE STUDY IN A SERIES OF HOMOGENOUSLY TREATED PATIENTS

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EBMT Myeloma Subcomittee redefined response criteria for MM treatment in 1998. In summary, negative immunofixation (IF) was established as a requisite for CR. The retrospective study of Spanish Registry of MM Transplantation, which was designed to validate the prognostic significance of CR defined by EBMT criteria, concluded that patients with negative IF constituted a subgroup with better prognosis than those with negative electrophoresis but positive IF. GEM-2000 protocol included, among its objectives, a prospective confirmation of the previously referred results. *Patients and Methods.* 1088 patients were included in this clinical trial, GEM-2000 protocol. All patients included in this protocol were treated with 6 alternative chemotherapy cycles (using VBMCP/VBAD), and either BUMEL or MEL200 as conditioning regimen. A second transplantation was performed if patients did not reach complete remission. Complete remission (CR) was defined when IF results were negative; when EEF^- but IF^+, the response was called Near Complete Remission (nCR). Other response categories were also evaluated: Partial Response (PR); Minor Response (MR); Stable Disease (SD) and Progression. Response was evaluated after chemotherapy, 1st or 2[nd] Stem Cell Transplantation in 740 patients (n of cases per response category at time of maximum response: CR=295 (39,9%); nCR= 129 (17,4%); PR=239 (32,3%); MR=23 (3,1%); SD=6 (0,8%); Progression= 49 (6,6%). MR and SD categories were not included in subsequent analysis due to the low number of patients. *Results* (Table). At median follow-up of 50,4 months, median was not reach and 67,5% of patients are still alive at 5 y. EFS and PFS were significantly better in the group of patients in CR than in group of ECR, and this was also superior to PR. Patients in Progression had a significantly worse outcome as compared to the rest of the patients($p<0,000$). Multivariate analysis confirmed the independent prognostic impact of response categorization (SLE OR for CR was125 $p=0.000$, for ECR OR was 8 $p=0,125$, for RP OR was 4,1 $p=0,02$; model $p<0.000$; X2 91,43). *Conclusion*. This large prospective study shows that response after transplant is a major prognostic factor that could be used a surrogate marker for predicting disease outcome. Patients with CR IF- have longer survival than patients with nCR, and these longer than PR patients.

Table. Summary of % Distribution and Survival for Category of Response.

	CR IF -	*nCR*	*PR*	*Progression*
Maximum Response (%)	40	16	32	7
OS median (range), months	NR	NR	66 (50-82)	14 (11-19)
OS, % 5 years	68	65	51	19
EFS median (range), months	60 (51-69)	49 (39-58)	36 (32-40)	10 (9-12)
EFS, % 5 years	50	29	21	0

S8: Pathophysiology - new treatment development

S8.1

DRUG RESISTANCE IN MYELOMA

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Drug resistance remains a major obstacle to prolonging the lives of myeloma patients. Recently, we have become more aware of the influence of the tumor microenvironment on tumor cell survival and growth. The central hypothesis of our research is that the bone marrow microenvironment provides a sanctuary for subpopulations of multiple myeloma (MM) cells to evade or circumvent drug-induced death and that this represents a form of *de novo* drug resistance. By definition, *de novo* drug resistance is associated with conditions that protect cells from initial drug exposure and therefore are present prior to drug selection. We have found that elements of the bone marrow microenvironment, including the extracellular matrix protein, fibronectin (FN) and bone marrow stroma (BMS), protect hematopoietic malignant cells from drug-induced cell death.[1-7] We propose that the tumor microenvironment protects tumor cells from drug-induced stress and cell death by two mechanisms: (A) a paracrine mechanism due to soluble cytokine and/or growth factors produced as a result of the tumor cell: environment interaction, and (B) a physical contact mechanism we have termed *cell adhesion mediated drug resistance (CAM-DR)*. We use the term *environment-mediated drug resistance or EMDR* to describe the combination of mechanisms that contribute to this form of *de novo* drug resistance. This form of *de novo* drug resistance associated with the bone marrow microenvironment presumably is responsible for minimal residual disease (MRD) in hematopoietic malignancies, including leukemias and multiple myeloma. In 1999, we reported that adhesion of MM cell lines to fibronectin (FN) via β1 integrins conferred a survival advantage against cytotoxic drugs by inhibiting drug-induced apoptosis.[1] We used the term *cell adhesion-mediated drug resistance* or CAM-DR to describe this phenotype and hypothesized that CAM-DR was responsible for MRD for MM following initial chemotherapy. In this case, myeloma cells that express α4β1 (VLA-4) and α5β1 (VLA-5) when adhered to fibronectin exhibited a transient drug resistance that reversed when cells were detached from fibronectin. Since that publication, we and others, have confirmed the CAM-DR phenotype in other hematologic cancers and solid tumors, including CLL, AML, breast and lung cancer.[1,2] These findings are consistent with our findings first reported in 1999, where MM cells adhered to fibronectin *in vitro* were resistant to a diverse spectrum of cytotoxic drugs.[1] Our laboratory has used microarray analysis to identify signal transduction pathways and gene products influenced by the interaction of MM cells with the extracellular matrix component, fibronectin that may contribute to CAM-DR.[4] Our laboratory found that of the 53 genes with a two-fold or greater increase in expression, 11 were reported to be regulated by the NF-κB family of transcription factors. In four of five MM cell lines studied, NF-κB was activated to a greater degree when cells were adhered to FN compared to cells in suspension. Furthermore, an increase in anti-apoptotic genes, including cIAP2, known to be regulated by NF-κB was observed. This study demonstrates that despite differences in genetic background, MM cell lines have common pathways influenced by FN adhesion that may contribute to CAM-DR. More recently we used microarray analysis, to compare genotypic and phenotypic properties of *de novo* and acquired resistance to melphalan in the isogenic MM cell line RPMI 8226.[4] We reported that the gene expression profile was less complex for the CAM-DR phenotype compared to the acquired drug resistant phenotype, and that MM cell adhesion to FN promotes cell survival by transiently altering key proteins involved in apoptosis. The most obvious change promoting cell survival was a decrease in the expression of the pro-apoptotic protein, Bim. We have validated this change at the protein level, and have observed this phenomenon in several different myeloma and leukemic cell lines. Most recently, we reported that FN adhesion reduces Bim levels by enhanced proteosome activation and that the reduction of Bim levels can be blocked by proteosome inhibitors. Thus, the use of Bortezomib in combination with other drugs may have increased activity due to the effects of Bortezomib on Bim levels. The bone marrow microenvironment consists of a diverse milieu of cytokines, growth factors, hormones and components of bone marrow stromal cells and extracellular matrices. Many of these factors may contribute to environment-mediated drug resistant (EMDR) phenotype, and indeed, many of these factors may collaborate or synergize to prevent drug-induced cell death. Several growth factors including IL-6, IGF-1, insulin and FGF are known to promote MM survival and pro-

liferation, and may contribute to EMDR. Although cellular adhesion to FN simplifies the model and allows for easier experimental manipulations of β1 integrin mediated pathways, it is unlikely that β1 integrin signaling represents the complete pathway of drug resistance associated with the bone marrow microenvironment. In order to address this concern we added complexity to our model of drug resistance, and recently we have studied myeloma cell survival by combining IL-6 and FN adhesion. We have generated preliminary data using MM cell lines demonstrating that interleukin-6 (IL-6) *cooperates* with β1 integrin receptors, in activating the Jak/Stat pathway. This cooperation is principally due to integrin recruitment of Stat3 to the IL-6 receptor and augmented phosphorylation of Stat3 by Jak2. We have also studied the role of bone marrow stroma (BMS) on myeloma survival with an emphasis on drug resistance. These studies, using a transwell model, demonstrate that both soluble factors (including IL-6) and cell adhesion confer a myeloma cell survival and induce drug resistance. Recently we demonstrated the involvement of Notch-1 signaling in bone marrow stroma-mediated *de novo* drug resistance in myeloma cells. The protection was associated with up-regulation of p21 and growth inhibition of cells. Overexpression of Notch-1 in U266 cells upregulated p21 and resulted in protection from drug induced apoptosis. In conclusion, these studies demonstrate that the microenvironment of the bone marrow is able to confer a myeloma cell survival advantage and drug resistance. This cell survival advantage is mediated by both soluble factors and cell adhesion molecules, including beta integrins. Interrupting myeloma cell: bone marrow microenvironment interaction and/or signal transduction mechanisms associated with these interactions represents a novel approach to enhancing treatment effect and possible patient survival.

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S8.2

TARGETING MYELOMA CELL GROWTH FACTORS IN MULTIPLE MYELOMA

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At the intramedullary stage, the survival of multiple myeloma cells (MMC) is strongly dependant on their microenvironment which produces myeloma growth factors (MGF). Key findings to understand the biology of multiple myeloma (MM) has been the identification of interleukin-6 (IL-6)[1] and Insulin Growth factor type I (IGF-1)[2] as major survival and proliferation factors for MMC. However, though IL-6 or IGF-1 are essential, they are not sufficient and additional MGF, produced by the microenvironment or the MMC themselves, are required to promote tumor growth together with them.[3] DNA arrays have proven to be useful to identify new MGF. Based on gene expression profile (GEP) data,[4] we have identified two new families of MGF which play a major role in the biology of the MM, and which are promising therapeutic targets. Those factors are members the Epidermal Growth Factor (EGF) family[5] and the B cell growth factors, BAFF (B-cell activating factor) and APRIL (A proliferation-inducing ligand).[6] EGF family members and ErbB receptors. The EGF receptor family comprises 4 members: ErbB1 (EGFR), ErbB2, ErbB3 and ErbB4. Expression and/or activation of ErbB receptors are altered in many epithelial tumors but this family of protein had never been involved in hematological malignancies. Using Affymetrix microarrays, we have provided a global picture of the expression of the 10 EGF-family members in MMC and throughout normal plasma cell (PC) differentiation.[7,8] We have shown that five genes (*AREG*, *TGF-α*, *NRG1*, *NRG2* and *NRG3*) are expressed by MMC purified from the BM of patients. Among them, two – *NRG2* and *NRG3* – are *myeloma genes*, i.e. they are significantly overexpressed in MMC compared to normal B cells, plasmablasts and bone marrow PC, whereas other three genes – *AREG*, *TGF-α* and *NRG1* – are *plasma cell genes*, i.e. they are expressed both in normal and malignant PC but not in B cells and plasmablasts. We further demonstrated that ErbB receptor expression is induced during normal PC differentiation (ErbB1-2) and oncogenesis (ErbB3-4).[7] The importance of ErbB receptor activation for MMC survival has been demonstrated by the finding that a pan-ErbB kinase inhibitor (PD169540, Pfizer) induced a dramatic apoptosis of primary MMC cultured *in vitro* for 5 days with their environment, in 10/14 patients .[5,7] When the ErbB inhibitor was combined with dexamethasone (dex) or an anti-IL-6 antibody, we found an almost complete elimination of the MMC present in the culture. A major observation was that the ErbB inhibitor did not affect the viability of other bone marrow cells present in the culture. More recently, we have demonstrated that syndecan-1, the main heparan sulphate (HS) proteoglycan present on MMC, plays a major role in the EGF/ErbB pathway.[8] Syndecan-1 concentrates high levels of HS-binding-EGF-ligands at the cell membrane which likely facilitates ErbB-activation. Only EGF family members able to bind HS chains - AREG, HB-EGF and NRG – could stimulate the growth of myeloma cell lines. Altogether, these data indicate that ErbBs are excellent candidates for targeted therapy and that inhibition of the EGF-family pathway may be useful in the treatment of MM. A phase I-II clinical trial using an anti-ErbB1 is antibody is currently ongoing at the university hospitals of Montpellier and Heidelberg to treat patients with MM in relapse after HDC. This work is a very good illustration of the interest of microarrays to design new treatments for MM. *BAFF, APRIL and their receptors*. Another example of the usefulness of microarrays to study the biological mechanisms of myeloma cell growth is provided by studies on BAFF and APRIL. BAFF is a TNF (tumor necrosis factor) family member involved in the survival of normal and malignant B cells. Using Affymetrix microarrays, we found that TACI and BCMA genes, each gene coding for a receptor of BAFF and APRIL, were overexpressed in malignant PC compared with their normal counterparts.[6] We also demonstrated that BAFF and APRIL can support the growth of MMC and conversely, an inhibitor of BAFF and APRIL can induce apoptosis of primary MMC.[6] Furthermore, GEP data of purified MMC from 65 newly-diagnosed patients were analyzed by supervised clustering of groups with higher (TACIhigh) vs. lower (TACIlow) TACI expression levels. TACIhigh MMC displayed a mature PC gene signature, indicating dependence on the BM environment. In contrast, the TACIlow group had a plasmablastic gene signature, suggesting a weaker dependence on the BM environment.[9] More recently, we

have found that APRIL is the main MMC growth factor, compared to BAFF (Moreaux, unpublished data). This is explained by the ability of APRIL to bind syndecan-1 through HS chains. In addition, the APRIL receptor, TACI, also binds syndecan-1 unlike BAFF-R or BCMA. Thus, the high syndecan-1 expression at the surface of MMC strongly favors APRIL binding to TACI and a major role of APRIL/TACI in MM biology (Moreaux, unpublished data). APRIL and BAFF are highly produced by osteoclasts unlike BM stromal cells ,[9] emphasizing the importance of bone cells to rigger MMC survival and proliferation. We have started a phase I-II trial using a receptor TACI coupled to Fc fragments of Ig (TACI-Ig, which blocks the effect of BAFF and APRIL) at the university hospital of Montpellier. This is the first clinical trial using this molecule. One can anticipate that GEP may help in identifying groups of patients who might benefit most from treatment with BAFF/APRIL inhibitors. *Heparanase, an enzyme modifying HS chains, is of major importance in MM disease*. Analyzing the GEP of whole BM cells of 39 patients (including the tumor cells and environment cells) and that of MMC purified from the BM of those 39 patients, we identified various genes that are mainly expressed by cells of the BM environment, in particular the gene encoding heparanase (HPSE).[10] HPSE is an enzyme that cleaves heparan sulphate chains into small bioactive fragments of 10-20 saccharides. It is overexpressed in numerous malignant tissues compared to their normal counterpart. In MM, two studies have demonstrated that HPSE stimulates angiogenesis and promotes metastasis of myeloma cells to bone in a murine model. Mechanisms of action of HPSE in MM are poorly known, but it is likely that HPSE regulates the function of the HS chains of syndecan-1. In addition, we have shown that HPSE controls syndecan-1 gene expression and shedding. Using Affymetrix microarrays, we found a high HPSE expression in the WBM samples, which was 7.6-fold higher than that found in the corresponding MMC.[10] Combining GEP and clinical data, we demonstrated that *HPSE* expression in the WBM of patients with MM is an indicator of poor prognosis. Patients with the highest *HPSE* mRNA expression in the WBM have a significantly shorter EFS and OAS than patients with the lowest *HPSE* expression. This is of major interest as it is the first study showing that a gene's expression mainly in the BM environment, i.e. *HSPE*, has prognostic value in MM. Again, this reinforces the importance of the microenvironment in this pathology. Of interest, clinical grade inhibitor of *HPSE*, actually highly sulfated HS chains, are already investigated in cancer diseases. In conclusion, the measurement of the expression of thousands of genes in hundreds of patient samples using microarrays has revealed novel molecularly defined subclasses of tumor, some of them predicting clinical behaviour. Furthermore, DNA microarrays proved to be very useful to improve our understanding of the myeloma pathogenesis, first, though a comparative analysis of tumor cells and their normal counterpart, and secondly, through a comprehensive analysis of the complex interactions between tumor cells and the BM microenvironment. The challenge will be now to translate this fundamental knowledge into new prognostic, diagnostic ant therapeutic tools that will improve treatment and outcome of patients with MM.

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S8.3

AKT AS A THERAPEUTIC TARGET IN MULTIPLE MYELOMA

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Multiple myeloma (MM) is a B-cell malignancy characterized by a clonal accumulation of malignant plasma cells in the bone marrow (BM). Although the availability of new agents such as thalidomide, bortezomib, and lenalidomide have led to improved responses and survival, MM remains incurable highlighting the need for development of new treatments. The BM microenvironment induces growth, survival, migration, and drug resistance in MM cells via at least two different mechanisms: first, adhesion of MM cells to fibronectin confers cell adhesion mediated drug resistance (CAM-DR); second, cytokine (ie, IL-6, IGF-1, VEGF, SDF-1α, BAFF, several TNF superfamily proteins, Wnt, and Notch family members)-induced signaling pathways, including MAPK kinase (MEK)/ERK; phosphatidylinositol 3-kinase (PI3-K/Akt); and/or Janus kinase 2 (JAK2)/STAT3 cascade. Among these signaling pathways, the PI3K/Akt may play crucial role in oncogenesis and drug resistance in MM cells. For example, previous studies have shown that PI3-K/Akt signaling mediates growth, survival, drug resistance, migration and cell cycle regulation in MM.[1-3] Specifically, dexamethasone (Dex)-induced cytotoxicity is blocked by PI3K/Akt activation. Cytokine induced Akt activation results in multiple biological down-stream effects in cancer cells, and in MM in particular. The effect on survival is multifactorial since Akt directly phosphorylates several components of the cell-death machinery. For example BAD phosphorylation prevents its binding to and inactivates the survival factor BCL-X_L. Similarly, Akt phosphorylates and inhibits the catalytic activity of caspase-9, a known pro-apoptotic protease. The anti-apoptotic effect of Akt is also mediated by phosphorylation of forkhead transcription factor (FKHR), which inhibits the nuclear translocation and activation of pro-apoptotic FKHR gene target proteins such as BIM, and FAS ligand. Moreover, activated Akt phosphorylates glycogen synthase kinase (GSK)-3β, and mammalian target of rapamycin (mTOR)which in turn impact growth and survival. Besides its direct anti-apoptotic effect, Akt influences two central regulators of cell death including nuclear factor of κB (NF-κB) and p53. In addition to the anti-apoptotic effect, Akt regulates cell cycle via phosphorylation and inhibition of the cyclin D1 kinase, preventing cyclin D1 degradation. Via activation and phosphorylation of mTOR, a serine/threonine kinase that serves as a molecular sensor regulating protein synthesis in response to nutrient availability, it enhances mRNA translation through the activation of p70 S6 kinase (RSK). Another mTOR effect is the inhibition of 4E-BP1 (PHAS-I), a translational repressor of mRNAs. The enhanced mRNA translation leads to an up-regulation of multiple proteins involved in cell cycle progression from G1 to S phase. Given the crucial role of PI3K/Akt in MM oncogenesis, AKT and its downstream molecules are obvious rational targets for novel therapeutics. Indeed, several classes of compounds target Akt either directly or indirectly. For example, Perifosine (Keryx Biopharmaceuticals, NY), a synthetic novel alkylphospholipid, targets cell membranes and inhibits Akt activation. We have demonstrated inhibition of Akt phosphorylation, and its downstream molecules, FKHRL1 and GSK3α/β/ resulting in significant cytotoxicity of MM cells in preclinical models.[4] This cytotoxic effect was mediated via JNK activation followed by caspase-8, 9, PARP cleavage. In addition, Perifosine was found to enhance the activity of several agents such as bortezomib and dexamethasone. Perifosine is orally bioavailable and is well tolerated with minimal side effects mainly gastrointestinal and fatigue. Clinical studies with Perifosine in combination with dexamethasone and bortezomib are currently underway. The downstream molecules of Akt including FKHR, GSK-3β, and mTOR are

also potential drugable target.[3] Rapamycin has been in clinical use for more than two decades, mostly as an immunosuppressive agent against allograft rejection. Rapamycin and two of its analogs, CCI-779 (Wyeth Ayerst, PA, USA). and RAD001 (Everolimus, Novartis, NJ) induce cytoreduction and G1 arrest in MM cells. In addition, rapamycin has demonstrated strong antiangiogenic activity.[5] A phase II study of intravenous CCI-779 in patients with relapsed MM is currently underway. Similarly, RAD001 has been tested as a single agent in MM and lymphomas and has demonstrated a favorable toxicity profile. The combination of rapamycin with other novel anti-MM agents such as lenalidomide, 17-AAG, and bortezomib have shown strong *in vitro* synergism and rapamycin analogs are now being studied in phase I/II combination studies in MM.[6,7] Another strategy currently under investigation is the use of protein kinase C (PKC) inhibitors. PKC family proteins are composed of 11 members of serine threonine kinases that mediate proliferation, survival, migration and angiogenesis in many malignancies. Enzastaurin (LY 317615; Eli Lilly and company, Indianapolis, IN) is one such oral PKC,βselective inhibitor. PKC overexpression has been reported in MM and its signaling pathways have been implicated in MM cell proliferation, anti-apoptosis, and migration. Preclinical studies have shown that Enzastaurin inhibits PKC activation resulting in inhibition of growth, survival, and migration in MM cells, as well as angiogenesis and adhesion, both *in vitro* and in an *in vivo* human MM xenograft model.[8] This oral PKC inhibitor has already been tested in clinical trials of patients with diffuse large B cell lymphoma and glioblastoma. Enzastaurin has demonstrated promising activity in lymphoma with a very favorable side effect profile comprising mainly of fatigue, peripheral edema and nausea. Because preclinical data in MM suggests strong synergism with bortezomib, we are about to test this combination in a phase I trial in patients with relapsed/refractory MM. Several agents in preclinical and clinical evaluation target Akt by their effects on receptor-ligand interaction which in turn activate Akt as a intracellular downstream target. Some such examples include IL-6, IGF-1, BAFF, and TACI inhibitors. Although monoclonal antibodies targeting IL-6 and its receptor have demonstrated cytostatic antitumor activity in preclinical studies, clinical trials using anti-IL6 monoclonal antibody failed to achieve clinical activity in MM. More recently; however, superantagonists to the IL-6 receptor have demonstrated strong anti-MM activity. For example, Sant7 demonstrated anti-MM activity and significantly potentiated the activity of Dex *in vivo* in a SCID-hu MM model. IGF-1 is another major regulator of cell survival and activator of PI3K/Akt and MAPK in MM. Based on its critical role in MM proliferation, a small-molecule IGF1R tyrosine kinase inhibitor, NVP-ADW742 (Novartis Pharma AG, Switzerland) has shown significant cytotoxicity *in vitro* and *in vivo* in MM.[9] In addition, IGF-IR inhibition led to a decrease in phosphorylation and activation of multiple key kinases and kinase targets involved in the PI3K/Akt pathway including Akt, IKK, FKHRL-1, p70S6k, and GSK3β. In addition, IGF-IR inhibition sensitized MM cells to other anticancer agents, including Doxorubicin, and Melphalan. These results provide the rationale for ongoing clinical trials of these IGF-IR inhibitors for the treatment of MM patients. Targeting growth and survival factors belonging to the TNF superfamily member including B cell-activating factor (BAFF) and transmembrane activator, calcium modulator and cyclophilin ligand interactor (TACI) represent yet another promising therapeutic strategy. MM cell lines and primary cells express BAFF and its receptors. Serum levels of BAFF are increased in patients with MM compared with healthy donors. Recent studies have indicated that the BM environment is the main source of BAFF for MM cells, especially monocytes, neutrophils and osteoclasts, and established its role in the localization and survival of MM cells in the BM milieu. Importantly, BAFF modulates the proliferation and survival of MM cells, associated with activation of PI-3K/Akt. Antibodies to both TACI and BAFF are currently in preclinical and clinical phases of evaluation. Small molecule inhibitors like the heat shock protein 90 (HSP90) inhibitors such as Geldanamycin, and its analog, 17-allylamino-17-demothoxy-geldanamycin (17-AAG) have demonstrated anti-MM activity.[10] This was attributed to a suppression of signaling events triggered by IGF-1 and IL6 at multiple levels, including suppression of cell surface receptor expression (IGF-R), and inhibition of downstream PI3K signaling mediators including Akt, and IKK. Multiple anti-Hsp90 compounds (17-AAG, KOS953, and IPI-504) are currently being tested in phase I and II clinical trials in patients with MM. In summary, the PI3K/Akt pathway is a critical regulator of cell survival, proliferation, migration, drug resistance, and angiogenesis in MM. Several direct and indirect inhibitors of this pathway have shown significant preclinical activity and clinical trials are ongoing, showing early promising activity. These include agents such as perifosine, enzastaurin, CCI-779, RAD001, and HSP90 inhibitors. Already, these are being combined with agents such as lenalidomide and bortezomib, and will form an important component of our armamentarium against MM in future clinical trials.

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S8.4

DNA VACCINES TO SUPPRESS MYELOMA

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Introduction. It is becoming clear that the immune system is capable of suppressing cancer in patients. Antibody therapy and adoptive transfer of T cells are both strategies which can provide clinical benefit following passive transfer. Allogeneic transplantation is able to cure patients with leukemia by activating donor T-cell attack against minor histocompatibility antigens. As is the case for infectious diseases, active immunity is preferable to passive transfer, since continuous attack on emergent cancer cells can be maintained. Based on experience mainly in end-stage patients with melanoma, the possibility of activating immunity by vaccination has been questioned.[1] Nevertheless, our increasing knowledge of immune pathways, together with novel vaccine design opportunities, are revealing the way to succeed. The challenge for vaccination varies with the disease setting. If the cancer cells have been ignored by the immune system, it will be necessary to prime a new response. On the other hand, if spontaneous immunity has been generated and subsequently down-regulated or evaded, the immune response must be restarted. Multiple myeloma is a tumor involving the immune system, and deleterious effects on immune capacity, due to disease and to chemotherapy, are evident. However, there is the advantage that, in contrast to some solid tumors, the neoplastic plasma cells are accessible to attack by circulating T cells. Current treatment usually involves autologous transplantation which can restore immune capacity. Alternatively, or in addition, new drugs or antibodies, able to remove tumor cells without excessive damage to normal lymphocytes, could provide an opportunity for vaccination. An ideal setting would be to vaccinate donors of allogeneic transplants, so that an immune response could be activated in an undamaged immune system, but this is not yet a common strategy

for myeloma. There is some evidence for pre-existing immunity against tumor antigens in myeloma, but it appears weak.[2] Mouse models of myeloma, although informative, are not closely related to human disease, making pre-clinical testing difficult. However, experience from models and from other cancers point to a requirement mainly for inducing T-cell attack, with several candidate target antigens now emerging.[3]

DNA fusion gene vaccines

Molecular biology has offered powerful tools for vaccine design and delivery. We have opted for DNA vaccines which are cassettes into which gene sequences encoding target antigens can be placed. In pre-clinical models we have developed the approach of fusion gene vaccines where weak tumor antigens are linked to strong antigens derived from microbes. Recognition of the microbial antigens then promotes immunity against the weak tumor antigen.[4] The microbial sequence can be diverse but we have selected a sequence derived from tetanus toxin (TT) which comprises the non-toxic Fragment C (FrC) sequence. The advantage of this is that the FrC induces high levels of T-cell help from an undamaged anti-TT repertoire, and that this helps the response to the attached tumor antigen. This fusion gene strategy has been validated for a range of tumor antigens.[4] Our first tumor antigen was the idiotypic Ig expressed by neoplastic B cells, including myeloma. We assembled the tumor-specific encoding variable region genes, V_H and V_L, as single chain Fv (scFv) and fused this to FrC. Our first trial of these vaccine in patients with B-cell lymphoma is now complete and we have induced antibody responses against FrC in 9/12 patients, and anti-idiotypic antibody in 2/12, with $CD4^+$ T cell responses also detectable. The two patients with anti-idiotypic antibodies remain in long term remission after 6 years with no evidence of disease. A similar small trial of 7 patients with myeloma post-autologous transplantation is also now proceeding. The data from the lymphoma trial are quite remarkable given that performance of naked DNA vaccines is highly influenced by the volume injected and, while this is no problem for mice, it is very difficult to scale up for patients. To circumvent this difficulty we have now tested electroporation at the time of the booster injection.[5] This has dramatically amplified all immune response pathways, and it is now in our clinical trial of a DNA fusion gene vaccine for patients with prostate cancer.

Modified vaccine design to induce $CD8^+$ T cell responses

Most tumor antigens are expressed within the cell and are only accessible as peptides in the MHC Class I groove. To attack these, $CD8^+$ cytolytic T cells (CTL) are required, and the fusion gene design has been modified to optimize induction of this response. We have minimized the FrC sequence to a single domain (DOM) to remove potentially competitive epitopes and placed the tumor target peptide sequence at the C-terminus of the DOM. In pre-clinical models this (DOM-peptide) design induces high levels of CTL able to kill tumor cells in a therapeutic setting.[4] Responses against HLA-A2-restricted peptides have been obtained in humanized mice. The clinical trial of patients with prostate cancer is using this design formatted for a peptide from prostate-specific membrane antigen.

Myeloma target

For myeloma, the cancer-testis antigens offer candidate targets for immune attack. There has been wide interest in the NY-ESO 1 antigen which is expressed by significant numbers of myeloma patients and in other cancers.[6] We have placed a known epitope able to bind to HLA-A2 into our new DOM-peptide design and so far have shown induction of high levels of NY-ESO 1-specific $CD8^+$ T cells in the humanized pre-clinical model. This sets the scene to extend our clinical trials of patients with myeloma by adding a second vaccine to the current scFv-FrC. Dual attack on two proteins expressed by cancer cells should increase efficacy and prevent evasion.

Future

The knowledge and required technology to develop highly effective DNA vaccines are available. Development in pre-clinical models provides a strong basis for clinical trials, where the modifications in delivery systems required for translating to patients can be tested. For all cancers including myeloma, there are already candidate antigens which can be targeted. What is needed is rapid pilot clinical trials to determine efficacy in patients at appropriate stages of disease, but this is difficult to achieve in the current regulatory climate.

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S8.5

NOVEL IMMUNOTHERAPY STRATEGIES IN MULTIPLE MYELOMA

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Novel targeted therapies are achieving responses in over 90% of the newly-diagnosed patients with Multiple Myeloma (MM) with one third of these patients achieving complete or near complete responses. However, ultimately patients experience disease progression and curative outcome still remains elusive. In this setting novel therapeutic interventions are being explored. Based on success of allogeneic transplant in achieving long term disease free survival, various vaccination strategies have been evaluated to maintain remissions achieved by conventional and novel agent therapies. Development of successful vaccine strategy in MM has been directed at two aspects. First to develop a successful vaccine strategy able to target MM cells with therapeutic efficacy; and second to improve the immune function in MM patients to allow robust responses to immune-based intervention. MM is associated with dysfunction in both humoral and cellular immunity.[1] However, both cellular and humoral immune responses have been observed against both viral and tumor antigens.[2] We have investigated various immunotherapeutic approaches using idiotype as a myeloma-specific antigen. In a study utilizing patient-specific Id protein coupled with KLH, the development of an anti-KLH response confirmed immune competence of the myeloma patients. Moreover, induction of Id-specific immune responses including generation of CTL specifically able to lyse MM cells, confirmed the ability to induce immune response to MM-specific antigen. To improve on these results we have investigated the role of dendritic cells (DCs), the most potent antigen-presenting cells (APCs) equipped with the necessary co-stimulatory, adhesion and MHC molecules, to effectively present MM-associated antigens and induce MM-specific immune responses. We have confirmed that the antigen presenting cells in MM patients are functional, supporting their use in clinical studies.[3] To optimize internalization and processing of myeloma protein, we evaluated various laboratory parameters including class of protein, duration of DC pulsing, DC maturational stage and mechanism of uptake using DCs from myeloma patients. These data indicate that cultured DCs from myeloma patients can efficiently and rapidly endocytose different classes of myeloma proteins providing support for using myeloma protein-pulsed DCs for generating *in vivo* anti-myeloma immune responses.[4] The clinical trials have investigated vaccination with DCs pulsed with tumor associated peptides or proteins in a variety of human cancers. We have evaluated Idiotype-pulsed DC vaccinations in MM confirming the feasibility of a dendritic cell-based vaccination, development of Id-specific immune responses and even occasional clinical responses.[5] However, robust clinical responses have not been observed, and the strategy targeting single known tumor associated antigens is subject to tumor cell resistance mediated by the down-regulation of that single gene product. Approaches being explored to circumvent this limitation are the DC pulsing with whole myeloma cell lysate, or fusing myeloma cell with DC. Preclinical results in both a murine model and with human cells confirm feasibility of presenting a wide array of myeloma-related antigens through DC-MM cell fusions and the development of CTLs able to lyse primary myeloma cells.[6] A clinical study of MM/DC fusion cell vaccination in which patients with MM undergo serial vaccination with DC/MM fusion cells with local GM-CSF (100 μg) on the day of vaccination and for 3 days thereafter, is ongoing. To date, 11 patients have been enrolled. The study has confirmed the feasibility of obtaining adequate number of MM/DC fusion cells for 3 or more vac-

cination; that the DC/MM fusion cells are functional; and that vaccinations are without significant toxicity. Although clinically defined responses have not been observed, number of patients have achieved stable disease and in majority of patients evidence of vaccine induced anti-MM immunity as demonstrated by at least 2 fold increase in IFNγ expression by CD4 and/or CD8 T cells in response to *ex vivo* exposure to autologous tumor lysate, is observed. These studies are also providing an opportunity to identify novel myeloma-associated antigens by screening myeloma cDNA expression library using the SEREX technique for eventual development of antigen cocktail. We have identified series for antigens using this technique as well as myeloma expression profile data and have begun to validate individual antigens such as Xbp-1 and CD138.[7,8] To improve the immune function, we have evaluated both humoral and cellular immunity in MM. The suppressed uninvolved immunoglobulins reflect the profound B cell immuneoparesis associated with active disease. Clinical studies demonstrating recovery of uninvolved immunoglobulins following effective therapy provides some information on the genesis of immune dysfunction. Reversible NKT cell dysfunction *in vivo* and their successful expansion *ex vivo* for adoptive transfer has been demonstrated.[9] Recently, we have also observed dysregulation of $CD4^+CD25^+$ T regulatory cells (T_{reg}) in MM.[10] The initiation of immune response is controlled by $CD4^+CD25^+$ T_{reg} cells which can modulate anti-tumor immune responses. This property of Treg cells to alter immune response, specifically the anti-tumor immune responses, may function as a barrier to cancer immunotherapy. We observed significant increase in $CD4^+CD25^+$ T cells in MM patient samples compared to normal donors (26% versus 14%, respectively). However, T_{reg} cells as measured by Foxp3 expression are significantly decreased in both MGUS and MM compared to normal donors. Additionally, T_{reg} cells in MM are dysfunctional. Even when added in similar proportions as those in normal control, they are unable to suppress anti-CD3-mediated T cell proliferation. This decreased number and function of T_{reg} cells in MM may partly account for the non-specific increase in $CD4^+CD25^+$ T cells, and thereby contributing to dysfunctional T cell responses. To target the mechanisms responsible for Treg cell dysfunction, we have evaluated the role of bone marrow (BM) microenvironment on T_{reg} both by cell-cell interactions as well as by production of soluble factors including cytokines and chemokines. Interestingly, IL-6 and its receptor produced in the BM milieu affect the ability of T_{reg} cells to suppress T cell proliferation. We have observed that the addition of IL-6 and/or soluble IL-6 receptor to T cell proliferation assay leads to loss of T_{reg} cell activity in normal donor cells (from inhibition of proliferation by 27% to increase in proliferation by 6% with IL-6/sIL-6R, p=0.01) Conversely, when T_{reg} cells from MM patients when treated with the anti-IL-6 antibody or IL-6 receptor super antagonist, sant 7, regains the ability to suppress T cell proliferation. We have also shown evidence of expansion of $Foxp3^+$ cell numbers in PBMC from MM patients following *in vitro* treatment with anti-IL-6 antibody. Understanding of the molecular basis of T_{reg} cell dysfunction is underway with a view to eventually target these mechanisms to improve immuno homeostasis in myeloma. Additionally, we are also identifying novel agents used for myeloma therapy such as hsp90 inhibitors that may affect immune responses;[11] conversely, novel immunomodulatory agents are being evaluated to provide T cell co-stimulation to improve immune response post vaccination.[12] In conclusion, these stepwise improvements in immunotherapeutic strategies directed at myeloma are likely to lead to immune, and more importantly, clinical responses that will eventually help achieve a cure in multiple myeloma.

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S8.6

THE PHARMACOGENETICS OF MYELOMA

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Introduction

Pharmacogenetics aims to define biologically relevant biomarkers, which correlate with both survival and side effects. It can be split into a number of areas. The first of these areas is the analysis of the tumour cells, defining biologically relevant biomarkers that are either predictive or prognostic. A newer area that is becoming increasingly important, is the study of inherited factors, often genetic, which not only predict the risk of side effects following treatment, but can also help predict outcome. The third area, which has been little explored to date, is the integration of inherited genetic variants, together with acquired genetic variation within the tumour cells.

Tumour cells

Cytogenetics has given us a great deal of insight into the biology of multiple myeloma. However, this is only effective in about 30% of all cases. FISH is applicable to most cases and is used to detect the recurrent cytogenetic features defining myeloma cells. Based on increasingly powerful clinical studies, it is now realised that the t(4;14) is a significant prognostic factor, which can predict response to autologous stem cell transplantation, as can the t(14;16). However, 13q-, known as a prognostic factor for many years, is less discriminatory using FISH than with cytogenetics. To date, mutational analysis has not been successfully applied as a prognostic factor. This reflects the tendency of mutation to occur late in the disease process with the percentage of presenting patients being low. The advent of gene CHIP technology has enabled us to look at global expression data and to integrate this with global copy number variation (CNV). The aim of expression analysis being to define a limited gene signature, which can identify outcome. Using this approach, it has been possible to identify differences between normal, MGUS and myeloma plasma cell. This information can be useful clinically because, at presentation, there is clearly a subgroup of patients, which has a signature more similar to MGUS, which has a favourable outcome and a group with a signature more similar to a cell line that has a worse outlook. This approach is being refined and reliable proliferation and response signatures are now being generated. These signatures are, however, some way from being fully integrated into clinical management and need evaluation in large clinical trials. Defining what constitutes a signature requires careful consideration as does the best way of generating one. One approach to defining signatures is to look at specific genetic subgroups and to use these 'genetic manipulations' as tools to dissect a relevant pathway or response outcome. A good recent example of this is the investigation of 16q-, which identified the loss of CYLD and activation of NFkB pathway. It has been reported that response to Velcade, which targets this pathway, can be affected by the presence of such a signature.

Inherited genetic variation

Since the completion of the genome project, we understand more about the nature of inherited genetic variation. As a consequence, it is now being realised that in addition to SNP variants, there is significant CNV and in addition, that there are long-range control mechanisms governing the activity of large regions of the genome. Inherited variation within a number of pathways has been shown to be important clinical-

ly. Examples of this included xenobiotic metaboliser genes, DNA repair variants together with a number of other pathways. An initial study was only able to look at very limited numbers of SNPs but now, as the technology has improved, and we are able to look at much higher numbers of variants, this approach has become increasingly m,ore clinically relevant. Inherited genetic variants are important in governing response and outcome following treatment, as well as in predicting side effects such as myelosuppression and other toxicity from treatments. We have carried out a large study, looking at the pharmacogenomics underlying the risk of thalidomide, associated VTE and we have used a 3000 component CHIP to analyse a range of hypotheses. The results of this study suggest that inherited variation in the immune response, probably in the endothelial cells, could influence VTE risk and may explain why aspirin is effective.

Integrating inherited cariation with tumour specific acquired genetic variation

While the general perception may be that inherited variation largely affects the risk of side effects, it is obvious that the tumour cells also carry these variants, and this may affect their behaviour. In addition, CNV and other genetic variation acquired by the tumour cells during progression, will specifically impact on this background, which also significantly affect tumour cell behaviour. It is, therefore, becoming increasingly important to be able to develop approaches for integrating the data describing inherited genetic variation and how this interacts with acquired tumour variation. The technology for carrying out pharmacogenomics is progressing rapidly. Once relevant variants are identified, it should be possible to design tumour specific CHIPS that will give clinical information in clinically relevant time periods allowing practising physicians to alter therapies accordingly, allowing us to truly practice 'personalised medicine'.

S9: Stem cell transplant

S9.1

OPTIMIZING STEM CELL TRANSPLANTATION: THE IFM EXPERIENCE

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During the past ten years, major advances in the treatment have improved the outlook in myeloma. The antitumor activity of thalidomide, bortezomib and lenalidomide has been discovered. In elderly patients, the combination of these new drugs with conventional chemotherapy has been reported to induce a high response rate and to improve overall survival. In young patients, these new drugs have been evaluated to improve the outcome of high dose therapy supported with autologous transplantation. This abstract will focus on the IFM experience of these new drugs in the transplantation setting: 1) during induction therapy, 2) combined with the high dose regimen, and 3) used as maintenance therapy post transplantation.

New drugs during the induction phase: The IFM 2005-01 trial

Patients eligible for transplantation should avoid alkylating agents to enable an adequate stem cell collection. The standard induction therapy has long been based on dexamethasone (DEX) alone or associated with vincristine and doxorubicin (the VAD regimen). Currently, the association of DEX with Thalidomide (THAL), Revlimid (REV) or Velcade (VEL) is being actively investigated. The association of bortezomib with DEX was evaluated in a pilot study of the Intergroupe Francophone du myélome and Harousseau *et al.* reported 67% of PR including 21% of CR. This association was compared with VAD in a large phase III trial (IFM 2005 01 trial). 480 patients with *de novo* symptomatic myeloma under the age of 65 years were enrolled. The analysis of the first 167 patients demonstrated that DEX-VEL significantly improved the response rate before high dose therapy (HDT) as compared with VAD (82% of PR including 43% of Very Good partial response versus 67% of PR including 26% of VGPR). Thus, although randomized trial are still ongoing, it is reasonable to speculate that the associations of these new drugs with DEX will increase the PR and CR rate before high dose therapy as compared with VAD. However, the impact of these improved induction regimen on the maximum response rate and overall survival after HDT is still unclear and will require a longer follow-up .

New drugs combined with the high dose regimen

In myeloma, the standard high dose regimen is melphalan alone in a dosage of 200 mg/m^2. Attempts to improve this regimen with the association of other drugs or TBI have failed to improve the antitumor response rate but have increased the hematological and non hematological toxities. A synergistic effect between Bortezomib and melphalan has been demonstrated *in vitro* and *in vivo*. Furthermore, the toxicity profile of these 2 drugs is not overlapping. Thus, the combination of bortezomib and high dose melphalan was a logical approach. We conducted a pilot study of melphalan (200 mg/m^2 on day –2) and bortezomib (1 mg/m^2 on days -6, -3, +1, +4) supported with autologous blood stem cells (day 0). Twenty five patients (non responding after induction therapy, n=18 ; or failing to achieve a near CR after a first transplant, n = 7) were enrolled. No toxic death occurred, the median durations of neutropenia (<500/mm^3) and thrombocytopenia (<20000/mm^3) were 7 and 1 day, respectively. The incidence of severe mucositis (grade 3/4) was 20%. Three months after transplantation an unexpected response rate was observed: 77% of near CR including 31% of true CR. Furthermore, 5/7 patients failing to achieve a near CR after the first transplant prepared with melphalan alone achieved a CR (n=4) or a near CR (n=1). Finally, this pilot study strongly suggests that the association of bortezomib and melphalan could improve the CR rate as compared with melphalan alone without additive toxicity. Since the achievement of CR has been shown to be the most important prognostic factor for survival after high dose therapy, this association could improve the overall survival. The IFM is currently testing the role of this association in a large multicentric trial.

New drugs as consolidation / maintenance therapy after transplantation

The role of maintenance therapy in myeloma remains controversial. Maintenance chemotherapy has failed to demonstrate any benefit. Most randomized studies and meta-analyses evaluating maintenance interferon showed a modest increase in progression free survival without

any, or with minimal, survival benefit after conventional or high dose therapy. Corticosteroid maintenance was found to prolong the duration of response, however the impact on survival was controversial. Thalidomide is an oral agent, with immunomodulatory properties, active in one-third of patients with refractory disease, with doses as low as 50 mg, without myelosuppressive toxicity. Thus, thalidomide was an attractive candidate for use in maintenance situations, particularly after high dose therapy. In 1999, the «Intergroupe Francophone du Myélome» (IFM) initiated the first randomized trial (IFM 99 02) designed to evaluate the role of thalidomide as maintenance treatment after transplantation. Two months after autologous stem cell transplantation, 597 patients under the age of 65 years were randomly assigned to receive no maintenance (arm A), pamidronate (arm B), or pamidronate plus thalidomide (arm C). Thalidomide was administered for a median duration of 15 months, in a mean dosage of 200 mg per day. A complete or very good partial response was achieved by 55 percent of patients in arm A, 57 percent in arm B, and 67 percent in arm C (p=0·03). The 3-year post-randomization probability of event-free-survival was 36 percent in arm A, 37 percent in arm B, and 52 percent in arm C (p<0·009). The 4-year post-diagnosis probability of overall survival was 77 percent in arm A, 74 percent in arm B, and 87 percent in arm C (p<0·04). The proportion of patients who had skeletal events requiring a specific therapy (chemotherapy, irradiation or surgery) was 24 percent in arm A, 21 percent in arm B, and 18 percent in arm C (p=0·4). Thus, thalidomide improves the overall survival in patients with myeloma and should be recommended. However, the survival benefit of thalidomide was not observed among patients already in CR after ASCT, suggesting that this benefit was not related to a maintenance effect. The survival benefit of thalidomide was only observed among patients failing to achieve CR after ASCT, suggesting that this benefit was due to the reduction of the residual tumor mass. Since thalidomide improves the survival by reducing the tumor mass (i. e. by a consolidation effect rather than by a pure maintenance effect) stopping thalidomide as soon as a very good partial response has been reached (2 or 3 months) could be an effective strategy in order to reduce the side effects and to avoid drug-resistance at time of relapse. Furthermore, thirty nine per cent of patients had to discontinue thalidomide due to drug-related adverse events. Peripheral neuropathy was the main reason for discontinuation. Thus, lenalidomide, a more potent analog of thalidomide without neurological toxicities, might be an attractive candidate for use in consolidation and maintenance situations post-ASCT. Consolidation/maintenance treatment with lenalidomide is currently evaluated in the IFM 2005-02 protocol.

Conclusions

New drugs have considerably modified the prognosis of elderly patients. Their impact in the high dose strategy of young patients could be more impressive: 80% of CR or near CR can be expected with the use of new induction and high dose regimens. Furthermore, effective consolidation / maintenance strategies will significantly prolong the duration of these CR. Whether such a CR rate, efficiently maintained, will be associated with a percentage of *cure* will be answered by ongoing studies.

S9.2

SINGLE VS DOUBLE AUTOLOGOUS TRANSPLANT

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S9.3

AUTOLOGOUS STEM CELL TRANSPLANTATION FOR MM: ANALYSIS OF PROGNOSTIC FACTORS

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Since 1983 high dose chemotherapy is evaluated to improve the survival of patients with advanced stage multiple myeloma. Since then high dose therapy has been significantly modified and especially the infusion of autologous stem cells and the improvement of supportive care have significantly improved the outcome of high dose chemotherapy for Multiple Myeloma. Numerous prognostic factors have been delineated in patients undergoing autologous stem cell transplantation for multiple myeloma. Patient-related, disease-related and treatment-related factors have been described to have a major impact on the outcome of this treatment modality. Already during the first studies patient age, renal function, co-existing amyloidosis and other co-morbidities have been found to be important patient-specific factors that influence the outcome of autologous stem cell transplantation. More recent approaches – pharmacogenomics – allow to define additional patient-related factors by analysing polymorphisms in genes involved in the transport of antitumor drugs or DNA repair genes which have recently been shown to also influence the outcome of autologous stem cell transplantation. Classical disease-related factors which were described to have an impact on the outcome of autologous stem cell transplantation in multiple myeloma were the isotype of the myeloma, LDH and β2-microglobulin. New techniques of molecular cytogenetics and gene expression profiling have allowed to define new disease-related factors associated with outcome of treatment in patients with multiple myeloma. It has been shown recently that among the five main immunoglobulin gene translocation groups (t(11;14), t(14;16), t(4;14), t(6;14) and t(11;14)), the t(14;16) and the t(4;14) translocation groups have a very poor prognosis. Gene expression profiling also attemps to find prognostic subgroups. Recently, it was shown that the overexpression of the gene CSK1b located on 1q21 is linked to poor prognosis. The higher number of gene copies, the poorer the prognosis. In addition, treatment-related factors also have a major impact on the outcome of autologous stem cell transplantation for multiple myeloma. The number of pre-transplant treatment regimens, the response to induction treatment and the disease status at transplantation – although not consistently have been identified as prognostic factors. The conditioning regimen administered prior to autologous stem cell transplantation was also found to have an impact on treatment outcome. Melphalan alone in a dosage of 200 mg/m^2 was found to be superior to TBI plus melphalan. In patients achieving less than a very good partial remission following stem cell transplantation a second timely performed transplant was shown to significantly improve response quality, event-free and overall survival. In line with these findings most of the studies have demonstrated achieving CR or near CR after transplant to improve outcome post-transplant. Also the source of transplant seems to play an important role. Peripheral blood stem cell transplantation was shown to be superior to bone marrow transplantation. Purging by positive selection of CD34$^+$ cells to reduce the burden among the infused cells has not improved the outcome but increased the rate of infectious complications post-transplant. The availability of novel agents (thalidomide, lenalidomide, bortezomib) already has and will even more in the future have a major impact on the regimes of autologous stem cell transplantation. Their inclusion in the induction therapy as well as their availability for consolidation or maintenance has already demonstrated improvement in response rates, event-free and overall survival post-transplant.

S9.4

IS THERE STILL PLACE FOR HIGH-DOSE THERAPY IN THE ELDERLY MYELOMA PATIENTS?

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Potential conflicts of interest

Two of the authors (A.P., M.B.) have received scientific advisory-board and lecture fees from Pharmion, Celgene, and Janssen-Cilag. M.B. has received research funding from Pharmion, Celgene, and Janssen-Cilag. The other authors declare that they have no conflict of interest. Multiple myeloma is the second most common hematological disease. At diagnosis, the majority of patients are older then 65 years: 28% are 65 to 74 years and 37% are older than 75.[1] In this elderly population oral melphalan and prednisone (MP) has been considered the standard. In patients younger than 65 years, melphalan 200 mg/m^2 (MEL200) supported by autologous stem-cell transplantation appears to be the treatment of choice. Several studies clearly demonstrated the superiority of MEL200 in terms of response rate and even-free survival when compared with conventional treatments. Results were less consistent when overall survival was examined.[2] The dose of 200 mg/m^2 may be poorly tolerated by elderly patients, while intermediate-dose melphalan (100-140 mg/m^2) appears more suitable. In an Italian phase III study, 194 patients were randomized to receive at diagnosis either 6 conventional courses of MP or intermediate-dose of melphalan 100 mg/m^2 (MEL100) with stem cell support. MEL100 induced higher response then MP (near-complete response rate of 25% versus 6%, p<0.0002) and increased event-free (EFS) and overall survival (OS) at 3 years (37% versus 16% and 77% versus 62%, respectively, p<0.001). A subgroup

analysis on patients aged 65 to 70 confirmed the superiority of MEL100 versus MP with a near-complete response of 25% versus 8% ($p<0.05$), an EFS at 3 years of 31% versus 18% ($p<0.01$) and an OS at 3 years of 73% versus 58% ($p<0.01$). Patients aged 65 to 70 treated with MEL100 had a median OS of 58 months (versus 37.2 months).[3] In another Italian prospective, randomized, phase III trial, MEL100 was compared with MEL200. After the first interim analysis preliminary results were presented. MEL200 resulted in a significantly higher very good partial response rate but this did not translate in a superior EFS and OS. The very good partial response rate was 38% in MEL200 arm and 22% in MEL100 arm ($p=0.011$). The 3 years EFS was 48% in the MEL200 arm and 31% in the MEL100 arm ($p=0.31$). The 3 years OS was 86% in the MEL200 group and 71% in the MEL100 ($p=0.51$).[4] The recent discovery of new drugs, such as thalidomide, lenalidomide and the proteasome inhibitor bortezomib have significantly increased the clinical efficacy of the old chemotherapy regimens. Bortezomib and thalidomide have been combined with MEL100, as conditioning before autologous hematopoietic cell infusions in advanced-stage myeloma patients. Responses occurred in 65% of patients, including 24% of very good partial remissions. Response rate was higher than that induced by the previous line of treatment in about 50% of patients.[5] For elderly patients non candidate to autologous transplant, the MP regimen remained the treatment of choice. Two randomized studies evaluated thalidomide plus MP regimen (MPT). In the Italian trial, oral MPT was compared with MP in patients aged 60-85 years.[6] The partial response rates were 76% in MPT patients and 47.6% in MP subjects, with a near-complete remission rates of 27.9% and 7.2% after MPT and MP, respectively. The 2-years EFS rates were 54% for MPT and 27% for MP ($p=0.0006$). The 3-year OS rates were 80% for MPT and 64% for MP ($p=0.19$). The French trial, comparing MPT with MP with MEL100 followed by autologous stem-cell transplantation, showed a higher partial response rate in the MPT and in the MEL100 arms, compared with MP (81% vs 73% vs 40%, respectively).[7] PFS was superior in the MPT patients compared with both MP ($p<0.001$) and autologous transplantation ($p=0.001$). Furthermore, OS was significantly improved in the MPT group in comparison with both MP ($p=0.001$) and autologous transplantation ($p=0.004$). In both studies, MPT was associated with a higher risk of grade 3–4 hematologic toxicity, infections, thromboembolic complications, peripheral neuropathy, constipation, and cardiac events. These data strongly support the use of MPT as standard of care in elderly patient with newly diagnosed MM. Lenalidomide appears to be an attractive alternative to thalidomide. In a phase I/II trial dosing, safety and efficacy of MP plus lenalidomide (MPR) have been evaluated in newly diagnosed elderly myeloma patients.[8] Aspirin was administered as antithrombotic prophylaxis. At the maximum tolerated dose (lenalidomide 10 mg plus melphalan 0.18 mg/kg), 85% of patients achieved at least a partial response with 23.8% of immunofixation negative complete remission. The 1-year EFS and OS were 92% and 100%. In the MPT historical controls, the corresponding 1-year event-free and overall survivals were 78% and 87.4%. Preliminary results showed that the EFS of patients with deletion of chromosome 13 or chromosomal translocation (4;14) was not significantly different from those who did not have show such abnormalities. By contrast, patients with high-levels of serum β2-microglobulin experienced a shorter EFS in comparison with those who showed low-levels of β2-microglobulin. Neutropenia and deep-vein thrombosis are the major complications with lenalidomide; the addition of aspirin markedly reduced the risk of thromboembolic events in newly diagnosed patients treated with lenalidomide in association with dexamethasone or chemotherapy. Although the optimal prophylaxis strategy has not been established, aspirin seems to be the preferred choice. Bortezomib has been added to MP regimen (VMP). The Spanish phase I/II trial of VMP showed encouraging results.[9] Partial response rate was 89%, including 32% immunofixation-negative complete remission. PFS at 16 months of VMP patients was significantly prolonged in comparison with historical controls treated with MP only (83% versus 51%, $p=0.002$), similarly OS at 16 months was improved (90% versus 62%, $p=0.001$). Interestingly, response rate, PFS and OS were similar among patients with or without retinoblastoma gene deletion or IgH translocations. Grade 3-4 adverse events were thrombocytopenia, neutropenia, peripheral neuropathy, infections and diarrhea. The treatment appeared more toxic in patients older then 75 years and during early cycles. The addition of thalidomide or bortezomib to the standard oral MP significantly increased response rate and EFS. In a multicenter phase I/II trial, dosing, safety, and efficacy of the 4-drug combination, bortezomib, melphalan, prednisone and thalidomide (VMPT) was determined in relapsed or refractory patients10. At the maximum tolerated dose of bortezomib (1.3 mg/m^2) 67% of patients achieved a partial response, including 43% who achieved at least a very good partial response. The 1-year PFS was 61%, and the 1-year survival from study entry was 84%. The most common grade 3 adverse events were hematologic toxicity (56%), infections (9%) Herpes Zooster reactivation (7%) and peripheral neuropathy (7%); no grade 4 toxicities were recorded. The incidence of neurotoxicity was unexpectedly low. Bortezomib induced transient thrombocytopenia and peripheral neuropathy. Pre-existing neuropathy or previous neurotoxic therapy increases the risk of peripheral neuropathy, which can be reduced or resolved by timely dose-adjustment of the drug. Bortezomib may enhance the incidence of infections, in particular Herpes Zoster reactivation, and prophylactic antiviral medication is highly recommended. *Conclusion*. High-dose melphalan followed by autologous stem cell transplantation in the younger patients and oral MP in the elderly have been considered the treatment of choice for the induction therapy of myeloma. In elderly patients or not candidate for autologous transplantation, the MPT combination has been considered the new standard of care, since two independent randomized trials demonstrate significant benefit from this approach.[4,8] In the near future, other regimens such as MPR[9] or VMP[10] or VMPT[11] might be introduced in the clinical practice. MPR showed similar efficacy with a lower incidence of adverse events. VMP appears equally attractive with a higher CR rate. VMPT showed significant efficacy in relapsed patients and might represent a new promising approach at diagnosis. Combinations incorporating thalidomide or lenalidomide or bortezomib with dexamethasone or doxorubicin seem to be better indicated as induction therapy before high-dose melphalan and autologous transplantation. These new induction regimens significantly increased the pre-transplant complete remission rate and might further improve the complete remission rate achieved after autologous transplantation. This benefit is likely to be translated in a prolonged remission duration. These new approaches might further improve the efficacy of high-dose therapy in younger patients and intermediate-dose therapy in elderly patients.

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S9.5

HIGH DOSE THERAPY FOR MULTIPLE MYELOMA - GETTING BEYOND HIGH DOSE MELPHALAN

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Introduction. Multiple myeloma (MM), is currently the most common indication for high dose therapy with autologous stem cell support in North America and Europe.[1] In North America alone more than 2000 transplant are performed each year, Table 1 summarizes the most commonly used conditioning regimens in North America, high dose melphalan is used in over 90% of patients with the most common regimen used being single agent melphalan at a median dose of 180 mg/m^2.[2]

Table 1. Characteristics of patients who underwent autologous transplantation for Multiple Myeloma for 1st remission consolidation between 2000 and 2004 in North America, reported to the CIBMTR.[2]

Characteristics of patients	*N (Eval) N(%)[b]*	*Median (range)[a],*
Number of patients		1371
Age, (median, range), years	1371	58 (22-80)
Missing (n=6)		
Time from Dx to SCT (months)	1371	7 (1-103)
Conditioning regimen	1367	
Lpam alone		1133 (83)
Lpam+TBI ± other		44 (3)
Lpam ± other (not TBI)		130 (10)
Others		60 (<1)
Dose of Melphalan 200, median (range), mg/m^2	1259	187 (0.9-278)
Missing (n=112)		
Type of second transplant	1371	
No planned 2nd tx		904 (67)
Planned 2nd auto		265 (19)
Planned 2nd allo		46 (3)
Missing		156 (11)
Planned post transplant treatment	1351	693 (51)
Missing (n=20)		

Lpam=melphalan; TBI=total body irradiation; Dx=diagnosis; SCT=stem cell transplant

The almost universal use of high dose melphalan as a conditioning regimen for ASCT in myeloma is due to various factors: 1) Known antimyeloma activity 2) Steep dose response curve 3) Limited extra medullary toxicity 4) Extensive experience with older and debilitated myeloma patients. Melphalan 200 mg/m^2 is now considered the gold standard conditioning regimen based on the IFM demonstrated in a randomized trial that patients treated with melphalan 200 mg/m^2 had similar complete response (CR) rates (35% vs 29%); but better median overall survival than patients treated with melphalan in combination with TBI (45 month survival 65% vs 45%).[3]

Improving High Dose Melphalan Therapy-Dose Intensification

Relapse after autografting remains the single most important cause of treatment failure in patients post-autologous transplant. Strategies aimed at improving the conditioning regimen have usually focused on intensifying the conditioning regimen either by increasing the dose of melphalan or by adding other alkylating agents as summarized in table 2, of these only tandem high dose therapy has been reported to improve outcomes in a randomized trial,[4] but this benefit is limited by the fact that many patients refuse to undergo the procedure, and the benefit seems to be limited to patients failing to achieve a greater than 90% reduction of their myeloma burden after the first high dose therapy. Various groups have explored dose intensification of melphalan with or without cytoprotectors such as amifostine. Phillips *et al.* demonstrated the feasibility of administering up to 280 mg/m^2 of melphalan with amifostine, however, significant mucosal toxicities were observed, and at the higher doses cardiotoxicity was dose limiting.[5] Moreau *et al.*, have explored using melphalan at a dose of 220 mg/m^2 as part of a tandem transplant strategy for patients with high risk myeloma as defined by cytogenetics and beta 2 microglobulin. Complete remissions were seen in 30% of patients with another 18% achieving at least a 90% reduction in tumor burden.[6]

Table 2. Transplant Outcomes Using Intensified Conditioning Regimens Compared to Melphalan 200 mg/m^2- Single Transplant Trials.

Strategy	*EFS months*	*Median %CR/%VGPR*	*Reference: & Comments*
Melphalan 200 vs	20	35/20	3 Randomized
Melphalan 140 + TBI	21	29/14	Trial
Melphalan 200 vs	22	43/NR	7 Registry
Busulfan-Melphalan vs	32	49/NR	Analysis
Melphalan + TBI	20	31/NR	
Melphalan 200 vs	19	32/NR	8,9
Melphalan + Holmium-DOTMP (<2400 cGy)	30	55/NR	Retrospective Analysis
Melphalan 200 vs Thiotepa busulfan	20	16/NR	10
cyclophosphamide	21	27/NR	Retrospective Analysis

Therefore, neither melphalan dose intensification nor addition of other alkylating agents have resulted in large increases in post-transplant CR rates, and novel conditioning regimens exploring different mechanisms of enhancing the antitumor effect of high dose melphalan need to be explored.

Improving High Dose Melphalan Therapy-Targeting the Stroma

The recent expansion of therapeutic options for MM can be partly attributed to a better understanding of the interactions between malignant plama cells and bone marrow microenvironment that includes stromal cells, extracellular adhesion molecules and secreted cytokines. Novel therapies targeting these interactions have shown promising responses and outcomes.[11] Arsenic trioxide (ATO) induces apoptosis even of drug-resistant MM cell lines and patient cells via caspase-9 activation, enhances MM cell apoptosis induced by dexamethasone, and can overcome the antiapoptotic effects of IL-6 by blocking both activation of STAT3 and upregulation of Mcl-1.[12] The induction of apoptosis by As_2O_3 is likely mediated via the production of ROS (reactive oxygen species) that damage mitochondria.[13,14] In addition to reducing ROS, the critical intracellular antioxidant GSH is also directly conjugated to arsenic and subsequently transported out of the cell by multidrug resistance efflux pumps. It follows that agents that deplete GSH will sensitize cells to As_2O_3-induced apoptosis.

Table 3. Preliminary Results of Arsenic Trioxide, Ascorbic Acid and High Dose Melphalan as Conditioning Regimen for Myeloma.

	No ATO	*ATO 0.15 mg/kg*	*ATO 0.25 mg/kg*
Number	8	10	7
Median days to engraftment	9	10	9
Median ATO level on day 0	0.2 ng/mL	26.3 ng/mL	46.2 ng/mL
Prior autograft	2	3	2
CR	0	1	0
PR	6	5	6
Progressed	2	4	1

One such agent is Ascorbic acid (AA).[12] Several clinical trials have shown the efficacy of ATO and AA in the treatment of relapsed and refractory multiple myeloma.[15-17] Based on encouraging reports of efficacy of combination of arsenic trioxide with ascorbic acid and conventional-dose melphalan (MAC) in relapsed multiple myeloma, we conducted a phase I/II study of arsenic trioxide + ascorbic acid + high-dose melphalan as conditioning regimen in myeloma patients undergoing an autologous transplant. This combination was well tolerated, did not adversely affect the engraftment and resulted in a response rate of 87%

after a median follow up of 7.1 months.[18] Table 3 summarizes the preliminary results of that regimen which demonstrated that arsenic trioxide and ascorbic acid can be safely combined with high dose melphalan without causing delays in engraftment or significant increases in toxicities. Bortezomib, a proteasome inhibitor, is an active agent in newly diagnosed and relapsed and refractory multiple myeloma in conventional therapy setting. Its proposed mechanisms of action include, cell cycle arrest at G1-S phase, direct apoptosis of steroid-resistant myeloma cells and inhibition of NFKappaB.[19] Preclinical data support the use of bortezomib in combination with melphalan. *in vitro*, exposure of highly melphalan-resistant myeloma cell lines, as well as bone marrow tumor cells from myeloma patients, to noncytotoxic concentrations of bortezomib increased sensitivity to melphalan, but this was not observed in normal bone marrow stem cells or peripheral-blood lymphocytes.[20,21] In one of the preclinical studies, subtoxic concentrations of PS-341 potently sensitized MM cell lines and patient cells to DNA-damaging activity of melphalan, including cells resistant to melphalan. Using gene expression profiling and proteomic analysis, the authors demonstrated that bortezomib inhibits genotoxic stress response pathways, which restores sensitivity to DNA-damaging chemotherapeutic agents. This study provided the framework for clinical use of this combination. Based on these data various Phase I and Phase II trials of bortezomib with high dose melphalan are currently ongoing. Targeted skeletal radiotherapy with Holmium-DOTMP was shown to be effective in delivering high doses of radiation to the marrow while sparing normal tissues as long as doses of less than 2400 mCi were administered.[8,9] Similar results have been obtained by Dispenzieri *et al.* using Samarium-EDTMP [22]

Optimizing High Dose Melphalan Therapy

It is a well known fact that patients tolerance and response to melphalan 200 is extremely heterogeneous, with some patients having no toxicities whatsoever and others having serious life threatening toxicities due to mucosal damage. To a certain degree this variability is probably due to genetic polymorphisms among enzymes such as GST (ref). However, the practice of dosing melphalan according to body surface area, with arbitrary modifications for overweight patients also suggests that melphalan dosing may be implicated as reported by Anaissie *et al.*[23] Lastly, Dimopoulos *et al.* have demonstrated that the extent of damage and repair seen in the p53 tumor-suppressor gene of peripheral blood lymphocytes after exposure to melphalan is an important predictor of outcome and could serve as a tool for developing a personalized or individually tailored conditioning regimen .[24]

Summary

High dose melphalan remains the gold standard to which all new conditioning regimens for autologous transplant in myeloma need to be compared to. However, the relatively low CR rate obtained with this conditioning behooves the field to prospectively explore new combinations. The advent of bortezomib, lenalinomide as well as other agents have opened a new era of conditioning regimens in myeloma in which both the stroma and the malignant cell are being targeted.

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S9.6

ALLOGENEIC TRANSPLANTATION IN MULTIPLE MYELOMA

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Introduction. Allogeneic Stem Cell Transplantation (ASCT) is probably the only treatment with curative potential for Multiple Myeloma (MM).This is due to the graft-versus-myeloma effect (GVM), mediated by immune competent donor lymphocytes, and at best illustrated by the induction of sustained remissions following donor lymphocyte infusions after ASCT.[1] However, the necessity of performing ASCT in MM is disputed as no survival advantage has been obtained compared to autologous SCT in particular when myeloablative conditioning for the ASCT is applied.[2] An important factor for this is the high treatment related mortality (TRM) associated with myeloablative conditioning.[3] Non-myeloablative ASCT (NMA) is associated with reduced acute toxicity, while anti-tumour activity is probably maintained. Although encouraging results have been reported, the role of NMA for myeloma is not yet established.[4] In the recently published prospective study by French IFM, high risk myeloma patients with an HLA-identical family

donor and treated with tandem autologous/NMA-ASCT had comparable PFS and OS to the patients with no donor that were treated with double autologous SCT.[5] In this study *in vivo* T-cell depletion was performed with high dose ATG as part of the non myeloablative conditioning regimen in all patients. The beneficial effect of *in vivo* T-cell depletion is the low incidence of acute and chronic GvHD; the detrimental effect is the elimination of the Graft versus Myeloma (GvM) effect. The importance of immune-competent donor T cells for GvM is illustrated by responses to DLI and the occurrence of chronic GVHD.[6] European study groups, including the Dutch Hemato-Oncology Association (HOVON), Spain's Programa para el estudio y tratamiento de las hemopatias malignas (PETHEMA), the multicenter Italian group lead by B.Bruno and the European Group for Blood and Marrow Transplantation (EBMT), are performing comparable prospective donor versus no-donor studies., however without *in vivo* or *in vitro* T cell depletion.

Single center experience with non-myeloablative allogeneic stem cell transplantation

Fifty-nine patients received NMA at the department of Hematology, University Medical Center Utrecht.7 The median age was 55 (range, 35-67). There were 42 males (71%) and 17 females (28%). The median follow-up duration of survivors was 25.2 months (range, 6.8-54.6) In 36 patients (61%), NMA was part of first-line treatment and in 23 patients (39%) it was part of salvage treatment. At the time of transplant, 9 patients (15%) were in CR and 40 patients (68%) were in PR. A full peripheral blood stem cell graft was given after conditioning with low dose TBI 200 cGy only. Relapsed patients without a previous autologous transplant between 2 and 6 months earlier were conditioned with low dose TBI and Fludarabine 30 mg/m^2 intravenously for 3 days. Immune suppression consisted of Cyclosporin A and Mycofenolmofetil for 3 months. In the absence of GvHD immune suppression was tapered and stopped after 6 months. Only 4% of the relapsed patients achieved a CR after NMA as opposed to 50% of patients who received tandem auto/allo as part of first line therapy. PFS of patients not achieving a CR was 13 months and has not been reached for patients achieving CR after NMA (Figure 1). Achievement of CR and chronic GvHD were associated with prolonged PFS and OS. TRM for the whole group was 9% (Figure 2). With all the restrictions of a single center experience these data illustrate the feasibility of NMA and associated acceptable TRM. In extensively pre-treated patients response rate and PFS are disappointing and in this category of patients new strategies need to be explored. The results achieved of NMA as part of first line treatment of MM are encouraging. However we must await the outcome of prospective donor versus no donor studies to better define the role of this treatment for myeloma.

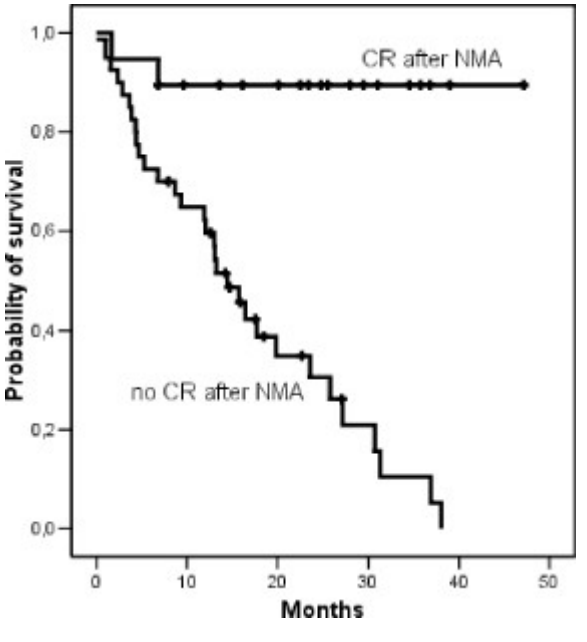

Figure 1. Progression Free Survival and response after nonmyeloablative Allo-SCT of patients treated at University Hospital Utrecht.

First results of the prospective Hovon 54 study, a donor versus no donor comparison.

Newly diagnosed MM patients, stage II or III, age 18-65 years, were eligible for inclusion in the HOVON-50 study. The patients, total 556, were randomly assigned to arm 3 cycles of VAD or the same regimen but with Thalidomide 200 - 400 mg orally, days 1-28 instead of Vincristine (TAD). After stem mobilization with CAD patients received High Melphalan (HDM) 200 mg/m^2 with autologous stem cell rescue. Patients with an HLA identical stem cell donor, without progressive disease, WHO performance 0-2 and without organ damage were eligible for the Hovon 54 study (Figure 3). Patients included in the Hovon 54 received nonmyeloablative Allo-SCT between 2 and 6 months following HDM. A full peripheral blood stem cell graft was given after conditioning with low dose TBI 200 cGy only. Immune suppression consisted of Cyclosporin A and Mycofenolmofetil for 3 months. In the absence of GvHD immune suppression was tapered and stopped after 6 months. Ninety-six patients were included. As of January 2007 data were available from 82 patients with a median age 53 years (33-65), including 56% males and 44% females. At 18 months overall survival was 79%. This compares favourable to a historical group of Hovon 24 patients who received a myeloablative Allo-SCT as part of first line therapy (Figure 4). Major courses of this inferior outcome of the Hovon 24 patients were the TRM of 33% and absence of a plateau in the OS curve even in the patients that achieved a CR after transplant.[3] At the workshop a complete analysis will be presented on all 96 patients treated in the Hovon 54 study with respect to OS, EFS, response and toxicity including comparison with Hovon 50 patients with no donor who received maintenance therapy following HDM.

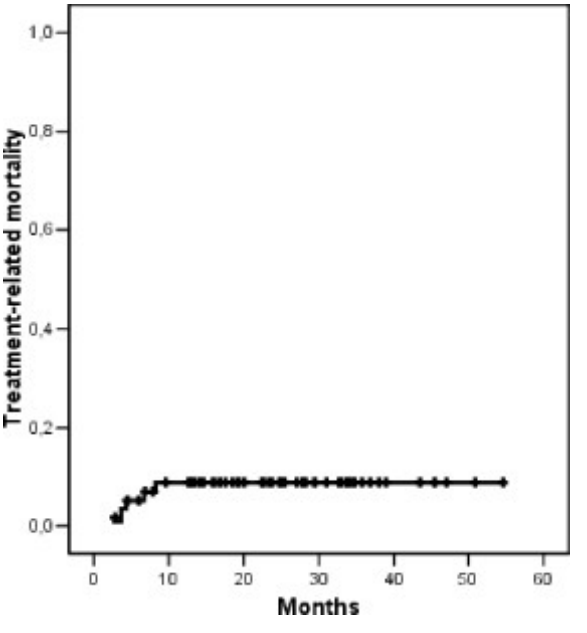

Figure 2. Treatment related mortality of patients treated at University Hospital Utrecht.

Future

In anticipation of the outcome of the prospective donor versus no donor comparisons it is necessary to explore new strategies which are aimed at stimulating the cytotoxic efficacy of the donor T cells towards the residual myeloma cells without enhancing GVHD. The suggestion that the novel anti-myeloma agents such as Bortezomib, thalidomide, and revlimid may preferentially stimulate the graft-versus-tumour effect and not GVHD is already incorporated in current transplant and DLI protocols.[8,9] In the Hovon 54 follow-up study, the Hovon 76 study, patients with an HLA identical donor are recruited from the Hovon 65/GMMG-HD4 study. These patients will receive a tandem autologous/ NMA transplantation followed by maintenance therapy with the novel immune modulating agent Lenalidomide.

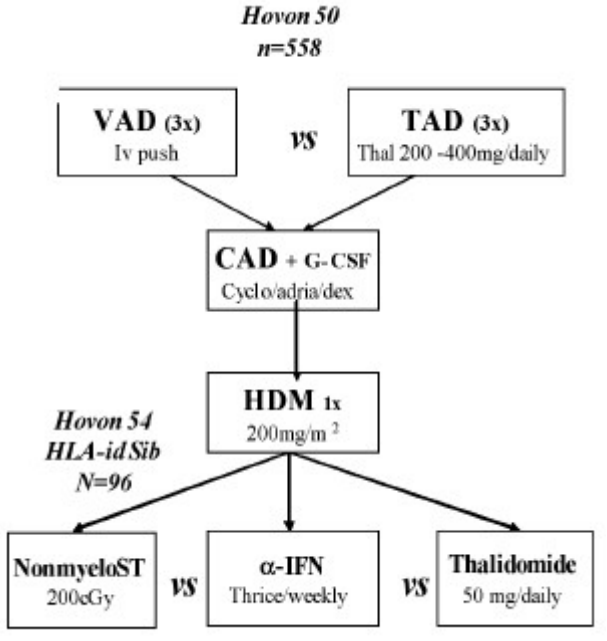

Figure 3. Flow chart of the HOVON 50 and Hovon 54 study.

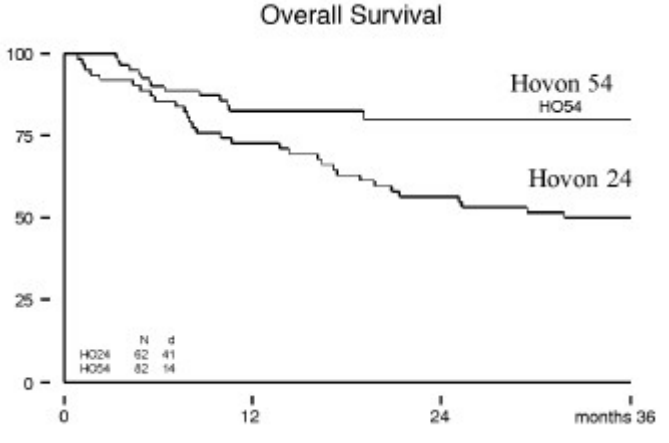

Figure 4. Overall survival curves of the Hovon 24 (myeloablative conditioning) and of the Hovon 54 study (nonmyeloablative conditioning).

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S9.7

THE ROLE OF DONOR-LYMPHOCYTE INFUSION AND OTHER POST-TRANSPLANT STRATEGIES AFTER ALLOGRAFTING IN MULTIPLE MYELOMA

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Donor-lymphocyte infusions (DLI) provide effective treatment for patients who experienced relapse after allogeneic stem cell transplantation.[1,2,3] Meanwhile the graft-versus-myeloma effect of DLI is well established. Several attempts had been made in the last years to improve the efficacy of DLI after allogeneic stem cell transplantation for multiple myeloma.

DLI for relapse

The most experience with DLI after allogeneic stem cell transplantation in patients with myeloma has been reported for relapse.[1,2,3] In those reports, response rates of 40-52% have been reported, and 22-28% of the responders achieved complete remission. One study reported an overall survival of 19 months and 23 months for those patients who achieved partial or complete remission, respectively. GvHD was the most significant complication after DLI, and the incidence of acute GvHD was reported to be 52 56% and of chronic GvHD 26-44%. However, the response to DLI-treatment was significantly correlated with the incidence and severity of GvHD.[3]

DLI as prophylactic treatment

Only few about prophylactic DLI, especially after non-myeloablative conditioning, were reported.[4,5,6] In those trials, DLI was given to prevent relapse or enhance remission status. In these trials, the incidence of GvHD was similar to DLI given for relapse, and in about 50-60% of the patients, an improvement of remission status could be observed. To reduce the risk of GvHD after DLI, strategies such as CD8-depleted DLI were investigated after allogeneic stem cell transplantation. With these strategies, acute GvHD grade II-IV was seen in 50% of the patients, and six out of ten patients with measurable disease experienced complete remission.[4]

DLI plus new agents

New available drugs with immunomodulatory properties have been investigated after allogeneic stem cell transplantation. Interferon-α alone induces in four of five patients after allograft a complete remission without GvHD.[7] In preclinical mice-models, the proteasome-inhibition inhibits T cell proliferation and acute GvHD while retaining the graft-versus-tumor effect.[8] In a clinical study investigating bortezomib as post-transplant strategies to enhance remission status, complete or partial remission could be in 32% and 50%, respectively.[9] Thalidomide as single agent for progressive myeloma after allogeneic stem cell transplantation induced in 29% of the patients a partial or very good partial remission.[10] In attempts to combine DLI with the new agents, one study investigated low-dose thalidomide (100 mg) in combination with DLI. The overall response rate was 67% with 22% complete remission. Interestingly, no grade II IV GvHD was seen, and only a minority of patients developed limited chronic GvHD.[11] Even in patients not responding to DLI, salvage treatment with thalidomide or bortezomib can induce complete or partial remission in 83% of the cases suggesting high efficacy of these drugs after allogeneic stem cell transplantation.[12]

Molecular targets for DLI

The major issue for further improvement of immunologically-based strategies post allogeneic trans¬plantation lies in the separation of the graft-versus-myeloma effect from the graft-versus-host reaction which would allow a more specific tumor-targeting without or with lesser risk of GvHD. Potential candidates for a more specific T-cell response are minor-histocompatibility antigens such as HA1. More recently, HA1-specific T-cells could be generated and induced complete remission in patients with relapsed multiple myeloma after allogeneic stem cell transplantation.[13] Another potential target for tumor-specific donor-T-cell response is the myeloma-specific idiotypic determinant of immunoglobulin-variable region, which has been used to immunize the donor prior to allogeneic stem cell transplantation in order to transplant a myeloma-specific T cell response.[14] In this study, two out of five patients remained disease-free after allografting for seven and eight years, respectively. In all patients immunoglobulin-specific T cell response was seen and persisted for 18 months. Another potential target is cancer-testis-antigens, especially MAGEC2 and MAGEA3, which are expressed in more than 50% of myeloma cells.[15] Antibody- and T cell response against cancer testis antigens was more frequently observed after allografting than after autologous transplantation while no antibody response was seen in patient and donor before transplantation. More recently, killer-immunoglobulin-like-receptor-ligand-donor/recipient-mismatch transplantation may be protective against relapse, suggesting a potential role of alloreactive NK-cells after allografting to enhance remission status and prevent relapse.[16] The importance of achieving molecular remission after allogeneic stem cell transplantation for long-term disease freedom has been shown in a retrospective EBMT-study.[17] In this study using high-sensitive patient-specific primers to monitor residual disease with PCR-technique, it could be shown that durable PCR-negativity after allografting had a cumulative risk of relapse at five years of 0%, in comparison to 33% for PCR-mixed patients and 100% for patients who never achieved PCR-negativity. Therefore, post-transplant strategies should not be used for relapsed patients only but also to target the molecular remission after allografting. A new donor-immune system after allografting offers a platform for numerous strategies or immunologically-based post-transplant strategies. Table 1 shows the possible treatment options after allografting in order to achieve molecular remission which seems to be a conditio sine qua non for curing the disease.

Table 1. Post-transplant strategies to enhance remission status or to treat relapse.

	ORR	CR	References
1. Donor lymphocyte infusion	40 – 67%	19 -30%	1, 2, 3
2. CD8-depleted donor-lymphocyte infusion	71%	43%	4
3. Thalidomide	29 – 83%	0 – 22%	10, 12
4. Bortezomib	80 – 100%	29 – 30%	9, 12
5. Interferon-a*	80%	80%	7
6. Thalidomide plus donor-lymphocyte infusion	67%	22%	11
7. Donor vaccination		3 out of 5 after allograft	14

** only five patients*

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S10: Other plasma cell dyscrasias

S10.1

MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE AND SMOLDERING MULTIPLE MYELOMA (SMM)

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Monoclonal gammopathy of undetermined significance (MGUS) is defined by a monoclonal immunoglobulin concentration in serum of 3 g/dL or less, fewer than 10% plasma cells in the bone marrow, and no evidence of end-organ damage (hypercalcemia, renal insufficiency, anemia, or lytic bone lesions) related to the proliferation of the clonal plasma cells. Monoclonal proteins have been reported without evidence of multiple myeloma (MM) or Waldenstrom's macroglobulinemia (WM) in approximately 3% of persons > 70 years of age in Sweden, the USA, and France. The first population-based study to detect monoclonal gammopathies examined 21,463 sera; 77% of the 28,038 enumerated residents of Olmsted County, Minnesota who were 50 years of age or older (Kyle, *et al.*, 2006). MGUS was identified in 3.2% of these patients. Age-adjusted rates were greater in men than in women (4.0% vs. 2.7%), while the prevalence was 5.3% among persons 70 years or older. Concentration of monoclonal immunoglobulins was < 1.0 g/dL in 63.5% and ≥ 2 g/dL in only 4.5%. Concentration of uninvolved immunoglobulins was reduced in 28% of 447 persons tested. Of 79 persons tested, 21% had a monoclonal urinary light chain. The prevalence rate remained almost constant throughout collection suggesting that patients who frequently seek medical care are at little or no greater risk for MGUS than those who do not. The prevalence was 4-fold higher in persons ≥ 80 years of age than those age 50-59 years. The prevalence was 2-fold higher than from the literature in persons ≥ 50 years of age and almost twice that previously reported in persons ≥ 70 years of age. MGUS is one of the most common premalignant disorders in the general population ≥ 50 years of age. The incidence of MGUS in African-Americans is greater than in Caucasians. A study of more than 4,000,000 African-American and white veterans in the United States reported that the prevalence of MGUS in African-Americans was 3.0-fold higher than in Caucasians. However, progression to multiple myeloma was virtually identical in both groups (17% among African-Americans, 15% among Caucasians) (Landgren, *et al.*, 2006). The outcome of patients with MGUS evaluated at the Mayo Clinic between 1956 and 1970 has been described (Kyle, *et al.*, 2004). Two-hundred-forty-one patients were followed for 3,579 person-years (median: 13.7 years; range: 0-39 years). During a follow-up of 33 years, only 14 (6%) were alive and had no substantial increase in monoclonal protein. The serum monoclonal protein increased to 3 g/dL or more in 25 patients (10%) but did not require chemotherapy for MM, WM, or AL (light chain amyloidosis). One-hundred and thirty-eight patients (57%) died without evidence of MM, WM, AL, or a related disorder (cardiac disease, cerebrovascular disease, or a non-plasma cell malignancy accounted for the major number of deaths). MM, WM, AL, or a lymphoproliferative disorder developed in 64 patients (27%) during a follow-up of 1-32 years (median 10.4 years). The actuarial rate of progression was 17% at 10 years, 34% at 20 years, and 39% at 25 years; a rate of approximately 1.5% per year. Forty-four (69%) of the 64 patients who progressed developed MM. Thirteen-hundred eighty-four patients with MGUS from the 11 counties from Southeastern Minnesota were evaluated at Mayo Clinic from 1960 to 1994 (Kyle, *et al.*, 2002). The median age at diagnosis was 72 years, which is 8 years older than the 241 patients in the referral population. The protein was IgG in 70%, IgM in 15%, IgA in 12%, and biclonal in 3%. Reduction of uninvolved immunoglobulins was found in 38% of 840 patients who were evaluated. A monoclonal light chain in the urine was found in 31% of the 418 patients who were tested. The 1,384 patients were followed for a total of 11,009 person-years (median: 15.4 years; range: 0-35 years). During follow-up, 115 patients (8%) developed MM, AL, lymphoma with an IgM serum protein, WM, plasmacytoma or chronic lymphocytic leukemia. The risk of progression was 10% at 10 years, 21% at 20 years, and 26% at 25 years; a rate of approximately 1% per year. An additional 32 patients were identified, in whom the serum M-protein value increased to > 3 g/dL or the percentage of bone marrow plasma cells increased to > 10%, but in whom symptomatic MM or WM did not develop. The number of patients with progression was 7.3 times the number expected. The risk of developing MM was increased 25-fold, WM 46-fold, and AL 8.4-fold. Characteristics of the 75 patients who developed MM were comparable with those of a cohort 1027 patients with newly-diagnosed MM who were referred to the Mayo Clinic between 1985 and 1998 (Kyle, *et al.*, 2003). The pathogenesis of progression of MGUS is not known. Fluorescence *in situ* hybridization (FISH) shows IgH translocations as well as deletion of chromosome 13 similar to the findings in multiple myeloma. Angiogenesis may also play a role in progression. Initial concentration of serum M protein, type of M protein (IgA or IgM are at higher risk for progression), and the free light chain (FLC) ratio are risk factors for progression. Patients with a combination of a serum M protein ≥ 1.5 g/dL, IgA or IgM MGUS and an abnormal serum FLC ratio had a risk of progression at 20 years of 58%, compared to 5% when none of the risk factors were present (Rajkumar, *et al.*, 2005). Patients with MGUS should be rechecked in 3 to 6 months in order to exclude an early multiple myeloma. If stable at that time, patient should be reevaluated at annual intervals or perhaps less frequently if no risk factors are present.

Smoldering (asymptomatic) Multiple Myeloma (SMM)

Smoldering multiple myeloma, first reported in 1980, is an asymptomatic plasma cell proliferative disorder associated with a high risk of progression to symptomatic MM. SMM is defined as a serum monoclonal protein ≥ 3 g/dL and/or bone marrow clonal plasma cells > 10%, but no end-organ damage. A study cohort of 276 patients was stratified into three prognostic groups at initial diagnosis: Group 1, bone marrow plasma cells 10% or greater and serum monoclonal protein ≥ 3 g/dL; Group 2, bone marrow plasma cells ≥ 10% but serum monoclonal protein < 3 g/dL; and Group 3, serum monoclonal protein ≥ 3 g/dL but bone marrow plasma cells < 10%. Criteria for SMM were fulfilled in 276 patients seen at Mayo Clinic from 1970-1995. The median age at diagnosis was 64 years (range 26-90 years) and only 2.9% were younger than 40 years. The median hemoglobin value was 13.0 g/dL. Of the monoclonal proteins, 74% were IgG, 22.5% were IgA, 0.5% IgD, and 3% were biclonal. Concentrations of uninvolved immunoglobulins were reduced in 83% of 230 patients who were studied. Fifty-three percent had a monoclonal light chain in the urine. Only 4 patients (1%) had an M-spike more than 1.0 g/24hours. During 2,131 cumulative years of follow-up (range: 0-29 years; median: 6.1 years) 85% died. Multiple myeloma developed in 158 persons (57%), while AL developed in 5 (2%). The cumulative probability of progression to active MM or AL was 51% at 5 years, 66% at 10 years, and 73% at 15 years; the median time to progression was 4.8 years. The overall risk of progression was 10% per year for the first 5 years, approximately 3% per year for the next 5 years, and 1.2% per year for the last 10 years. The risk of progression to active MM was 522 times that expected while the risk of AL was increased 50-fold. On multivariate analysis, the 3 prognostic groups based on the serum monoclonal protein size and the number of bone marrow plasma cells were the most important risk factors for progression. The current standard of care for SMM is close follow-up at 4- to 6-month intervals.

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S10.2

SOLITARY BONE AND EXTRAMEDULLARY PLASMACYTOMA

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Solitary Bone Plasmacytoma

Among patients with myeloma and bone destruction, approximately 2% show only a solitary lytic lesion, that had usually caused localized bone pain. For rational planning of treatment, selected procedures are required to confirm the diagnosis and to exclude multiple myeloma with as much certainty as possible. These include needle biopsy evidence of monoclonal plasma cells, no other evidence of myeloma on bone survey or random marrow aspirate, and no other complication (anemia, renal impairment) that might be due to myeloma. Despite normal bone survey, magnetic resonance imaging (MRI) of thoracic and lumbar spine has been useful since Moulopoulos *et al.* detected unexpected bone lesions in 4 of 12 patients considered initially to have SPB.[1] Thus, the diagnosis of SPB requires a negative MRI of thoracolumbar spine, with the potential value of MRI of other parts of the skeleton or of PET scans unclear at this time. Electrophoretic studies are also essential. While 75% of patients have a monoclonal protein in serum or urine, the level has been low in most patients so that serum M protein concentrations are usually < 1.0 g/dL (our highest 2.2 g/dL), urine Bence Jones protein < 0.2 g/d (our highest 0.7 g/day).[2,3] For the 25% of patients with apparent *nonsecretory* disease, free light chain assays have revealed high free kappa or lambda light chain levels in approximately two-thirds of patients.[4] Levels of uninvolved serum immunoglobulins are preserved, as only 3 of 76 patients at our center had low values, each of whom considered to have occult systemic disease because of early disease progression.[5] High doses of radiotherapy to the solitary lesion remain the treatment of choice since there is local control in virtually all patients and the prospect of cure for many patients. Too few trials of large numbers of patients have been conducted to define the optimum dose, but Table 1 summarizes the experience of several single institution studies. Following a dose of ≥ 40 Gy, local disease progression was nonexistent or rare in all trials so that the practice at our and other centers has been to recommend 45 Gy in 25 fractions over 5 weeks. The United Kingdom Myeloma Forum has recommended 40 Gy in 20 fractions to encompass the tumor mass plus a margin of at least 2 cm beyond disease detectable by MRI; for bulkier disease > 5 cm, 45-50 Gy in 25 fractions was advised.[9] Surgery is not indicated for SBP, but some patients require decompressive laminectomy, spine fusion or intramedullary rod fixation of a long bone. Adjuvant chemotherapy has been given without benefit in most studies. The role of intensive therapy supported by autologous stem cells is not clear but is difficult to assess in view of the long disease course and known cure fraction. A major early endpoint associated with prolonged disease stability and long survival has been disappearance of monoclonal protein following radiation therapy, a change that usually occurs within 6 months. Serial assessment of free monoclonal light chain levels in patients with apparent *nonsecretory* disease allows more patients to be assessed in this manner. Figure 1 depicts the different outcomes at our center among 76 patients with or without resolution of myeloma protein (currently by immunofixation) as an index of effective myeloma control. Myeloma protein persisted in most patients as a reflection of residual disease and was associated with earlier evolution of multiple myeloma. Since none of 11 patients with serum M protein > 1.0 g/dL showed disappearance of abnormal protein after radiotherapy, all patients with such levels probably have multiple myeloma even though asymptomatic, especially since evolution of myeloma occurred after a median 2.0 years. In such patients, control of the local symptomatic lesion should be followed by some form of cytostatic therapy, such as with alpha interferon or thalidomide, until disease progression. The same approach seems reasonable for other patients with lower myeloma protein values that persist after radiotherapy in whom the median time to progression was 2.7 years. Thus, among 76 patients staged by current and older procedures, one-half have developed multiple myeloma within 2 years. Yet, the median survival of all patients was 11.6 years with 30% of deaths due to unrelated diseases; the potential cure fraction based on analyses of cause-specific survival was approximately 35%. Various prognostic factors associated with progression of multiple myeloma have been observed by others, such as spine disease, older age, bulky disease, M protein level, etc., but we could not confirm that any of these factors were harmful. With progressive advances in staging that include MRI and sensitive techniques to detect clonal disease in marrow, the diagnosis of SPB will be made less frequently. When one also requires that uninvolved immunoglobulins be preserved, and that serum M protein be ≤ 1.0 gm for effective long-term control, a true SBP should be associated with a higher cure fraction after effective radiotherapy.

Table 1. Radiotherapy for solitary plasmacytoma of bone

	No.	*Dose (Gy)*	*10-year DFS (%)*	*OS (Yrs)*
Wilder[6]	60	30 – 70	38	11
Frassica[6]	46	<12 – 70	25	9
Tsang[7]	32	<30 – 50	36	10
Bolek[8]	27	28 – 60	46	10

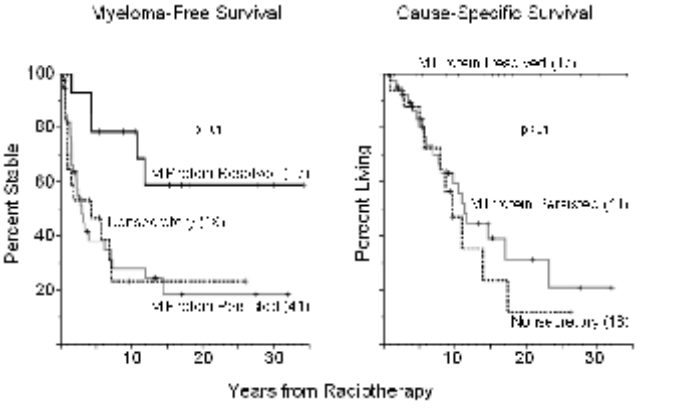

Figure 1. Myeloma-free survival (A) and cause-specific survival (B) correlated with response of SPB to radiotherapy among 76 patients treated at M.D. Anderson Cancer Center.

Extramedullary plasmacytoma

A solitary extramedullary plasmacytoma (EMP) is a rare disorder characterized by a mass of clonal plasma cells unrelated to underlying bone destruction and with no other evidence of multiple myeloma. Careful phenotypic studies are essential to distinguish this process from a reactive plasmacytoma or lymphoma (MALT, marginal zone). Thus, surgical biopsy is useful to affirm plasma cells with CD38 and monoclonal cytoplasmic light chain expression without markers suggestive of lymphoma. As with SBP, there should not be marrow plasmacytosis and bone survey should be normal. Approximately 85% of lesions involve the mucosa of head and neck; the presence of bone destruction with sinus involvement suggests that this subgroup represents a SBP of a sinus bone. Other sites of EMP include GI tract, lung, bladder, thyroid, testis among others. CT or MRI are necessary to define the extent of an EMP but the role of MRI of spine or of PET scan has not been studied carefully. Less than one-fourth of 25 patients at our center showed monoclonal globulin in serum or urine by standard studies and levels of uninvolved immunoglobulins have been preserved. Free light chain levels in serum should be assessed even though their value remains unclear. As with SBP, EMP are highly radiosensitive so that local control is usually achieved and approximately one-half of our patients have remained stable for more than 10 years.[10] At most centers, radiotherapy doses have ranged from 35-45 Gy, and our practice has been to include prophylactic irradiation of regional lymph nodes when oral cavity, pharynx, larynx or parotid are involved, but not for nasal cavity or maxillary sinus disease. Neither surgery nor adjuvant chemotherapy appear justified.[11] Rare patients have shown local recurrence and the disease-free and cause-specific survival after 10 years have been approximately 60% for patients at our center. One-fourth of patients developed multiple myeloma after a median of approximately 1 year presumably from occult disease elsewhere. No prognostic factors have been identified in part because a monoclonal immunoglobulin is present infrequently and modern staging procedures have not been conducted in many patients. *Conclusion*. SBP and EMP represent uncommon solitary plasmacytomas that are radiosensitive and usually associated with long-term stability. More sensitive techniques for detection and follow up (MRI, PET, free light chain assays) should permit clearer staging so that fewer patients will meet the criteria for diagnosis and the cure fraction will be higher after radiotherapy.

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S10.3

AL AMYLOIDOSIS: DIAGNOSIS AND PROGNOSIS

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Systemic AL amyloidosis is a plasma cell disorder characterized by the overproduction and tissue deposition of a monoclonal immunoglobulin light chain (LC), or fragments containing the LC variable region and a portion of the constant region. The deposits are composed of amyloid fibrils, presenting a cross beta supersecondary structure. The process of amyloid deposition produces tissue damage and eventually organ failure, leading to death in untreated patients.[1] The optimal management of patients with AL amyloidosis requires early diagnosis, correct amyloid typing, effective treatment, tight follow-up and careful supportive therapy. One of the most important determinants of outcome is early diagnosis, as severe amyloid organ disease may preclude the use of potentially effective treatment regimens. The systemic involvement affecting vital organs such as heart, kidney and liver renders these patients particularly fragile and sensitive to the toxicity of chemotherapy. Early diagnosis depends on the level of alertness of the physician: any patient with nephrotic-range proteinuria, unexplained right-sided heart failure, progressive peripheral neuropathy, unexplained hepatomegaly or functional hyposplenism, orthostatic hypotension and other manifestations of autonomic neuropathy with weight loss should be screened for amyloidosis. The diagnosis of AL is biopsy based and requires the presence of deposits with apple green birefringence after Congo red staining, or the prototypic, nonbranching, 10-nm diameter fibrillar structures by electron microscopy. Fine-needle aspiration of abdominal fat is innocuous, fast, inexpensive, and sensitive (87%).[2] The method is based on the almost constant involvement of subcutaneous adipose tissue in AA, AL and ATTR forms of systemic amyloidosis, and probably in other systemic amyloidoses as well. Amyloid is found both in the walls of small vessels and around the individual fat cells. False-positive Congo red-stained biopsies occur because of overstaining and inexperienced review. If the abdominal fat is negative, the second choice biopsy site at the Pavia Amyloid Center is the minor labial salivary glands. Renal and hepatic biopsies carry a small risk of bleeding and often require overnight hospitalization. Once the diagnosis of amyloidosis has been established histologically, the type must be determined because the prognosis and treatment depend on the biochemical amyloid forms. There are specific treatments available for some systemic amyloidoses, this means that exact and safe determination of the type of amyloid deposit in the individual patient is critical. This can be accomplished using immunohistochemistry, immunoelectron microscopy [3] or by biochemical methods which are applicable also to formalin-fixed tissue samples.[4] Immunohistochemistry is usually reliable for identifying or ruling out AA amyloidosis, but is frequently not diagnostic with respect to AL amyloidosis. Immunoelectron microscopy and biochemical methods provide definitive results; however, they are labor-intensive and require expertise. If these techniques are not available, the DNA analysis should be performed upfront in order to exclude the hereditary amyloidoses whose clinical presentation is consistent with the patient's manifestations. In order to characterize the amyloidosis of AL type, a plasma cell clone should be documented. The demonstration of the clone requires sensitive techniques. The bone marrow should always be examined, bearing in mind that, typically, the amyloid plasma cell clone infiltrates the bone marrow to a modest extent (median bone marrow plasma cell percentage 7%), often requiring anti-light chain immunohistochemistry/immunofluorescence for κ and λ light chains to be identified. Accordingly, also the circulating monoclonal protein is usually present at low concentration, being missed by screening serum electrophoresis in approximately 50% of patients. Therefore, all patients with a clinical suspicion of AL amyloidosis should undergo sensitive immunofixation electrophoresis of serum and urine that is able to detect a monoclonal protein in up to 97% of patients.[2] The quantification of serum-free light chains (FLC) may complement immunofixation and represents now an irreplaceable tool for monitoring response to therapy. Evidence of progression or regression of amyloid deposits can be obtained from serum amyloid P (SAP) component scintigraphy. Due to the relatively high prevalence of a monoclonal protein in the adult population the possibility of a chance coexistence of a monoclonal protein in a patient with hereditary amyloidosis should always be considered.[5] Clinically, it is difficult to distinguish AL from reactive, familial, and senile systemic forms of amyloidosis, because of their overlapping clinical presentations and the lack of an informative family history in half of the patients with hereditary amyloidosis. Since mistyping of amyloidosis may have catastrophic therapeutic consequences, such as transplanting hematopoietic stem cells instead of liver, great care should be devoted to the diagnostic process. When two possible sources of amyloid have been identified, patients should be referred to centers specializing in amyloidosis for further evaluation. The prognosis of AL amyloidosis has significantly improved in the last decade due to earlier diagnosis and more effective specific and supportive treatments. The median survival of patients with AL ranges from approximately 2 years to 3.9 years, depending in part on the treatment center and the nature of referral pattern. The median survival of 822 patients with AL amyloidosis followed in Pavia is 47 months. Recently we have investigated the factors affecting renal survival in patients with amyloid renal involvement: serum creatinine, proteinuria and young age at diagnosis predicted the progression to dialysis. Response to chemotherapy prolongs both renal and overall survival. The most frequent cause of death in these patients is progression of amyloid cardiomyopathy. Actually, most patients with AL amyloidosis die of cardiac complications (~ 75% in our 822 patient population), either congestive heart failure or sudden death. Cox multivariate analysis showed that the only 2 significant independent prognostic factors were response to therapy (protective), and cardiac involvement. Median survival of patients with heart involvement was significantly shorter than that of patients without cardiac amyloidosis (24 vs 81 months, $p<0.001$), and patients who obtained a hematologic response to chemotherapy survived longer than other patients (median 96 vs 20 months, $p<0.001$). Our recent data indicate that patients with cardiac AL amyloidosis who achieve hematologic response to chemotherapy have a better outcome than non-responsive patients (median survival 68 months vs 11 months, $p<0.001$) irrespective of the severity of heart involvement at diagnosis. Elevated serum cardiac troponins are related to poor prognosis in AL patients[6] and our group reported that the serum N-terminal portion of natriuretic peptide type B (NT-proBNP) is a sensitive marker of myocardial dysfunction in AL and a powerful prognostic determinant.[7] These two cardiac biomarkers were used to develop a reliable staging system for AL patients that can be used to stratify patients in randomized clinical trials and to compare outcomes between therapeutic interventions when randomized clinical trials are not available.[8] NT-proBNP clearance relies almost exclusively on glomerular filtration while natriuretic peptide type B (BNP) is eliminated from plasma through both glomerular filtration and clearance receptors that promote its degradation. Therefore, it is likely that BNP will prove to be a more reliable marker of cardiac dysfunction than NT-proBNP in AL patients with advanced renal disease. In most of the patients, the reduction of the circulating FLC concentration induced by chemotherapy translates into a reduction of serum NT-proBNP level and improving of heart failure, often before any reduction in amyloid load can be demonstrated at echocardiography.[9] This observation indicates that serum NT-proBNP can be used as a marker of cardiac response to therapy. It has

been reported that normalization of FLC levels after peripheral blood stem cell transplantation predicted both complete hematologic response and organ response.[10] The concurrent quantification of the FLC and of NT-proBNP in patients with cardiac amyloidosis allows titration of the anticlone treatment improving the toxicity-benefit ratio and allowing a prompt change of therapy in the case of an inadequate response.

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S10.4

AL AMYLOIDOSIS: RESPONSE, ASSESSMENT, AND TREATMENT

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The ability to accurately assess the response to treatment in AL is critical, since without a consistent method, it next to impossible to assess the efficacy of competing therapies currently used for this disease. Radiolabeled amyloid P component with iodine 123 or iodine 131 is a useful imaging agent for detecting amyloid deposits. Serialized scans have been used to assess the response to therapy and have demonstrated regression of established amyloid deposits after successful interruption of immunoglobulin light chain production. The SAP scan does not distinguish AL from other forms of amyloidosis, but imaging will detect deposits in the spleen, liver, and kidneys in 87%, 60% and 25% of patients, respectively. Because of the cardiac blood pool, the SAP scan is not generally useful in detecting myocardial amyloid deposits and is usually used in conjunction with echocardiography. Plasma clearance of radiolabeled amyloid P component has been shown to correlate with survival. Rapid plasma clearance is associated with a high body burden of amyloid. Imaging studies do not correlate well with the clinical degree of organ dysfunction. As an example, hepatic involvement with an SAP component scan is common, but palpable hepatomegaly in AL is only seen in approximately 15% of patients.[1] Recently, magnetic resonance imaging has been used in an attempt to further define myocardial involvement with amyloid. The characteristic features of cardiac amyloidosis by magnetic resonance imaging include impaired biventricular systolic function, thickened atrioventricular valves, bi-atrial enlargement, increased atrioseptal thickness, and increased left ventricular mass.[2] It is also presumed that serialized MRIs may be useful in monitoring the response in AL. The immunoglobulin free light chain assay has been found to be important in both classifying the type of amyloidosis and providing a method for assessing response. It has also been found to be of prognostic value. The free light chain assay has been incorporated into the response criteria, and its high sensitivity has improved the ability to monitor the status of patients with AL. The absolute level of free light chain achieved after therapy predicts survival. Normalization of free light chain levels after transplantation predicted both organ response and complete hematologic response. In this study, the percent free light chain reduction did not predict for survival, but the absolute level of free light chain achieved after therapy did.[3] As in many malignancies, response to treatment has a profound effect on survival. Hematologic responders have superior survival to nonresponders, even in a landmark analysis to correct for early mortality. Organ responses, however, are time-dependent and renal responses have a median time to response of a year and can be delayed up to 36 months after transplantation. Hematologic responses can be seen in as many as two-thirds of patients with complete hematologic responses in a third. The definition of a complete hematologic response requires a negative immunofixation of serum and of urine as well as an immunoglobulin free light chain ratio that is in the normal range. A partial hematologic response requires a 50% reduction of a serum and urine M protein, if they are measurable, and a 50% reduction in the level of the abnormal free light chain, whether it be kappa or lambda. Response rates appear to be dependent on the dose of melphalan administered as part of conditioning. At 200 mg/m^2, complete hematologic response rates are seen in 55%; and at lower doses in only 35%.[4] Response to therapy in patients with AL can be defined either by improved organ function or by hematologic responses comparable to those seen in patients with myeloma. All patients who have a measurable serum or urine monoclonal protein should be monitored for changes in the size of the peak after a therapeutic intervention. For patients who only have a free light chain, the nephelometric free light chain assay is essential in monitoring for a decrease in circulating free light chain levels. In one study,[5] chemotherapy resulted in a significant reduction in amyloidogenic free light chains, and patients with a normalized kappa to lambda ratio had an improved prognosis. Free light chain responses also seemed to parallel decreases in the NT-pro-BNP levels in cardiac responders to therapy. For patients in whom free light chains decreased by more than 50%, the NT-pro-BNP concentration decreased by a median of 48%, whereas in patients without a free light chain decrease, the NT-pro-BNP concentration increased by 47%.[6] The NT-pro-BNP decrease was greater in complete responders than partial responders. A decrease in circulating free light chains and NT-pro-BNP level translates into improved survival. Organ-based response criteria have been defined for patients with amyloidosis and reported in a consensus paper. Fundamentally, for renal amyloid, a 50% decrease in 24-hour urine albumin excretion is required, for hepatic involvement a 50% reduction in an increased serum alkaline phosphatase concentration is required. Echocardiographic response and progression of amyloid is difficult to assess because of variability in estimates of the septal wall thickness. Neurologic responses, although uncommon, can be documented by electromyography. Achievement of a hematologic response is an important predictor of prolonged survival after high-dose therapy for patients with AL. The degree of response is important because those people who achieve a complete response have a better survival than those who achieve a >50% reduction in light chain, and both groups do better than patients who fail to achieve a 50% reduction. The hematologic response is a good surrogate marker for survival. One unanswered question is whether patients who do not achieve a complete response should receive some form of maintenance or consolidation chemotherapy to try and further depress their light chain levels. In a study from the National Amyloidosis Center in Great Britain,[7] free light chain concentrations were measured in 262 patients, 137 who received cytotoxic chemotherapy. The five-year survival in patients who had a 50% reduction was 88%. Those who did not have a 50% reduction had a five-year survival of only 39%. This suggests that a 50% reduction in the urinary protein is an important and valid endpoint in assessing outcome. Sanchorawala [8] and colleagues assessed serum free light chain responses after high-dose melphalan and autologous stem cell transplantation. A complete response rate of 41% was seen. In this study, if the free light chain concentration decreased by more than 90%, the likelihood of clinical improvement was greater and longer survival was noted regardless of whether the patient fulfilled strict complete response criteria. In a review of autologous stem cell transplantation from the UK, a reduction in the serum free light chain proteins was seen in >50% in 83% of evaluable patients. This hematologic response translated to an overall median survival of 8.5 years for those patients who survived over 100 days.[9]

Therapy

A controversy in the treatment of AL is the role of stem cell transplant compared to conventional chemotherapy to suppress the amyloidogenic light chain. The Italian Amyloidosis Treatment Group reported the use of melphalan 0.22 mg/kg and dexamethasone 40 mg both for four days every 28 days. Forty-six patients were treated. The response rate was 67%, 33% complete responses, 48% organ responses, only two treatment-related deaths in the first 100 days, and a projected median survival of 5.1 years. The IFM presented a small phase 3 study at the American

Society of Hematology in 2005 randomizing patients to this regimen or to high-dose therapy with stem cell transplant. The complete response rate, objective response rate, organ response rate, and overall survival were no different between the two groups. The combination of thalidomide with dexamethasone was reported in 31 patients to produce a 48% hematologic response rate and a 26% organ response rate. The combination of cyclophosphamide, thalidomide, and reduced dose dexamethasone has been reported in 75 patients to produce a hematologic response of 75% and an organ response of 21 to 27%. Lenalidomide, when combined with dexamethasone, has been reported to be active in amyloidosis, producing hematologic responses in 60% and organ responses in 30%. The median time on therapy is 5.3 months. Bortezomib appears to be active in amyloidosis. When 18 patients were treated, hematologic response was seen in 77%, complete in 16%, and organ responses in 27%. Transplantation for amyloidosis has been used because it is efficacious in the treatment of multiple myeloma, and AL patients have a low tumor mass. Stringent patient selection is required, and only 25% of patients seen at Mayo Clinic are eligible for high-dose therapy. Of 270 transplanted patients, 94 achieved a complete response and 105 a partial response. Median survival is 80 months. The chemotherapy dose has an impact on survival. Patients receiving higher-dose chemotherapy tend to have higher response rates. Patients with three or more organs involved have a treatment-related mortality that approaches 30%. When the BNP is >170 picograms/mL pretransplant, the median survival is significantly shorter at 25 months.[10] Mayo 100-day mortality is 11% but has fallen to 8% in calendar year 2006. Optimal therapy for AL has yet to be defined.

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S10.5

THE ROLE OF HIGH-DOSE THERAPY IN AL-AMYLOIDOSIS

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Systemic light-chain (AL-)amyloidosis is treated by eliminating the clonal cells producing the free light chains (FLC) that cause the disease, allowing patients to survive and amyloid organ damage to stabilize or improve. Only one-third of newly diagnosed untreated patients with systemic AL-amyloidosis have sufficient organ reserves to tolerate high-dose melphalan with stem cell transplant (SCT). Post-SCT, two-thirds of patients experience at least a partial hematologic response (a 50% reduction in clonal plasma cells) but only half of the responders achieve a complete hematologic response, the optimal outcome with respect to survival and organ recovery. The remaining one third of patients do not respond and are treatment failures with a poor prognosis. Considering that the clonal plasma cells in AL-amyloidosis are indolent and non-proliferative, lack of response is less surprising than the fact that 30% of patients achieve complete hematologic responses. How melphalan is so effective is unclear. The early experience with SCT in AL-amyloidosis was characterized by a 20% to 40% treatment-related mortality due to toxicities such as sudden cardiac death, intractable hypotension and massive gastrointestinal hemorrhage.[1] Improved patient selection and risk-adapted melphalan dosing have reduced treatment-related mortality to less than 5% at our center and elsewhere. Risk-adapted melphalan dosing aims to reduce treatment-related mortality, and is based on observed differences in melphalan-related toxicity and age-related survival in patients with AL-amyloidosis. This approach allows SCT to be a treatment platform not a high-risk strategy. Recently, we combined the platform of risk-adapted melphalan and SCT with adjuvant therapy (thalidomide and dexamethasone) in those failing to achieve a complete hematologic response post-SCT.[2] We are now evaluating the same approach with adjuvant bortezomib and dexamethasone. In order to appreciate the role of high-dose melphalan and SCT in the treatment of systemic AL-amyloidosis one must consider the low-intensity therapies and the importance of patient selection. The goal remains eliminating the clonal plasma cells and FLC and supporting the patient over the time needed to achieve a hematologic response and organ stabilization or improvement. The conundrum of low-intensity therapy is that, though usually well tolerated, it is often ineffective because progression of amyloid organ-disease continues. Monthly oral melphalan and prednisone was the first therapy to show benefit in phase III trials.[3] Median survival was prolonged from 8 to 18 months and for the 20% who responded and survived more than 3.5 years there was a 20% incidence of myelodysplasia creating a risk of secondary leukemia. A multi-center phase II trial testing pulse dexamethasone followed by maintenance dexamethasone and alpha-interferon led to hematologic responses in 53% of patients; 24% achieved a complete response and median survival was 31 months.[4] In a phase II trial using monthly oral melphalan and dexamethasone, the response rate was 67% with 33% complete responses.[5] There were 2 treatment-related deaths in the first 100 days of therapy and 1 patient subsequently developed myelodysplasia but median survival exceeded 4 years. Many consider oral melphalan and dexamethasone the standard therapy for non-SCT patients. The combination is easily administered, usually for 6 to 12 months depending on the response of the FLC, and is similar to melphalan-based SCT except for the risk of myelodysplasia and secondary leukemia. In those failing melphalan and dexamethasone, clinical trials using bortezomib or lenalidomide should be sought; both agents are active. Thalidomide is active but difficult for patients to tolerate.[6] The development of risk-adapted melphalan dosing and the application of SCT as initial therapy were both based on clinical trials. In the largest phase II clinical trial of high-dose melphalan and SCT, untreated patients enrolled within a year of diagnosis were stratified and randomized to initial SCT or SCT after 2 cycles of oral melphalan and prednisone.[7] The 100-day treatment-related mortality was 20% and 12% died in association with stem cell mobilization. At 5 years the overall survival was 50% for immediate and 39% for delayed SCT. Fewer patients randomized to initial oral therapy underwent SCT due to progression of disease; this affected patients with cardiac involvement disproportionately. Post-SCT survival was a function of the number of affected organ systems and the presence of cardiac involvement. With 4 years follow-up, median survival had not been reached for patients with either 1 or 2 major organ systems involved (of heart, kidneys, liver/GI tract and peripheral nervous system) or no symptomatic cardiac involvement,. In contrast, for those with ≥ 3 organs involved or cardiac involvement, median survivals ranged from 5 to 10 months highlighting the importance of patient selection. Patients at high risk of dying in SCT were those with symptomatic 3- or 4-organ system involvement or cardiac amyloid associated with recurrent pleural effusions, cardiac syncope or symptomatic arrhythmias. In a multicenter randomized prospective phase III trial, SCT was compared with oral melphalan and dexamethasone.[8] Comparisons of response rates and survival between those alive at least 3 months post-SCT and those who completed at least 3 months of oral melphalan and dexamethasone showed no difference. For both groups the hematologic response rates were 65%. Median survival was 48 months for SCT and 58 months for oral therapy. Surprisingly no cases of myelodysplasia were reported in the oral melphalan group. This phase III trial did not clearly define a standard therapy for AL. However, a case-cohort analysis has shown a survival advantage in good performance-status patients treated with SCT and quality of life improves in patients who respond to transplant.[9,10] The difference between 12 or 18 months of oral melphalan and dexametha-

sone and high-dose melpahlan with SCT is that, as in myeloma, transplant is not a final therapy but rather a platform for therapy with low risk of myelodysplasia. It is a useful initial therapy to which adjuvant treatments can be added in order to improve response rates. In a phase II trial we tested the combined approach of SCT and adjuvant thalidomide and dexamethasone.[2] Patients received SCT with risk-adapted melphalan dosing, and those not achieving a hematologic complete response at 3 months post-SCT received 9 months of thalidomide (50-200 mg nightly) and dexamethasone (20 mg/m^2, 1-3 pulses monthly). Treatment-related mortality was 4.4% (2 of 45 patients) and at 3 months post-SCT 61% of patients had hematologic responses. Nearly half of those on adjuvant therapy had improved responses at 12 months including 6 who achieved complete responses. The response rate at 12 months was 77% with 38% complete responses; there was no difference in response rate based on the dose of melphalan. With a median follow-up of 29 months, median survival has not been reached. Further study of such combined approaches is warranted, employing SCT as a platform for therapy. Blood stem cells have been mobilized with granulocyte colony-stimulating factor (G-CSF) and rare deaths have been reported during mobilization in patients with symptomatic cardiac amyloid or multisystem disease. Catastrophic complications though rare include a pulmonary syndrome associated with hypoxia and rupture of the spleen requiring emergent surgery. Currently we recommend that G-CSF be given at 6 mcg/kg every 12 hours with collection beginning on day 5, and employ in-hospital monitored mobilization for patients at risk of hypoxia, hypotension or syncope. In patients achieving complete or near complete hematologic responses, organ recovery can be variable. The liver can regenerate, regress to normal size and regain normal function. Peripheral and autonomic nervous system involvement can be reversed. Proteinuria can decline dramatically over months and years but creatinine clearance rarely improves and the kidneys remain at risk from non-amyloid insults. Recovery from cardiac involvement remains problematic. Only 20% of cardiac patients with hematologic responses show objective improvement by echocardiogram. With serial studies showing brain natriuretic peptide (BNP) decline in conjunction with FLC response, we now know that less injury or strain occurs as the precursor protein is eliminated. Despite complete hematologic responses, patients with cardiac amyloid can experience cardiac dysfunction and sudden arrhythmic events. The utility of low-dose anti-arrhythmic agents or anticoagulation in the management of cardiac patients remains to be prospectively defined. High-dose melphalan with SCT for AL-amyloidosis is effective in patients with limited disease when they are treated at centers with low treatment-related mortality. It provides a platform for testing novel adjuvant therapies post-SCT with the goal of maximizing the complete hematologic response rate. Its role may change if outcomes with low-intensity therapies improve as novel agents are tested in combination with traditional ones such as cyclophosphamide, melphalan and dexamethasone. The development of anti-plasma cell monoclonal antibody therapy will also have a major impact on treatment strategies and patient outcomes.[11] Patients with systemic AL-amyloidosis should be treated whenever possible on clinical trials in order to advance our understanding and management of the disease.

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S10.6

LIGHT CHAIN DEPOSITION DISEASE

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Light chain deposition disease (LCDD) is a plasma cell dyscrasia characterized by non-amyloid deposits in various organs. It is the most common form of monoclonal immunoglobulin deposition disease which includes light heavy chain deposition disease (LHCDD) and heavy chain deposition disease (HCDD). Light chain deposition disease was first described by Randall *et al.* in 1976 but non-amyloidotic kidney disease resembling diabetes had been reported since the 1950's.[1] The true incidence of LCDD is unknown. Autopsy data of myeloma patients suggest the rate of LCDD is approximately 5%.[2] This is compared to 9% for AL amyloidosis and 32% for cast nephropathy in the same study. The rate appears to be much higher amongst patients with a monoclonal gammopathy who underwent a kidney biopsy. In this study, LCDD was found in 11.6% and LHCDD in 4.1%.[3] Rates of cast nephropathy and AL amyloidosis were found in only 10.7% of the patients. Light chain deposition disease occurs in the sixth decade of life. The age however ranges from 28 to 94 years.[4] There may be a slightly higher incidence in males. Nearly every patient presents with renal manifestations. This includes renal insufficiency, proteinuria and hypertension. Extrarenal manifestation occurs in about 35% of the patients. Nearly every organ can be involved with the most commonly reported being the heart and liver. Lung, gut, peripheral nerves, autonomic nervous system, muscle, salivary gland, carpel tunnel and brain may become involved.[5] There is a definite predilection for kappa light chain in LCDD. Nearly 74% of patients with LCDD have a monoclonal kappa light chain. Proportion may be higher in smaller series. There also appears to be an overrepresentation of the kI subtype in this disease. Protein analysis revealed in increased hydrophobicity in these light chains.[6] One study suggests the mutations are at the somatic level and appears to be concentrated in the CDR regions of the gene. Many patients with LCDD also have multiple myeloma. The rate of myeloma varies from 37% to 65%.[4,7] This variation may explain the differences in life expectancy reported in these patients in the literature. The median survival varies from 18 months to over 5 years. The myeloma rate in the study with the longest survival was 37% compared with over 50% in studies with shorter median survivals. However, another study suggests the histologic pattern may be more important in determining survival. In this study, the presence of myeloma did not affect survival but patients who had LHCDD had a significantly shorter survival.[8] There is a great variation in the treatment of LCDD. Some patients with renal limited disease without myeloma may not receive any disease directed therapy. Others are given steroids and cytotoxic agents similar to those used in the treatment of multiple myeloma with varying success. One study suggests those who received vincristine-doxorubicin-dexamethasone/ methylprednisolone may have a better outcome. These patients were also more likely to have multiple myeloma.[4] High dose melphalan with stem cell rescue has also been used in these patients. The experience has been small but so far appears to be beneficial with a low treatment mortality rate. Royer *et al.* reported their experience with 11 patients, 10 of whom had multiple myeloma.[9] After stem cell transplant, 6 patients achieved hematologic complete response and 2 had very good partial response. Responders were also noted to have organ response including improvement in heart, liver and kidney function. Three patients relapsed after stem cell transplant and 1 died as a result of progressive myeloma. Boston University reported their experience with stem cell transplant in 6 LCDD patients without myeloma.[10] Complete

hematologic response was achieved in 86%. All had normalized their serum free light chain levels. All were alive at a median follow-up of 12 months. It appears that high dose melphalan followed by autologous stem cell transplant may be the treatment of choice for these patients regardless of their myeloma status. It provides the patient with the highest chance of achieving hematologic CR with a low treatment related mortality rate.

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S10.7

WHAT'S NEW ABOUT THE POEMS SYNDROME?

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POEMS syndrome is defined by the presence of a peripheral neuropathy (P), a monoclonal plasma cell disorder (M), and other paraneoplastic features, the most common of which include organomegaly (O), endocrinopathy (E), skin changes (S), papilledema, edema, effusions, ascites, and thrombocytosis.[1,2] Virtually all patients will have either at least one sclerotic bone lesion, an elevation in plasma levels of vascular endothelial growth factor (VEGF), or co-existent Castleman's disease. Not all features of the disease are required to make the diagnosis, and early recognition is important to reduce morbidity. Other names for the syndrome include osteosclerotic myeloma, Crow-Fukase Syndrome, or Takatsuki syndrome. Though the pathophysiologic mechanism is not well understood, there is a correlation between treating the underlying clonal plasmaproliferative disorder and clinical improvement. This observation clearly links the plasma cell clone to the peripheral neuropathy and other clinical features, though the mechanism is not yet fully elucidated. Pro-angiogenic and pro-inflammatory cytokines have been shown to track with disease course, and VEGF is considered to be the best putative candidate for underlying pathogenesis.[3,4] Emerging cytokine and molecular data, including cytogenetic findings will be reviewed.[5] Because a progressive neuropathy is often the dominant feature of the disease, patients are often labeled as having chronic inflammatory demyelinating polyneuropathy (CIDP). Clues that differentiate CIDP from POEMS syndrome are the presence of other paraneoplastic features of the acronym/syndrome and a lack of response to standard CIDP therapies, i.e. intravenous gammaglobulin and plasmapheresis. Instead, the mainstays of therapy for patients with POEMS include irradiation, corticosteroids, and alkylator-based therapy, including high dose chemotherapy with peripheral blood stem cell transplant.[6] Data on novel therapies like bevacizumab and immunomodulatory therapies will be discussed.[7]

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S10.8

PARAPROTEINEMIA RELATED NEUROPATHIES

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Introduction. Even if the association of neuropathy with monoclonal gammopathy has been known for several years, the clinical and pathogenetic relevance of this association as well as its therapeutical implication are not completely established. This is not a marginal problem as: a) monoclonal gammopathy, which in 75% of cases are or of undetermined significance and therefore asymptomatic not requiring *per se* any treatment, can be found in 1% of the population above 50 years and in 3% of those above 70 years, and b) a symptomatic neuropathy can be found in at least 8% of patients with monoclonal gammopathy, so that the prevalence of this neuropathy in the population above 50 years may be of 80 per 100.000, representing, after diabetes, one of the leading causes of neuropathy in aged people. Monoclonal gammopathy may result from malignant lymphoproliferative diseases including multiple myeloma or solitary plasmocytoma, Waldenstrom's macroglobulinemia (WM), other IgM secreting lymphoma or chronic lymphocytic leukaemia, as well as from primary amyloidosis (AL) and cryoglobulinemia. In most instances monoclonal gammopathy is not associated with any of the above mentioned disorders and is *per se* totally asymptomatic, being named benign monoclonal gammopathy or, more appropriately, monoclonal gammopathy of undetermined significance (MGUS) for the possible, though infrequent (approximately 1% per year), evolution into malignant forms (Kyle & Rajkumar 2003). In these patients neuropathy is often the only clinical manifestation of the underlying haematological disorders. The prevalence of a symptomatic neuropathy in patients with monoclonal gammopathy varies according to the haematological diseases and, for the same disease, from series to series, depending on the criteria used to define the presence of neuropathy. In two large series of patients with MGUS for instance, the prevalence of a symptomatic neuropathy ranged from 8% to 36% of patients being significantly higher in patients with IgM than with IgG or IgA MGUS, reinforcing the hypothesis, at least for IgM monoclonal gammopathy, of a possible pathogenetic role of IgM M-proteins in the neuropathy. This issue has bee recently reviewed by a panel of experts (Hadden *et al.* 2006).

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 of them have however an IgM MGUS whose only clinical manifestation is the neuropathy leading a panel of haematologist to include them in a clinically distinct group that they proposed to name IgM- related disorders (Owen *et al.* 2003). Different forms of neuropathies have been associated with IgM monoclonal gammopathy, possibly reflecting the different mechanisms involved in their pathogenesis (Nobile-Orazio 1998): cranial nerve palsies, mononeuropathies or mononeuritis multiplex have been reported in WM and lymphoma and were related to lymphoplasmacytic infiltration of nerves, amyloid deposition, cryoglobulinemic vasculitis or microangiopathy of

endoneurial vessels. The vast majority of these patients, as well as of those with IgM MGUS, have however a chronic progressive, symmetric and predominantly distal neuropathy which was occasionally related to endoneurial accumulation of the M-protein, or diffuse microangiopathy but most frequently to a reac¬tivity of the M-protein with a number of neural antigens including MAG, cytoskeletal proteins, chondroitin sulfate C, sulfatide and several gangliosides. Overall these reactivities are found in at least two thirds of patients with neuropathy and IgM monoclonal gammopathy being more frequent in MGUS (84%) than WM (38%) (Nobile-Orazio *et al*. 1998). Some of these IgM reactivities have been associated with homogeneous neuropathy features, which will be here briefly reviewed. *Neuropathy associated with anti-MAG IgM*. In almost 50% of patients with neuropathy associated with IgM monoclonal gammopathy the M-protein react with MAG and other nerve glycoconjugates sharing with MAG the HNK-1 carbohydrate epitope (Nobile-Orazio *et al.* 1998). Almost 80% of patients with anti-MAG IgM have IgM MGUS while most remaining patients have an otherwise asymptomatic WM. The neuropathy in patients with high anti-MAG IgM antibodies is quite homogeneous, mostly affecting men in the sixties or seventies. The neuropathy is characterized by a distal and symmetric, predominantly deep sensory involvement, with gait ataxia and postural tremor in the upper limbs. Motor impairment is usually less prominent and often appears later. The neuropathy usually runs a slowly progressive course with most of the patients having a long-term favourable functional prognosis with only a minority of them becoming disabled after several years (Nobile-Orazio *et al.* 2000). Electrophysiological and morphological studies are consistent with a demyelinating neuropathy. Several data support the pathogenetic role of anti-MAG IgM in the neuropathy: 1) high titres of anti-MAG IgM antibodies are almost invariably associated with an homogeneous clinical pattern and predict the development of neuropathy in asymptomatic patients with IgM monoclonal gammopathy; 2) pathological studies on nerve biopsies show segmental demyelination with abnormally spaced myelin lamellae and deposits of IgM M-protein and complement on nerve myelin, i.e. the target of the anti-neural reactivity; 3) therapeutical reduction of anti-MAG IgM most often correlates with clinical improvement; 4) complement mediated demyelination of nerve has been experimentally induced in animals by intraneural or systemic injection of anti-MAG IgM M-proteins. Several therapies directed at reducing the presumably pathogenic IgM paraprotein or B-cell clone have been used in these patients, including steroids, plasma exchange, cytotoxic agents, high-dose intravenous immunoglobulin (IVIg) and interferon-α Even if almost 50% of patients have been reported to improve, at least temporarily, after one of more of these therapies' their effect on the long-term prognosis of the neuropathy remains unclear as in only few studies the follow-up exceeded two years. This data would be particularly important in consideration of the usually slow progression and relatively favourable prognosis of the neuropathy associated with anti-MAG IgM, and the frequent adverse effects of most of these therapies. In addition very few controlled trials have been performed in these patients with only one showing a modest short-term efficacy of IVIg so that there is insufficient evidence to recommend any particular immunotherapy in this neuropathy (Lunn *et al.* 2003). The preliminary positive effect on the neuropathy reported with the humanised monoclonal antibody (Rituximab) directed against the CD20 antigen on B-lymphocytes has been now confirmed by a randomized trial (Dalakas *et al.* 2006). *Neuropathy with other anti-nerve reactivities*. Several other anti-neural reactivities of IgM M-proteins have been reported in patients with IgM related neuropathies. Anti-sulfatide IgM have been reported in several patients with neuropathy, half of whom had IgM monoclonal gammopathy, and were initially associated with chronic progressive, predominantly sensory axonal neuropathy or with painful small fiber neuropathy with normal electrophysiological studies, while in subsequent reports this reactivity has been associated with sensorimotor demyelinating neuropathy. Morphological studies on sural nerve biopsy showed in some patients deposits of the M-protein and comple¬ment. Few data are available on the clinical response to treatment in these patients so that their strict association with a dysimmune neuropathy mainly supports the possible pathogenetic relevance of these antibodies. IgM reactivity with the ganglioside *GM1* has been originally reported in patients with IgM monoclonal gammopathy and a peculiar neuropathy named multifocal motor neuropathy, even if the vast majority of subsequently reported patients with these antibodies did not to have an IgM monoclonal gammopathy. More recently a number of patients have been reported with neuropathy associated with an IgM monoclonal gammopathy reacting with *gangliosides containing disialosyl groups including GQ1b, GD1b, GT1b, GD3 and GD2* (Willison *et al.* (2001). Most of these patients had a chronic sensory neuropathy with prominent ataxia, usually mild or no weakness, recurrent ophthalmoplegia and cold agglutinin activity of the M-protein that often bind to the Pr2 antigen on red cell membranes. Willison proposed for this syndrome the acronym CANOMAD (Chronic Ataxic Neuropathy with Ophthalmoplegia, M-protein, cold Agglutinins and anti-Disyalosil antibodies). In most of these patients electrophysiological and morphological studies were consistent with a demyelinating process. In none of them myelin deposits of the M-protein were found in sural nerve, but in one inflammatory cells infiltrates were found reminiscent of CIDP. This may explain the reported improvement of some of these patients after IVIg therapy. Monoclonal IgM reactivities with other gangliosides have been occasionally reported in these patients including *GD1a*, *GM2* and *GD1b*. The possible pathogenetic and clinical relevance of these and other even less frequent IgM reactivities remains unclear as in none of these patients IgM deposits were found in sural nerves and little is known on their response to immune therapies. At the same time the very small number or reported observations does not permit to clarify the clinical phenotype of these reactivities. *Neuropathy with IgM not reacting with neural antigens*. In approximately one third of the patients with neuropathy associated with IgM monoclonal gammopathy no reactivity of the M- protein with any of the above mentioned nerve antigens could be detected. This is particularly true for patients with *WM or lymphoma*, two thirds of whom have no detectable reactivity. Several other mechanisms have been implicated in the pathogenesis of the neuropathy in this group of patients including vasculitis of vasa nervorum or intravasal precipita¬tion of immunoglobulins when the IgM M-protein is a cryoglobulin, direct lymphoplasmacytic infiltration of nerves, hyper viscosity, microangiopathy of vasa nervorum, endoneurial accumulation of the M-protein or amyloidosis. As already mentioned, these mechanisms are often associated with a mononeuropathy or mononeuritis multiplex, but can also underlie a symmetric polyneuropathy. Whatever is the mechanism for the neuropathy in this heterogeneous group, this is also often strictly related to the M-protein or to its producing cells explaining why treatment directed at reducing IgM M-protein production may result in clinical improvement. Since a malignant lymphoproliferative disease with concomitant life-threatening extra-neurological impairment affects most of these patients, an aggressive chemotherapy under the supervision of competent haematologists is usually required.

Neuropathy and IgG monoclonal gammopathy

While neuropathy associated with IgM monoclonal gammopathy is well characterised, less clear is the relationship between the neuropathy and IgG M-protein. Some patients have *multiple myeloma* in which neuropathy is occasionally the presenting symptom but more frequently occurs in patients with an established disease, with a prevalence of 3% to 13%. The neuropathy associated with multiple myeloma is clinically heterogeneous (Kelly 1998) probably reflecting the presence of different pathogenetic mechanism. In approximately half of the patients the neuropathy is caused by light chain amyloidosis with a predominantly sensory distal impairment, postural hypotension and other signs of autonomic impairment, and is often associated with signs of systemic amyloidosis including malabsorbtion, cardiac and renal dysfunction. Nerve or nerve root direct infiltration by myeloma or compression by bone lesions cause an asymmetric mono or multineuropathy or radiculopathy often characterized by excruciating pain. Neuropathy also occurs as a complication of therapy used in myeloma including vincristine, thalidomide and, more recently, bortezomib. More typical are the features of the neuropathy associated with *osteosclerotic myeloma* where neuropathy is found in up to 50% of the patients and where it is often the presenting symptom of the disease. These patients often have a severely disabling predominantly motor demyelinating neuropathy frequently starting with sensory symptoms. This neuropathy is sometime associated with other non-neurological manifestations including, organomegaly, endocrinopathy, lymphoadenopathy, ascites, peripheral oedema and a very typical brown reddish *tanned* colour of the skin. This constellation of symptoms has been collected under the eponym of POEMS (*P*olyneuropathy, *O*rganomegaly, *E*ndocrinopathy, *M*-protein and *S*kin changes) and has been occasionally reported also in patients with non-malignant gammopathies (Dispenzieri *et al.* 2003). The clinical and pathogenetic relevance of this association is supported by the improvement of the neuropathy observed in more than half of the patients who respond to the treatment of the osteosclerotic lesion(s) which include local radiotherapy or resection of the tumor and a variable combination of steroids and melphalan, and more recently autologous peripheral blood stem cell transplantation (Dispenzieri *et al.* 2004). The majority of patients with neuropathy and IgG M-protein have an IgG MGUS, which is found during the work-up or the follow-up of the neuropathy. The prevalence of a symptomatic neuropathy is however lower (3%) than IgM MGUS (15%) (Nobile-Orazio *et al.* 2002), possibly explaining the lower representation of IgG in large series of patients with neuropathy and MGUS. Several forms of neuropathy have been associated with IgG MGUS even

if in more recent studies, almost half of the patients had a chronic demyelinating neuropathy clinically and therapeutically indistinguishable from chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) (Hadden *et al.* 2006) while the remaining had a predominantly sensory axonal or mixed neuropathy. The possible pathogenetic role of IgG M-proteins in the neuropathy remains however unclear as in very few patients reactivity of IgG M-proteins with neural antigens or endoneurial deposits of IgG have been reported, while in over 50% of them the M-protein become manifest several months to years after onset of the neuropathy. Still the improvement observed with immunotherapy not only in patients with a CIDP-like neuropathy but also in some of those with an axonal neuropathy suggest that even if the presence of IgG MGUS might not be primarily pathogenetic, its finding may represent a marker of a possibly dysimmune origin of the neuropathy.

Neuropathy and IgA monoclonal gammopathy

Only few patients with neuropathy and IgA monoclonal gammopathy have been reported representing in most large of patients with neuropathy and monoclonal gammopathy a very small proportion of the patients. Some of these patients have myeloma or a POEMS syndrome (see above) while a few of them had IgA MGUS. The clinical and electrophysiological features of the neuropathy in these patients are quite heterogeneous (Nobile-Orazio *et al.* 2002) making it impossible to identify a prevailing type of presentation except that the neuropathy was chronic progressive in all but one patient who had an acute onset. As in the case of neuropathy associated with IgG MGUS there is little evidence that IgA M-proteins have a primary pathogenic role in the neuropathy since antineural reactivity or endoneurial deposits of IgA M-proteins have been occasionally reported. Few patients have been reported to improve with immune therapies but their limited number and the consequent elevated risk of a publication bias are not sufficient to justify the assumption that the identification of an IgA M-protein reveal a dysimmune origin of the neuropathy which might benefit of immune therapies.

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S11: Oral presentations III

S11.1

HIGH-RESOLUTION MAPPING OF COMMON GAINS AND LOSSES IN MYELOMA

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Introduction. To identify novel genetic factors contributing to the pathogenesis and prognosis of multiple myeloma (MM) we have initiated a comprehensive genomic screen using array-based comparative genomic hybridization (aCGH). *Materials and Methods*. Genomic copy number changes were assessed in 68 MM samples using the Agilent Human Genome 44B microarray (Agilent Technologies). We focused on defining common breakpoint regions and minimally deleted and amplified regions, MDR and MAR respectively, which are not well defined in MM. Genes contained within the MDR and MAR were cross-referenced with a list of ~2100 genes mutated and implicated in neoplasia. *Results*. A large number of recurrent breakpoints and copy number gains/losses were identified. Known aberrations such as the recurrent trisomies, 1p loss, 1q gain and 13 monosomy were observed and agrees with existing FISH data on these patients. We identified 35 recurrent breakpoints that either occurred within a specific genomic window of ~35 kb or breakpoints that occurred within a specific gene. Six of the identified breakpoints occurred in more than 4 patients and one was present in 6 patients. We identified 99 minimal regions of copy number change that encompassed 352 genes implicated in cancer. Interestingly, we identified nearly equal numbers of MDR and MAR, 49 and 50 respectively. The high-resolution of this aCGH platform allowed the identification of 43 recurrent regions of copy number change below 1.0 Mb. Furthermore, 24 are below 0.5 Mb and 9 are below 0.2 Mb. The number of aberrations per tumor ranged from 2 to 58. The presence of a complex genome (more than 20 aberrations) is associated with a worse prognosis (24 months versus 88 months, log-rank p=0.0016). This effect is not simply a dichotomy of hyper and non-hyperdiploid MM as the incidence of a complex genome was equally distributed between both subgroups. *Conclusions*. This study has identified a number of recurrent breakpoint regions and copy number changes that were not previously characterized. The high resolution of our aCGH platform has identified 24 recurrent gains or losses smaller than 1.0 Mb that contain genes previously implicated in cancer.

S11.2

MICROCHIPS FOR OPTIMIZED FISH SCREENING IN MYELOMA

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Introduction. Interphase fluorescence *in situ* hybridization (FISH) is a sensitive diagnostic tool used for the detection of chromosomal abnormalities on cell-by-cell basis, that can predict prognosis and response to therapy. However, the cost-per-test and the technical complexity of current FISH protocols has compromised its widespread utilization. Lab-on-a-chip devices miniaturize, integrate and automate conventional analytical techniques onto microfluidic platforms. Since microchannels permit sophisticated levels of fluid control, these devices can reduce analysis times, lower reagent consumption, and minimize human intervention. *Materials and methods*. We present both glass and PDMS microfluidic platforms that standardize much of the FISH protocol offering repeatable results that are accurate, cost-effective and easy to obtain in a clinical setting. Furthermore, we examine on-chip methods to enhance the hybridization portion of FISH; specifically, mechanical or electrokinetic pumping. To verify the robustness of our microchip FISH protocols, multiple probe and cell combinations were tested. *Results*. Compared to conventional methods, these first implementations of on-chip FISH provide a 10-fold higher throughput and a 10-fold reduction in the cost of testing, enabling the simultaneous assessment of several chromosomal abnormalities or patients. In addition, the two methods of on-chip agi-

tation improve the hybridization rate and are currently being optimized. We also demonstrate that the time limiting mechanisms during hybridization can be minimized even further using microchip methods. *Conclusions*. It is increasingly essential that diagnostic tests determine the type and extent of chromosomal abnormalities for more informed diagnosis and for appropriate choice of treatment strategies. On-chip FISH technology allows chromosomal analysis in hours as opposed to days. Further, the on-chip FISH technique introduced here was 10 times more cost-effective than conventional methods with the potential to be fully integrated and automated. This technology will make wide-spread genetic testing of myeloma patients more accessible in a clinical setting. This work was supported by the Natural Sciences and Engineering Research Council (NSERC), the Informatics Circle of Research Excellence (iCORE), the Alberta Ingenuity Fund, a Western Economic Diversification grant, and the Canadian Institutes of Health Research (CIHR).

S11.3

MOLECULAR PROGNOSIS IN MULTIPLE MYELOMA: THE IFM EXPERIENCE

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Introduction. Survival of patients with multiple myeloma is highly heterogeneous from periods of few weeks to more than ten years. We used functional genomics to develop a gene classifier of survival in these patients. *Materials and Methods*. Malignant plasma cells from 250 myeloma patients at diagnosis were examined for gene-expression profile. Supervised methods were used to identify individual genes that predicted survival based on their expression in a training group of 182 patients. The survival gene-predictor built from these genes was validated in an independent test group of 68 patients. *Results*. The 15 most stable genes associated with the length of survival were used to calculate a risk score and to stratify patients in low-risk, intermediate-risk and high-risk. The 15-gene classifier was highly predictive of survival in the training group (p<0.001) and in the test group (p<0.001). The Kaplan Meier estimates of rates of survival at 3 years were 95.1 percent (95 percent confidence interval, 88.4 to 97.9), 81.3 percent (95 percent confidence interval, 66.5 - 90.1) and 47.4 (95 percent confidence interval, 33.5 to 60.1) respectively in patients having a low, intermediate or high risk. In a multivariate Cox proportional–hazards analysis, the 15-gene classifier performed significantly better (p<0.0001) than serum beta2-microglobulin > 5.5 mg/L (p=0.03) while t(4;14) was not statistically significant. Combination of both independent parameters was very powerful to identify highest risk patients (20 out of 250) with a 3-year survival rate of 26.1 percent (95 percent confidence interval, 8.6 to 47.9). *Conclusions*. Gene-expression-based classification provides a robust and accurate tool to predict survival after high dose therapy for multiple myeloma. This method might serve for a more personalized treatment strategy.

S11.4

GENE EXPRESSION PROFILES AS PROGNOSTIC FACTORS FOR HIGH-DOSE THERAPY AND BORTEZOMIB IN PATIENTS WITH MULTIPLE MYELOMA

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Background. The standard treatment of newly diagnosed multiple myeloma (MM) is based on induction treatment followed by high-dose melphalan. CR/nCR percentages range from 20-50% with event free survival (EFS) ranging from 18 months to 28 months. The 5-year survival rates are 25% to 50%, however all patients eventually relapse and succumb to the disease. Classical unfavourable prognostic factors include high serum β2-microglobulin and chromosome aberrations such as-13/13q-, t(4;14) and t(14;16). Bortezomib, a proteasome inhibitor, and Thalidomide, an anti-angiogenic and immunomodulatory drug, have recently shown a remarkable effect in patients with relapsed or refractory MM with 30-40% response rates. In combination with Dexamethasone and/ or other conventional agents overall response rates of 50-70% can be achieved. In newly diagnosed patients the response rates vary from 70 - 85%. Moreover, Bortezomib was found to overcome poor prognostic factors like a high β2-microglobulin and/ or deletion of chromosome 13. However, 15-30% of newly diagnosed patients do not respond to Bortezomib or Thalidomide. Secondly, 30% of the patients treated with these novel agents have to stop prematurely because of intolerable side effects, such as polyneuropathy, thrombocytopenia, thrombosis and gastro-intestinal symptoms. *Aims.* In order to develop new, genetic prognostic factors for clinical response and toxicity associated with Bortezomib and Thalidomide, we have started to analyze gene expression profiles of myeloma specific genes in plasma cells purified from bone marrow from myeloma patients at diagnosis who have been treated in a prospective randomized trial, HOVON 65. This large multicenter, prospective, randomized phase III trial compares Bortezomib in combination with Adriamycin, Dexamethasone (PAD, arm A) followed by HDM followed by maintenance with Bortezomib vs. VAD (arm B) followed by HDM and maintenance with Thalidomide (HOVON65/GMMG-HD4). This cooperative trial in the Netherlands, Belgium and Germany has recruited over 400 patients since April 2005 and will include 800 patients. *Methods.* Gene expression profiling of CD138 magnetic cell selected (MACS) myeloma plasma cells were performed using Affymetrix GeneChip Human Genome U133 plus 2.0 arrays. Data obtained from micro-array studies were submitted to Cox regression analysis and multifactorial analysis with the clinical data set from these patients. *Results*. We will present an unsupervised cluster (SAM) analysis based on the array results from the first cohort of 130 patients. The analysis shows that the majority of cases can be identified according to the TC classification. The initial results of clinical outcome of these cases will be presented.

S11.5

COMPARTMENT-SPECIFIC BIOLUMINESCENCE IMAGING (CS-BLI): A HIGH-THROUGHPUT APPROACH TO IDENTIFY NOVEL ANTI-MYELOMA THERAPIES THAT OVERCOME THE PROTECTION OF STROMAL CELLS

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Introduction. The bone marrow (BM) microenvironment (including bone marrow stromal cells; BMSCs) attenuates multiple myeloma (MM) cell response to conventional treatments. Novel agents (*e.g.* thalidomide derivatives and proteasome inhibitors) are able to overcome this BM-derived protection and consecquently have better clinical activity in relapsed/refractory MM. MM-BMSCs interactions are therefore important for testing potential anti-MM compounds. However, conventional assays used in high-throughput drug screening (e.g MTT assays) are typically not amenable to co-cultures. Conversely, assays often applied in MM-stromal co-cultures (*e.g.* 3H-thymidine incorporation) have limitations that preclude their high-throughput application. *Materials/Methods/Results*. To address this void in anti-MM drug development, we established a compartment-specific bioluminescence imaging (CS-BLI) assay, where the tumor cell compartment (*e.g.* MM cells) is engineered to stably express luciferase (Luc) and can be co-cultured with Luc-negative accessory cells of the tumor milieu (*e.g.* BMSCs). Addition of luciferin to the culture generates bioluminescence signal directly proportional to number of viable Luc+ cells, thus allowing for selective and sensitive quantification of the viable MM tumor cell compartment. We established that CS-BLI exhibits high linear correlation (R2>0.99) between bioluminescent signal and detection of (as few as 1500) viable MM cells; provides sensitive and specific detection of MM cell viability both in the presence and absence of BMSCs; yields results consistent with conventional drug sensitivity assays both in the presence or absence of BMSCs. We applied CS-BLI with variety of permutations, including different tumors types (*e.g.* MM, leukemia, lymphoma and solid tumor cells) and accessory cells (different BMSC types, fibroblasts etc.). We confirmed that CS-BLI provides results consistent with prior data, i.e. that stroma protects MM cells from Dex, alkylators and anthracyclines, but not against bortezomib and hsp90 inhibitors and confirmed the feasibility of using CS-BLI in high-throughput formats. *Conclusions*. CS-BLI is able to overcome the key limitations that have precluded the establishment of high-throughput screening for testing new drugs in tumor-stroma co-cultures. Its application provides a powerful tool to identify new and, hopefully, more effective classes of drugs which are active despite the effects of the microenvironment.

S11.6

IN VITRO AND *IN VIVO* ANTI-MYELOMA ACTIVITY OF PRLX, AN ORALLY-BIOAVAILABLE AGENT AGAINST MUTANT RAS-TRANSFORMED CELLS

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Introduction. Ras mutations occur in 40-60% of multiple myeloma (MM) patients and are implicated in progression to advanced MM (including plasma cell leukemia/extramedullary lesions). The small molecule PRLX (Prolexys Pharmaceuticals) was identified in the context of *synthetic lethal* chemical screening for genotype-selective cytotoxicity against cells transformed with forced expression of mutant Ras (but not against isogenic normal cell counterparts) and was tested for possible anti-MM activity *in vitro* and *in vivo*. *Materials, Methods and Results*. MTT survival assays showed that 34 of 46 human MM cell lines (74%) responded to 48hr treatment with sub-uM PRLX concentrations (achievable in preclinical pharmacokinetic studies). (24 MM lines had IC50 values <300 nM). PRLX anti-MM activity compared favorably with its *in vitro* activity against other neoplasias (leukemias, lymphomas, and solid tumors) and was not restricted to cells with known Ras mutations. Importantly, PRLX was active against MM cells resistant to conventional (Dex, alkylators, anthracyclines) and/or novel (*e.g.* lenalidomide, CC-4047, bortezomib, multitargeted kinase inhibitors) anti-MM treatments. Cell death commitment assays revealed that a pharmacologically relevant 5hr pulse with 300 nM PRLX is sufficient to commit MM-1S, NCI-H929 and OPM-2 MM cells to cell death. Importantly, co-culture with BMSCs did not protect MM cells against PRLX (at doses non-toxic to BMSCs). Gene expression profiles (with Affymetrix U133 2.0plus oligonucleotide microarrays) showed early (<2hr) of PRLX-induced modulation of broad spectrum of genes involved in regulation of cellular bioenergetics. The *in vivo* anti-MM activity of PRLX was evaluated in SCID-beige mice sublethally irradiated with 300 rad, subsequently injected i.v. with 1×10^6 OPM-2 MM cells (which led to diffuse medullary and extramedullary lesions). Mice were randomly assigned to receive, by oral gavage, either PRLX 100 mg/kg (n=14) or vehicle only (n=14), on cyclical schedule of 5 days-on/2 days-off treatment. After 47 days of oral PRXL administration, median overall survival was not reached in PRLX-treated cohort (12/14 mice still alive) vs. 35 days (95% CI: 23-47 days) in the control group (0/14 alive at day 47) (Kaplan-Meier analysis, $p<0.0001$, log-rank test). *Conclusions*. PRLX represents a promising novel orally bioavailable agent that merits further evaluation for possible clinical trials for advanced MM patients.

S11.7

CATHEPSIN G IS UPREGULATED IN PATIENTS TREATED WITH IMIDS

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Introduction. Despite impressive response rates induced by IMiDs in patients with multiple myeloma, one of the major side effects of this therapy is the increased risk of thromboembolic events (TEE). Cathepsin G is a serine proteinase present in the azurophilic granules of polymorphonuclear neutrophils (PMNs) and is a potent platelet activator with potency similar to thrombin. In the current study we investigated the effect of IMiDs (CC-4047, CC-5013) on cathepsin G. Methods and *Results*. $CD34^+$ hematopoietic progenitors were cultured under conditions (IL-3, IL-6 and SCF) supporting the development of granulocytes and treated with CC-4047 or DMSO (control). RNA and protein were extracted for the analysis of cathepsin G. A significant up-regulation of cathepsin G (7.7 fold) was detected in oligonucleotide gene array analysis after 3 days of treatment with CC-4047 as compared to control group. These results were confirmed by RT-PCR, which showed a 3.7 and 7.6 fold mRNA-increase on day 6 and day 10 of culture compared to vehicle control. Measuring cathepsin G in supernatant by ELISA revealed a 1.5 and 1.6 fold up-regulation on day 6 and 10, respectively. Next we analyzed the cathepsin G levels of patients (n=10) treated with CC-5013 before treatment and on day 15 of each cycle. We observed a continuous significant increase of the mRNA levels over the course of treatment (baseline: 1, cycle 2: 1.7 fold, cycle 3: 4.8 fold, cycle 4: 20.7 fold). These data were confirmed by measuring cathepsin G levels in patient serum by ELISA. Cathepsin G significantly increased from a baseline mean of 53 ng/mL to 77.5 ng/mL (cycle 2), 129.2 ng/mL (cycle 3) and 145.5 ng/mL (cycle 4). *Conclusions*. These results show that IMiDs upregulate the potent platelet activator cathepsin G in hematopoietic cells and thereby might contribute to the development of thromboembolic events in patients receiving IMiD treatment. These results might explain why aspirin is effective in preventing TEE in patients receiving IMiDs. Further studies are needed to determine if cathepsin G helps to predict thromboembolic events. Inhibition of cathepsin G might be a potential therapeutic target for preventing the hypercoagulable state induced by IMiD treatment.

S11.8

MELPHALAN-PREDNISONE-THALIDOMIDE (MP-T) IS ALSO SUPERIOR TO MELPHALAN-PREDNISONE (MP) IN PATIENTS 75 YEARS OF AGE OR OLDER WITH UNTREATED MULTIPLE MYELOMA (MM). PRELIMINARY RESULTS OF THE RANDOMIZED, DOUBLE-BLIND, PLACEBO CONTROLLED IFM 01-01 TRIAL

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Introduction. The MP-T combination has been shown to be the standard treatment in newly diagnosed MM patients (pts) aged 65 to 75 years (Facon *et al.*; JCO 2006; 24, A1). However, no specific therapeutic recommendation exists for pts older than 75 years regarding the benefit of adding Thalidomide to MP and these pts have frequently been excluded from large clinical trials, although they represent more than 20% of MM pts. *Methods.* The IFM 01-01 trial was initiated in 04/2002. Patients > 75 years with untreated MM were randomized to receive MP (Melphalan 0.2 mg/kg/d + Prednisone 2 mg/kg/d day1-4, 12 courses at 6-weeks intervals) + placebo (MP-placebo) vs MP + daily Thalidomide 100mg/d (MP-T). No anti-VTE prophylaxis was given. The primary endpoint was overall survival (OS). Secondary end points were progression-free survival (PFS), response to treatment and toxicity. A first interim analysis was performed after the inclusion of 150 patients and a data safety monitoring board recommended a second analysis after the accrual of 200 patients. We here present the preliminary results of this analysis. *Results*. At the reference date of November 1, 2006, 232 pts were randomised. In all, 200 pts were analysed (100 per group), with 33.5% of pts >80 years (median age, 78.4 years). There were no differences between the 2 groups regarding baseline characteristics. Data were analysed on an intent-to-treat basis. After the completion of therapy the rates of partial response and very good partial response were 31% and 8% respectively with MP-placebo vs 61% and 22% respectively with MP-T. The median PFS time was 19 months (95%-CI 14.6-21.5) with MP-placebo vs 24.1 months (95%-CI 19.4-29.7) with MP-T ($p=0.004$ log-rank test). In the MP-T arm, 43/100 pts stopped treatment due to toxicity (10 due to neuropathy) versus 11/100 in the MP-placebo arm. Toxicity (Grade 2-4) included peripheral neuropathy (18%), somnolence (7%) and DVT (7%) with MP-T, vs 6%, 6% and 1% respectively, with MP-placebo. Final results including OS data will be presented at the meeting. *Conclusion*. MPT is an effective combination with acceptable toxicity in patients with MM older than 75 years of age, with a significant improvement in PFS.

S12: Debate II

S12.1

SHOULD AUTOLOGOUS TRANSPLANT BE PART OF THE PRIMARY TREATMENT IN MM?

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Background. During the past 15 years, there has been tremendous progress in the primary and salvage management of patients afflicted with multiple myeloma (MM). Several randomized and historically controlled trials have demonstrated that melphalan (MEL)-based high-dose therapy with autologous hematopoietic cell support effects, in comparison to standard-dose regimens, superior rates of complete response (CR) and, consequently, extended event-free survival (EFS), which translated into overall survival (OS) prolongation in some but not all studies. Tandem autotransplants have further improved clinical outcome in the IFM94 trial. Over the past 5-7 years, several new agents, especially immuno-modulatory drugs [thalidomide and lenalidomide] and the proteasome inhibitor bortezomib, have demonstrated marked salvage potential. When applied up-front, in combination with DEX, each other and especially MEL, unexpectedly high CR rates approaching those after MEL transplants have been reported. Follow-up is too short to be certain about EFS and OS duration. Thus, there is enormous controversy regarding the role, if any, of MEL transplants. Herein we report our collective front-line experience with Total Therapy (TT) regimens, employing as their backbone MEL200-based tandem transplants. As a result of incorporating, in a step-wise fashion, novel agents and treatment principles, all 3 clinical outcome measures (CR, EFS and OS) have been steadily improved. Outcome results will be examined in the context of state-of-the-art molecular genetic features, especially gene expression profiling (GEP) of highly purified plasma cells. *Patients and Methods.* Accruals to TT1, TT2 and TT3 regimens involved 231, 686 and 303 patients during the time periods of August, 1990 to June, 1995; October, 1998 to February, 2004; and February, 2004 to July, 2006. TT1 was a phase II study employing induction therapy with 3 cycles of VAD, HDC-TX for PBSC collection and EDAP; followed by MEL 200-based tandem transplant (in absence of PR after first transplant, MEL140+TBI 8.5Gy was applied); followed by IFN maintenance. TT2 was a phase III study randomizing patients up-front to ± thalidomide (THAL) and introduced more intensive induction therapy (VAD, DCEP, CAD with PBSC collection, DCEP); followed by MEL200-based tandem transplant (MEL140 for age >70yr and creatinine 3 mg/dL); followed by consolidation therapy with D-PACE q 3 mo for 4 cycles; and IFN maintenance with DEX pulsing during the first year. TT3 introduced bortezomib (V) into an abbreviated 2-cycle induction prior to and consolidation treatment after MEL200-based tandem transplants. Median follow-up times of living patients are 12 years with TT1, 4.8 years with TT2, and 1.7 with TT3. *Results*. *With TT1*, 62 patients remain alive (17% at 15 years); 31 remain event-free (7% at 15 years including 16 of 94 (41%) that initially achieved CR. Currently alive patients less frequently had cytogenetic abnormalities (CA)at baseline (p=0.002), post-enrollment (p<0.001)and at relapse (p=0.004); elevations of CRP (p=0.003) and LDH (p=0.029)m, anemia (p=0.029); and they more oftrn had completed 2 transplants within 12 months 9p=0.019). Post-enrollment overall survival (OS) and event-free survival (EFS) were superior in the absence of CA of the hypodiploidy or deletion 13 variety (p<0.001, p=0.037) and in the presence of low CRP at baseline (p=0.001, p=0.017). Post-relapse survival was longer in the absence of CA at relapse (p<0.001), IgA isotype (p=0.002), ISS stage 3 (p=0.014) and when patients and two protocol-based transplants prior to relapse)p=0.038).Ten-year EFS and OS could be accomplished in 15% and 33% of patients, respectively, when all agents available in 1089, especially MEL, were applied together up-front for the management of MM. GEP data available in 18 >10yr survivors revealed 14 to belong to the MGUS-like subgroup of MM. *With TT2*, the 323 randomized to THAL had superior CR at 48 months (61% v 42%; p<.0001) and EFS (median, 5.9 yrs v 4.2 yrs; p=0.006) but comparable OS (median, NR v 7.1 yrs; p=0.38). When analyzed overall regardless of study arm, 436 patients remain alive (54% at 7 years); 325 remain event-free (37% at 7 years) including 205 of 341 (60%) that initially achieved CR. The overall median OS has not been reached at 8 years, whereas the median overall EFS is 4.6 years; and the median duration from onset of CR (median time to onset, 0.80 years) is 6.0 years. As with TT1, OS and EFS were strongly influenced by the presence at baseline of any CA, so that 7-year rates were 76%/53% in the absence and 50%/40% in the presence of CA (p<.0001, p=0.0006). *GEP data*, available in 351 patients at baseline, revealed 13% to have high-risk MM (70 gene model) whose 7-year OS/EFS rates are 32%/20% compared to 63%/36% for the remainder (p<0.0001, p<0.0001). According to a 7 subgroup model, 7-year OS/EFS rates are 74%/41% in CCND1-1, 89%/23% in CCND1-2, 58%/48% in HYPERDIPLOIDY (HY), 78%/54% in LOW BONE (LB), 70%/44% in MYELOID (MY), 57%/35% in MAF/MAFB (MF), 41%/18% in FGFR3/MMSET (MS) and 20%/15% in PROLIFERATION (PR); significant differences were thus observed among the former 4 and the latter 3 subgroups (p<0.0001, p=0.0001). Comparing 51 paired relapse and baseline samples, all 8 patients in the PR group relapsed with such signature; conversion at relapse to PR was noted in 1/12 with MS, 2/11 with HY and 7/11 with MY. We also analyzed the *importance of CR* and timely application of a second scheduled MEL transplant on clinical outcome. Among 326 patients with both standard prognostic factor (SPF) and GEP information, CR was beneficial in the model that included GEP only in the high-risk subgroup of 13% (HR=0.22; p<0.001). A *second transplant* was an independent favourable prognostic feature in both risk groups, regardless of whether CR was achieved. With TT3, 303 patients were enrolled between February 2004 and July 2006. As a result of shorter induction and consolidation therapies before and after tandem transplants, the compliance rate was markedly improved over TT2: 84% v 66% completed both transplants (p<0.0001); treatment-related mortality (TRM) was similar at 5%, although more patients on TT3 were at least 65 years old (p=0.01). At 24 months, 84% v 68% had achieved n-CR including CR in 59% v 44% (both p<0.0001). TT3 effected superior EFS (24-mo estimates: 84% v 75%, p=0.02; <65yr: 86% v 76%, p=0.008) while OS was similar (86% v 85%; <65yr: 88% v 85%, p=0.16). In younger patients with GEP-based high-risk MM, 24-mo estimates with TT3 v TT2 were 62% v 27% for EFS (p=0.006) and 74% v 43% for OS (p=0.06). Compared to the THAL arm of TT2, fewer patients on TT3 experienced grade >2 tremor, constipation, syncope and thromboembolic events. Thus, compared to TT2, added bortezomib and shortened induction in TT3 increase tandem transplant compliance, effected higher CR rates and extended EFS with a strong trend for superior OS in younger patients with high-risk MM, with reduced toxicity and similar TRM. *MEL-based autotransplants vis a vis novel agent combinations.* As with TT programs for children with acute leukemia, performed under the auspices of St. Jude Children Hospital, the Arkansas data with TT regimens for MM have improved steadily as a result of introducing, based on salvage trial results, new agents and concepts into the front-line management of patients with MM. Patient characteristics have remained similar since 1989 with regard to the frequency of CA (one-third) and most other prognostically relevant baseline features, except a significant increase in the proportion of patients >=65yr that has risen from 10% to 20% to almost 30% in TT1 to TT2 to TT3. The OS extension from a median of 5.7 yr to >8 years is remarkable, as the 10-yr projection with TT2 will likely exceed the 33% with TT1 significantly (possibly exceeding 45%). CR and EFS, and in a high-risk subgroup, OS results with TT3 already surpass those with TT2. In this scenario, we are very concerned about abandoning the *workhorse* of MEL-based high-dose therapy requiring autologous stem cell support. Unlike other hematologic malignancies, MM is characterized from the outset, and even at the MGUS stage, by profound genetic complexity representing disease evolution and transformation when patients present for their initial therapy. Thus, multiple targets have to be *drugged*, which can be accomplished both with novel seemingly non-genotoxic and with genotoxic therapies such as PACE and MEL. Moreover, recognizing the importance of inactivating the *cancer stem-cell* also in MM (which seems to have a pre-plasma cell phenotype and is likely quiescent), the TT concept of wisely applying the entire treatment armamentarium up-front seems to be well-founded. In fact, analysis of survival in relationship to the length of uninterrupted remission revealed that patients without relapse for 5 years had 12-yr OS rates of 66% v 30% (ref). As many practicing physicians have begun to employ bortezomib and immuno-modulatory agents in combination up-front, concern is growing and justified as to the salvageability of such patients and whether delayed MEL-based interventions subsequently will lead to cumulative survival experiences reported here. *The Arkansas approach to further improving MM therapy is taking:* the following direction toward individualizing treatment: 1. In an extended TT3 trial of an additional 100 patients with evaluable baseline and 48hr post-bortezomib single agent GEP data, validate (a) the high-risk MM baseline signature; and (b) the favorable effect of bortezomib suppression of a micro-environment-associated gene. 2. In a pilot TT4 protocol for previously treated patients with high-risk MM, we will determine whether rapidly and frequently re-cycled stem-cell-supported high-dose DTPACE (cycle length 18-21 days) can increase CR rate beyond 80% and markedly improve 2-yr CR and EFS rates. 3. Pilot standard-dose MEL-VTD-type therapies in high host risk settings (age >70yr) and examine results in context of GEP-defined risk groups. 4. Validate pilot high-dose MEL300

(3 fractions) plus VTD as consolidation for previously treated patients with standard risk. 5. Evaluate, in patients with MRI-defined focal lesions, whether regional or systemic administration of MSC can increase osteoblast (OB) number and function and thereby exert further anti-MM effects. *Conclusion*. The treatment armamentarium for MM has been greatly expanded. Rather than repeating a *partisanship*-like approach of new versus old, the Arkansas data with TT programs have provided compelling evidence that 10-yr survivorship can be expected in nearly one-half of all patients treated up-front with all available agents and principles. In order to safeguard against loss of long-term EFS and OS results, trials need to be conducted in the context of the best available prognostic markers. The uniquely important role of CR in GEP-defined high-risk MM is consistent with the prospect of curing hitherto notoriously fatal MM entity, analogous to DLCL with the introduction of doxorubicin. The lack of EFS and OS implications of M-protein-defined CR in good-risk patients has been partially traced to a MGUS-like GEP subtype of MM in which such precursor lesion is re-established; in addition, the persistence of MRI-defined focal lesions in clinical CR is suggestive of dormant non-M-protein secreting MM-stem?-cells that are responsible for disease recurrence.

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S12.2

SHOULD AUTOLOGOUS TRANSPLANT BE PART OF THE PRIMARY TREATMENT IN MM?

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Present standard of care for young patients with multiple myeloma (MM) is high dose therapy (HDT) with autotransplantation using blood as the source of autologous stem cells. This therapeutic approach can produce long-term survival (19% of the patients that we treated between 1985 and 1990, now with a median 15.8 year follow-up). In addition, it has been demonstrated to significantly prolong survival when compared with standard dose therapy (SDT) in prospective randomised trials conducted by French (IFM), English (MRC) and Italian groups.[1-3] However, the situation is somewhat more complex. Indeed, in other randomised studies, namely a large US study, our MAG 91 study and the study of the Spanish group Pethema, superiority of HDT over SDT in terms of overall survival (OS) was not demonstrated.[4-6] The very nature of these contradictory data indicates that large numbers of patients would need to be studied to demonstrate the potential advantage of HDT and suggest that the improvement in survival, if any, is of limited duration. The different results of the randomized studies can be explained in part by differences in their design. In the Spanish study, for example, only patients responding to an initial conventional regimen were randomized whereas the others randomly assigned patients to HDT or SDT upfront. Other significant differences in these study designs include the duration of SDT, which was highly variable, and patient age (patients were older in the Italian and in the MAG studies). In the IFM90, MRCVII and Italian studies, SDT was delivered for one year (2 to 18 courses), until the maximal response was attained (1 to 9 cycles, median 5) and to a maximum of 6 monthly courses of melphalan and prednisone, respectively. In the US study, all patients received 4 cycles of a VAD-like regimen, high dose cytoxan and then one year of VBMCP therapy. In the MAG study, SDT was maintained until a stable plateau phase was achieved (1 to 26 courses, median 12). Notably, OS of conventionally treated patients was shorter in the 3 first studies (44, 42 and 37 months in median, respectively) than in the 2 others (53 and 49 months, respectively). This apparent correlation between SDT duration and survival is not surprising since, as we have learnt from many pionering works, a too short duration of SDT may mean that the slow response that occurs in some patients, and that usually translates into long remission, is not observed. Patients' age also deserves consideration. Upper limits were 70 years in the US and Italian studies as compared to 65 years in the others. Median age was about 55 years in all trials except in the MAG and in the Italian studies in which it was 61 and 64 years, respectively. In the English study, although randomization used an algorithm based on age (<55 years vs.≥ 55 years), results according to this planned subdivision were not reported.[2] In contrast, the IFM investigators reported the benefit of HDT on survival only in the subgroup of patients who were 60 years or younger (1). Thus, not only the *negative* MAG study but also the *positive* IFM trial highlighted the issue of patients' age suggesting that the survival advantage of HDT, if any, is likely only to concern the younger age brackets. Of note, conclusions of the Italian study, which suggested the superiority of an intermediate-dose treatment (tandem melphalan 100 mg/m^2) over a MP regimen, were not confirmed by a second similarly designed randomized trial (see below). Most of the previously mentioned trials compared HDT rescued by SDT to SDT alone. A protocol comparing HDT with SDT followed by rescue HDT is likely to be a better way of assessing the true merit of the two therapies. In the so-called MAG 90 study, we did that *early vs. late* comparison in young MM patients that were randomly assigned to receive HDT either as first line therapy or as rescue treatment in case of primary resistance to a standard dose regimen or at relapse in responders.[7] Importantly, OS results were quite similar, providing additional argument that HDT performed early is not necessarily part of the primary treatment even for young MM patients. A meta-analysis of data from 575 patients included in the 3 previously mentioned French randomized studies did not provide evidence of an OS advantage of HDT as compared to SDT.[8] This was confirmed by a recently published review and meta-analysis of all controlled trials comparing HDT with single auto-transplantation and SDT for newly diagnosed MM patients.[9] It indicated a significant progression-free survival (PFS) benefit but no OS benefit at all for HDT. Importantly, this study included a careful comparison of treatment-related mortality (TRM), which varied between 0 and 5% in SDT arms and between 2.1 and 12% in HDT arms. Overall, the risk of developing TRM with HDT was increased about three fold, which is all the more noteworthy considering that all the randomized trials enrolled a somewhat selected populations excluding, for instance, patients with overt renal insufficiency. In unselected patients, TRM may be even higher when using HDT rather than SDT. Pushing the *more is better* concept, B.Barlogie developed *total therapy* programs including tandem transplantation. This prompted the design of prospective randomized trials comparing a single versus double HDT sequence. The main one, the IFM 94 trial, which enrolled 399 young patients showed superior survival for tandem HDT.[10] OS in the double HDT arm (58 months in median) was statistically better than OS in the single HDT arm (48 months) but, surprisingly, was similar to the OS (57 months) of the (single) HDT arm of the previous *HDT vs SDT* IFM90 study. Although another randomized study, conducted in our group, suggested a survival advantage for double transplant,[11]overall data did not provide evidence for a benefit sufficient enough to overcome the risks and constraints (including cost) of using tandem transplantation instead of single transplant as primary treatment of young myeloma patients. Even using tandem transplant, there is no plateau in survival curves and present modalities of HDT do not to lead to cure. Accordingly, it is critical to assess quality of life achieved after this treatment. Analyzing average time without symptoms, treatment and treatment toxicity - the so-called TwiSTT –, we suggested a better quality of life for HDT up-front as compared to SDT and to late HDT.[6,7] This was based on treatment duration and obviously did not hold true for the very intensive *total therapy* type programs in which tandem transplant is preceded by an induction period and followed by consolidation cycles which generally involves over 2 years on therapy, without taking into account the maintenance treatment.[12] Novel anti-myeloma agents (thalidomide, bortezomib and lenalidomide) that have transformed the therapeutic options in refractory disease have been integrated into therapy for newly diagnosed patients only recently. However, we already

know that combination of these drugs with corticosteroids, alkylating agents and/or other chemotherapeutic drugs can produce impressive improvements over the historical response rates for *classical* SDT, with complete remission (CR) rates of up to 30%.[13,14] Moroever, the most recent randomized trial conducted by the IFM investigators, involving patients aged between 65 and 75 years (including 41% over 70 years), demonstrated that an up-front 12-month oral treatment combining MP plus thalidomide is not only superior to MP alone but also to a tandem melphalan (100 mg/m^2) regimen.[15] Impressively, the OS of the patients who were randomly assigned to the MP plus Thalidomide arm (>55 months in median) appeared greater than the OS of the patients who received double transplant in the IFM94 study, who were significantly younger (52 years in mean)!!! Thus, new SDT regimens can produced very good response rates that are likely to be translated into longer remission duration compared to *old* SDT regimens. In contrast, incorporating novel anti-myeloma agents into pre- per or post-HDT regimen does not appear to confer any survival advantage. Indeed, the additional benefit of performing HDT in good responders to initial chemotherapy is questionable, as illustrated by the Spanish Pethema study.[5] In addition, in two randomized comparisons of thalidomide or bortezomib including regimen to a VAD-like regimen as pre-HDT induction treatment, response rates were better at the time of HDT but similar at 6 months post-HDT for patients in the new drug arm as compared to patients in the VAD arm.[16,17] Finally, incorporation of thalidomide into an intensive HDT program was evaluated through a large randomized study that showed an increase in the frequency of CR and an extension of EFS but more adverse effects and no improvement in OS.[12] It is time to question the *more is better* dogma which, unfortunately, produce true CR in only a minority of patients, if any, and is not curative. The recent increase in our therapeutic arsenal now gives us the opportunity to apply another strategy: namely, long-term disease control through the sequential use of combinations of novel and old anti-myeloma agents. This strategy is already challenging the current standard of upfront HDT in that new SDT modalities are likely to produce even higher response rates than with sequential HDT. Of course, while evaluating these new regimens through well-designed randomized studies, we should pursue our efforts aimed at better understanding the oncogenic mechanisms of the different forms of the disease, in an attempt to design adapted targeted therapies.

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S13: Current and future perspectives in multiple myeloma

S13.1

THE IMPORTANCE OF NEW AND EVOLVING RESPONSE CRITERIA

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Why new International Uniform Response criteria are important

With the introduction of thalidomide, bortezomib (Velcade) and Lenalidomide (Revlimid) important new questions have emerged.[1]

- What is the best choice for frontline therapy?
- Are complete response (CR) and very good partial response (VGPR) predictive of improved outcome?
- Is level of response pre-autotransplant important?
- Which patients benefit most from double autotransplant?
- Is benefit with maintenance mostly influenced by pre-maintenance response?

Addressing these and many other issues requires accurate, widely accepted and reproducible criteria for response assessment.[2,3] With this in mind, the IMWG (International Myeloma Working Group) set out to:

- Clarify the critical endpoints
- Introduce stricter criteria for high level response: sCR (stringent complete response) and VGPR (very good partial response)
- Clarify and improve details and correct inconsistencies in prior response criteria
- Incorporate the serum FLC (FREELITE) assay into response assessment especially for patients with oligosecretory and non secretory disease.
- Provide discrete recommendations for monitoring and follow-up to assess response status and/or development of a new event.

The new response criteria are listed in Table 1.

Practical details, which make a difference

Full eligibility and evaluability within a clinical trial dramatically improves the quality of the outcome assessment. The new criteria eliminate the need for confirmation of response after 6-weeks. The main concern is to eliminate laboratory error. Thus, a confirmatory test is required to document maximum response, but this can be at any time up to the 6-week time point and but prior to initiation of stem cell harvesting and/or any alternate therapy. More major emphasis is placed upon documenting time to progression (TTP) and duration of response (DOR), which are true clinical endpoints. In addition, a distinction is made between progressive disease associated with ≥ 25% M-component increase and other related numeric changes versus true clinical relapse. Protocols can be structured with planned interventions (or not) at the time of biochemical relapse (as in the past) and/or at clinical relapse with development of a new bone lesion, plasmacytoma or hypercalcemia. Data related to both types of relapse can be captured.

Implications of achieving sCR, CR and/or VGPR

Response ≥ VGPR occurs much more frequently with novel therapy approaches. Is it worth added toxicities to achieve VGPR, CR or SCR versus just PR? Does higher-level response translate into better outcomes? More protocols are designed to ask these kinds of questions. (4) For example, in a recent IFM trial comparing VAD with Velcade/ dexamethasone for induction, more patients achieved ≥ VGPR with Velcade/ dexamethasone and thus did not need to proceed with a second autotransplant.[5] Conversely, with a prior thalidomide/ dexamethasone trial for frontline therapy the ≥ VGPR rate was the same versus VAD when evaluated post a single autotransplant.[6] Having clear response criteria available is incredibly helpful in these situations. Likewise, with Revlimid/dexamethasone for frontline therapy it is important to note that, with longer-term follow-up, 67% of patients continuing on Rev/Dex maintenance ultimately achieved ≥ VGPR (versus 38% after first 4 cycles) as compared with 58% in a non-randomized cohort receiving a single auto transplant.[7] These types of higher-level discriminations are increasingly important in assessing *best therapy* options, balancing efficacy and toxicities.

Introduction of the serum FLC assay

Three aspects pertaining to the introduction of the serum FLC assay deserve emphasis. First, the serum FLC assay (FREELITE, The Binding Site, Birmingham, UK) is a highly sensitive marker of light chains in circulation that are unbound to intact immunoglobulin, and the FLC ratio is an excellent indicator of clonality. Thus, normalizing of serum FLC ratio is a stricter indicator of CR, and may correlate well with extended response duration.[8] Note that in patients with renal insufficiency, the levels of both the kappa and lambda may remain elevated, but the ratio normalizes with achievement of CR. Second, in order to minimize chance of error, FLC response is not assessable for patients who start with low baseline serum FLC assay levels below 10mg/dL (< 100mg/L). Third, although the serum FLC is a very reliable test, it is important to closely monitor laboratory variation.[9] Strict guidelines are required with regard to usage times for the serum FLC assay kits. It should also be noted that serum FLC assay testing might be useful in the prognostic and response evaluation of patients who also have a measurable serum and/or urine M-component in the future, given its recently reported prognostic value in monoclonal gammopathy of undetermined significance (MGUS).[10]

Table 1. International Myeloma Working Group (IMWG) Uniform Response Criteria*.

Response Category	*Criteria*
sCR	Stringent Complete Response CR as below, plus: FLC ratio normal No clonal plasma cells (by immunotesting)
CR	Complete Response Immunofixation negative serum and/or urine ≤ 5% bone marrow plasma cells Disappearance of any plasmacytomas
VGPR	Very Good Partial Response ≥ 90% reduction in serum M-protein Urine M-protein < 100 mg/ 24-hours Immunofixation positive
PR	Partial Response ≥ 50% reduction in serum M-protein ≥ 90% reduction in urine M-protein and < 200 mg/ 24-hrs.
SD	Stable Disease Not meeting any of the above criteria nor progressive disease

** See Reference 3 for full details*

Survival end points

The most useful early endpoints are progression-free survival (PFS) and time to progression (TTP). Progression-free survival is the time from start of treatment to myeloma progression or death from any cause. This is the best overall assessment of treatment benefit. Time to progression is the time from start of treatment to disease progression with deaths due to causes other than myeloma progression not counted, but censored. This is the most direct assessment of treatment durability without considering other toxicities, events, or non-myeloma related deaths. Both PFS and TTP are the best surrogates for overall survival. It is important to realize that several factors complicate the use of overall survival as the ultimate endpoint. In 2007, the typical survival will be ≥ 5 years and the advent of new agents may extend this possibly substantially. Thus, the sequential documentation of response, TTP and PFS with each treatment strategy becomes increasingly important to attempt to assess the incremental benefit. It is essential to track TTP, PFS plus recorded toxicities and data related to both convenience and quality of life. Because anticipated survival is now frequently ≥ 5 years permanent toxicities are especially problematic and need to be kept at a minimum. For example, neuropathy caused by one agent can not only cause long-term impaired quality of life, but also reduce the ability to use and benefit from subsequent potentially neurotoxic agents. An important secondary endpoint is the ability to tolerate multiple sequential therapies. Baseline molecular evaluation is also recommended within the trial set-

ting. Risk stratification has been a goal for some time. However, the introduction of the novel therapies has forced a re-evaluation of preconceived notions about risk. It may be that novel combinations can overcome risks associated with 13q-; t (4;14) and other abnormalities. Further studies are required. For now it is most important to include both molecular and other potential prognostic tools, such as imaging, within trials to allow prospective validation for new therapies. As treatment for myeloma steadily improves it is increasingly important to have the framework of the International Uniform Response Criteria, plus accurate diagnostic, prognostic and stratification criteria to asses the best outcomes.

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S13.2

INDIVIDUALIZING TREATMENT IN THE AREA OF MULTIPLE NOVEL AGENTS

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The recent discovery of new drugs with singular mechanisms of action, together with the better understanding of MM cell biology, which has lead to the identification of new relevant prognostic factors, will probably make it possible to develop individualized treatment in the near future. Accordingly MM should no longer be considered as a single entity and as occurs in other haematological malignancies (ALL, AML, NHL) treatment should be tailored to the different MM subtypes. As a brainstorming exercise I will share with you my personal view on different alternatives for treatment individualization in MM patients. Upon dealing with a newly diagnosed patient , as a first step I would stratify their treatment according to two elements: age and risk factors. The age (>65 or 70 years) will differentiate between transplant and non transplant candidates, while risk factors (poor cytogenetics, high S-phase, advanced ISS with renal failure or disease progression under induction treatment) would separate high vs standard risk patients.

The newly diagnosed standard risk patient and transplant candidate

The *dream goal* for these young patients should be their eventual cure and until then to achieve long survival (>10 years). 1.a *Induction treatment*: Novel drug combinations appear to be superior to conventional chemotherapy (VAD like regimens) as de-bulky pretransplant (Trx) therapy. Using schemes with Bortezomib, Lenalidomide or Thalidomide, the majority of patients respond (>80%) with 10%-30% CR. Moreover, these schemes do not affect stem cell collection. Interestingly, in six pilot studies based on Bortezomib regimens it was observed that the CR rate was improved following autologous Transplant (ASCT), which suggests that both approaches (induction with novel agents and ASCT) are complementary rather than alternatives. Nevertheless, the benefit in terms of EFS and OS remains to be seen. In addition the German /Dutch group as well as the Fermand group have shown that the initial advantage of a Thalidomide based regimen (TAD) vs VAD used as de-bulky treatment was overcome after transplant. Data on lenalidomide are still scantly. 1b. *Autologous stem cell transplantation*: One or two: Following 4-6 cycles of the above mentioned de-bulky regimens all responding patients (refractory patients will be considered separately) will proceed to an ASCT with standard Melphalan 200 mg/m^2. I would discourage the use of a double transplant for two reasons: i) Only patients achieving < VGPR with the first transplant benefit from the second and ii) Similar efficacy is obtained upon using Thalidomide as consolidation/maintenance post-transplant therapy. 1c. *Maintenance treatment*: The oral formulation of both thalidomide and Lenalidomide makes them ideal maintenance drugs but the final benefit should be balanced against toxicity. The French group (IFM) has recently shown that Thalidomide maintenance is clearly superior to No- maintenance or Pamidronate alone in terms of EFS and OS. This has been confirmed by the Australian group using Thalidomide and Prednisone as compared to Prednisone alone. Of note, the Arkansas group has also observed that the use of Thalidomide as part of induction and maintenance phases was associated with longer EFS, although this does not translate into a prolonged OS. This raises an important concern about whether the continuous use of novel agents may induce relapses more resistant to salvage therapy leading to shorter survival after relapse. 1d. *Treatment of relapse*: In order to individualize treatment of relapsing patients after transplant, I would separate them into 3 groups: early relapse (<1 year), intermediate (1-3 years) late (>3years) relapse. If the relapse occurs within the first year after transplant I would try to rescue the patient with novel agents but, in order to overcome drug resistance, I would use them in alternating cycles of two combinations of non–cross resistant agents (i.e Velcade/Adria/Dex alternating with Thal or Len /Cyclo /Dex). If CR is achieved I would proceed to an Allo-transplant with reduced intensity conditioning regimen (RIC-Allo). If relapse occurs between 1-3 years after transplant I would support rescue with novel agents but used in a sequential (not simultaneous) manner: first one line of treatment (different from the one used as induction) and only when disease progression, occurs to shift to the second and subsequent lines. Within this category of patients, for those that are young (<55 years) and had suboptimal response to the first line I would re-consider the possibility of a RIC-Allo-Trx. Finally if the relapse has occurred > 3 years after the first Trx, I would favour reinduction followed by a second ASCT.

The newly diagnosed high risk patient and transplant candidate

As mentioned above high risk patients can be considered as those with high proliferative activity of PC (LI or S-phase) and advanced stage (ISS III) but particularly those with poor cytogenetics. Patients with renal failure and disease progression under induction therapy will be discussed separately. One possibility is to use an approach similar to that proposed above for early relapses (induction with non-cross resistant agents, including two novel drugs, followed by a tandem transplant: ASCT and RIC-Allo) . It should be noted that new agents appear to overcome the adverse influence of these cytogenetic abnormalities, at least during induction. A second possibility would be the use of targeted therapies in patients with specific genetic lesions, such as FGFR kinase inhibitors in t(4;14) or cyclin–dependent kinase inhibitors. However, since MM is a multi-step process these latter approaches should be complemented with standard treatments. Ideally these patients should be included on experimental clinical trials.

The newly diagnosed elderly patient or non transplant candidate

The goal in these patients should be to prolong survival and to maintain a good performance avoiding hospitalisation as much as possible. 3a. *Induction treatment*: Recent data demonstrate that the combination of any of the new agents (Thalidomide, Lenalidomide and Bortezomib) with MP yields a higher RR, prolonged EFS and, at least in one of the studies, longer OS as compared to MP. However, the higher efficacy of these new regimens should be balanced against their higher toxicity and more frequent hospital visits. Again, an individualised treatment approach would probably be valuable. Patients <80 years and good PS can be candidates for standard induction with MP-Thal or MP-V or MP-L. The choice between these 3 options could be based on different factors such as antecedent / risk of DVT or PN or renal function or distance from the Hospital. The duration of treatment perhaps could be reduced from the *standard 12 months of MP* to six cycles of these more active combinations. In very elderly patients (>80y) or poor PS, I would favour modified schemes with a lower dose of thalidomide (100 mg, maximum

200mg); Lenalidomide (15-20 mg) or Velcade (1 mg/m^2 or a weekly schedule). 3b. *Maintenance treatment*: Although there is no data to support it, maintenance treatment for 1 year with low doses of an oral IMID is attractive. Nevertheless, this should be counterbalanced by the risk of inducing more resistant relapses. 3c. *Treatment of relapse*: Decision at relapse must take into account the general condition of the patient. If he is suitable for further treatment, alternative schemes, different from the one used as induction, should be given, and ideally considered for inclusion in experimental clinical trials. If the condition is poor, I would favour palliative treatment with oral cyclophosphamide and Prednisone.

The primary refractory patient

Within this category it is critical to distinguish between the non-responding non-progressing patients and those with progressive disease under induction therapy. The former category shows a similar outcome to responding patients and it is therefore important to recognise them in order to avoid unnecessary toxicity with a non-useful treatment. Truly refractory (progressive) patients have until now been considered candidates for direct rescue treatment with high dose therapy, but in our experience, the survival with this approach is rather poor. This is a high risk category and I would favour the use of a cocktail of non-cross resistant drugs followed by a double transplant (Auto and RIC- Allo).

The patient with renal insufficiency

In patients with renal insufficiency VAD was the treatment of choice because in contrast to alkylating-based regimens, it does not require dose adjustment . Recent reports indicate that Bortezomib ± Dexamethasone is highly efficient in this setting, including patients on dialysis, and does not require dose adjustment. Although thalidomide has been used for >6 years, no specific information on its efficacy and toxicity in patients with renal failure is available. For lenalidomide, recent guideline recommendations have been generated . If the patient responds to initial therapy and he is a transplant candidate, renal failure can be reversed in up to 43% of cases. However the toxicity of ASCT is higher than in standard MM patients (TRM: 29%) and we discourage ASCT if the patient has Cr>5mg/dL, Hb <9g/dL and PS ≥3.

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S13.3

EUROPEAN MYELOMA NETWORK: THE PRESENT AND THE FUTURE

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The European Myeloma Network (EMN) was initiated in 2003 in order to allow scientists and physicians with interest in Multiple Myeloma and related disorders (MM) to share their experience and develop cooperative studies.

Constitution and goals

EMN is a non-profit organisation which has been founded in Copenhagen, Denmark. Today more than a hundred scientists or institutions from EU member states are registered EMN members with interest in MM. Since 2006, the EMN has been officially appointed as a Working Group of the European Haematology Association (EHA). Initially, 4 Working Parties were organized 1) Clinical Trials; 2) Allogeneic Stem Cell Transplantation and Immunotherapy; 3) New Drugs; 4) Pathogenesis and Standardization. The goal of WP's 1 & 2 was to acquire project grants from the European Framework programs for pre-clinical and clinical research. The aim of WP 3 is to develop a cooperative clinical setting for clinical trials with new drugs. WP 4 is a program for standardisation in diagnostic and research tools. Support is obtained from unrestricted grants from non-commercial organisations, government and pharmaceutical companies. Also, the International Myeloma Foundation has provided support.

Research programs

In 2006 the EMN successfully applied for a 3 year grant from the 6th FW program of the EU, resulting in the MSCNET project entitled *Myeloma Stem Cell Network: A translational programme identifying and targeting the early myeloma cell hierarchy*. This program is coordinated by H.E. Johnsen from Aalborg Hospital, Aarhus University in Denmark with participation of Salamanca, Heidelberg, Southampton, Groningen, Montpellier, Rotterdam and Brussels. In 2007, an application was made for the FW 7 program, i.e. *EMNBIO: An European Myeloma Network (EMN) of Biobanks* which encompasses an EMN strategy for developing standards and norms for existing and future biosamples from patients suffering from multiple myeloma and related disorders, coordinated by D. Hose and HE Johnsen. Also, an application was submitted called *EMNOMICS: A European Myeloma Network for Translational Science: Developing a genomic platform for the detection of prognostic and predictive biomarkers in multiple myeloma* coordinated by G. Morgan.

Quality control

Several Workshops have been organised on the application of diagnostic techniques aiming at standardisation of (molecular) tools between European centers which will improve the comparability of test results. A first Workshop dedicated to FISH was held in London and a quality control program for FISH in myeloma is being developed by H. Avet Loiseau from Nantes, involving many laboratories in Europe. Another Workshop has been organised by A Rawnstrom and A Orfao on the use of Flow Cytometry to characterise myeloma immunophenotypes and a second one on practical aspects in Leeds 2007. An important focus has been initiated during the first Workshop on Gene Expression Profiling in myeloma, held in Heidelberg in 2007. This Workshop brought together several groups who will attempt to define sampling procedures, plasma cell purification methods, statistical analysis and to organise combined analyses of samples obtained from patients in clinical trials. Follow-up Workshops are planned to accomplish this goal.

Clinical trials

In 2006 preparations were made to organise an independent prospective clinical trial in Waldenström's disease. This phase II trial of Bortezomib combined with Rituximab and Dexamethasone in previously untreated patients is exclusively sponsored by EMN and 15 centers from 10 European countries participate in the protocol, which accrued the first patient in 2007 (coordinated by M. Dimopoulos). Also, EMN facilitated the development of the Celgene-sponsored trial of Lenalidomid combined with MP in previously untreated elderly patients with myeloma. Overviews of Phase I/II trials with new agents in various European centers were prepared by A. Palumbo. EMN will increase efforts to accrue future clinical trials with new agents in multiple myeloma based on its strong position with leading myeloma centers among its participants.

Consensus

The European Myeloma Network wants to develop a position statement on the use of prognostic and predictive factors in myeloma and to

develop general guidelines for the diagnosis and treatment of myeloma. It is expected that a consensus paper can be prepared in 2007. In conclusion, EMN has been started to combine efforts in clinical and laboratory research in multiple myeloma and to define common standards of diagnosis and care. The network is gaining momentum and will use the strenght of participating centers to improve the quality of myeloma research in Europe. Visit: www.myeloma-europe.org

S13.4

FUTURE PERSPECTIVES IN THE MANAGEMENT OF MYELOMA

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Future translational research in multiple myeloma (MM) will focus on the development of molecularly-based combination therapies to achieve high frequency and durable responses in the majority of patients. Combination therapies have proven to be curative in childhood acute lymphoblastic leukemia, Hodgkins disease, and testicular cancer among others. Already cytogenetic abnormalities known to carry adverse prognostic import to conventional and high dose therapies do not apply to novel therapies (bortezomib, lenalidomide), which will serve as platform drugs for future combined therapies. Two major advances are making this goal possible. First, recent advances in genomics and proteomics in MM have allowed for advances in our understanding of disease pathogenesis, identified novel therapeutic targets, allowed for molecular classification, and provided the scientific rationale for combining targeted therapies to increase tumor cell cytotoxicity and abrogate drug resistance. Specifically, gene microarray profiling has shown major differences between normal plasma cells versus those from monoclonal gammopathy of unclear significance (MGUS) and MM, with further modulations within MM and progression to plasma cell leukemia. These studies identify genetic changes associated with progression of MGUS to MM, and provide the basis for RNA based prognostic classification systems. Integration of comparative genomic hybridization (aCGH) with expression profiling data has allowed for further identification of new therapeutic targets, as well as DNA based classification systems. Excitingly those genes transcribed can be systematically overexpressed and knocked out first in cancer and then in MM model systems, in order to identify genes implicated in cause or progression of MM. Gene products on the cell surface are targeted by monoclonal antibodies or vaccines, whereas prototype small molecule inhibitors can be synthesized against intracellular targets. Second, there is now an increased understanding of how adhesion of MM cells further impacts gene expression in MM cells, as well as in bone marrow stromal cells (BMSCs). Specifically, adhesion of MM cells to the BMSCs upregulates genes for growth, survival, and drug resistance in tumor cells; adhesion molecules on MM and BMSCs; and cytokines in BMSCs. These modulations have been demonstrated using both *in vitro* models of MM cells bound to BMSCs; as well as *in vivo* in xenograft models by injecting fluorochrome labeled MM cells directly into human bone grafts within SCID mice (SCID-hu). These systems have delineated genetic changes and sequelae induced when MM cells bind to the BM; and conversely, validated the ability of novel agents to abrogate these genetic changes and induce tumor cell cytotoxicity even in the BM milieu. Within the BM, plasmacytoid dendritic cells promote proliferation and survival of patient MM cells for generation of cell lines, as well as genomic/proteomic and drug sensitivity profiling of self renewing MM cells. A genetically based mouse model of human MM has been generated by overexpression of XBP-1 spliced isoform (XBP-1s), a transcription factor required for plasma cell differentiation which is also highly expressed at a gene and protein level in MM cells versus normal plasma cells. In this new model, mice transgenic for Eu-directed (XBP-1s) develop pathognomonic features of monoclonal gammopathy of unclear significance (MGUS) which progressed to MM with time, including serum monoclonal protein, bone marrow plasmacytosis, renal disease, and lytic bone disease. Genomic analysis of premalignant B cells and MM cells showed dysregulation of genes with known relevance to human MM including cyclin D1, MAF, MAFB; and uncovered pathogenetic insights into MCL-1 and FOS/JUN. This model identifies genetic changes mediating the development of MGUS and progression to MM; overlay of addition genetic abnormalities (p16, p53) offers the opportunity both to shorten latency time of this model and define their role in MM pathogenesis. This model also provides a unique system both for identifying novel targets and validating novel targeted therapies. As a result of these advances in oncogenomics on the one hand and increased understanding of the role of the BM in the pathogenesis of MM on the other, a new treatment paradigm targeting the tumor cell and its BM microenvironment to overcome drug resistance and improve patient outcome has now been developed in MM. Thalidomide, lenalidomide, and Bortezomib are three agents which target the tumor cell in its microenvironment in both laboratory and animal models which have rapidly translated from the bench to the bedside. These studies serve as a testament to the power of collaborations between academia, pharmaceuticals, Food and Drug Administration, National Cancer Institute, and Advocacy groups to rapidly identify therapeutic targets in the MM cell and its BM microenvironment, use laboratory and animal models of human MM to validate novel agents directed at these targets, and then design clinical trials evaluating these agents which ultimately lead to their rapid FDA approval. Each was first shown to achieve responses in relapsed refractory MM both alone and combined with Dexamethasone, and was then combined with conventional Dexamethasone therapy as initial therapy for newly diagnosed patients eligible for high dose therapy and stem cell transplantation. Most excitingly, each has now been combined with conventional melphalan and prednisone therapy, the gold standard initial therapy for elderly patients with newly diagnosed MM, and achieved increased extent and frequency of response, as well as prolonged progression free and overall survival. Ongoing and future efforts are identifying next generation therapies in MM on the one hand, and using oncogenomics to inform the design of combination trials on the other. Examples of promising novel targeted therapies include agents targeting the tumor cell surface (CD40, CS-1, FGFR3), cytokines (VEGF, BAFF), and intracellular targets (MEK, PKC, NF-kB, IKK, cyclin D, proteasomes). Having defined novel agents directed at these targets which can induce cytotoxicity against MM cells in the BM in both *in vitro* and *in vivo* models, we have gone on to define combination therapies to enhance cytotoxicity and overcome drug resistance. Microarray profiling shows that Bortezomib induces hsp 90 gene transcripts in human MM cells, providing the rationale for our preclinical functional validation studies showing that combining Bortezomib with hsp 90 inhibitor 17AAG can enhance cytotoxicity. Two phase I/II clinical trials of 17AAG have defined optimal single agent dose; an ongoing trial combining Bortezomib and 17AAG already demonstrates that the combination can overcome Bortezomib resistance, and a phase III trial comparing Bortezomib with Bortezomib and hsp 90 inhibitor is soon to begin. Apoptosis induced by Bortezomib is mediated primarily via caspase 9 and by lenalidomide via caspase 8. Already clinical trials have shown that combined Bortezomib and lenalidomide achieves responses in the majority of patients resistant to either agent alone, with a favorable side effect profile, and an ongoing trial is evaluating this combination as initial therapy of newly diagnosed MM. Bortezomib inhibits growth (Erk), survival (Jak/STAT), and migration (PKC) signaling, but activates Akt; our preclinical studies show synergistic MM cytotoxicity combining Akt inhibitor perifosine with Bortezomib. We have nearly completed a clinical trial of single agent Akt inhibitor perifosine in MM, and a phase II trial evaluating this combination is rapidly accruing. Importantly, the histone deacetylase inhibitors tubacin or LBH can block protein degradation via the aggresome autophagy pathway; blockade of the proteasome with Bortezomib upregulates aggresome activity, providing the preclinical rationale for combining these agents to enhance tumor cytotoxicity. A phase II trial of LBH is now beginning in MM, with a combination trial to quickly follow. Lenalidomide has been combined with steroids, proteasome inhibitors, mTOR inhibitors, humanized monoclonal antibodies, and Akt inhibitors; our preclinical studies also suggest synergistic cytotoxicity, and clinical trials are either ongoing or soon to begin. In contrast, multiple combinations have been evaluated and found to be antagonistic, i.e. IKK and Akt inhibitors; therefore, such combinations are not moving forward to the clinic. At present the minority of phase III clinical trials are successful, but the use of oncogenomics in this fashion to inform clinical protocol design should markedly enhance success rate. Correlative science of patient samples on clinical protocols will be necessary to define mechanisms of response versus resistance, select patients most likely to respond, and design next generation more potent targeted agents. For example, microarray profiling, as well as as qualitative and quantitative assessment of proteasome inhibition, can readily be done of MM cells in patients on treatment protocols. Once promising cocktails of therapies are defined using preclinical models, they will rapidly translate to derived phase I and II clinical trials to identify those which achieve high extent and frequency of responses. It will then be possible to carry out phase III clinical trials treating newly diagnosed patients with these regimens, collect autologous peripheral blood stem cells, and then randomize patients to receive early versus late stem cell transplantation. These studies will define durability of response on the one hand, and the contribution of stem cell transplant on the other. This new paradigm for overcoming drug resistance and improving patient outcome in MM has great promise not only to change the natural history of MM,

but also to serve as a model for targeted therapeutics directed to improve outcome of patients with other hematologic malignancies and solid tumors as well.

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WM1: Genetics, pathophysiology and staging system

WM1.1

BIOLOGIC AND CLINICAL OVERLAP OF IGM-SECRETING LYMPHOMAS; FOCUS ON WALDENSTROM'S MACROGLOBULINAEMIA (WM)

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Virtually any B-cell lymphoma (B-NHL) may secrete a monoclonal immunoglobulin (Ig) that could be of the IgM class. Waldenstrom's macroglobulinaemia (WM) is the paragon of IgM-secreting B-NHL given that, by definition, a serum monoclonal IgM component is always present. In a series of 130 IgM-secreting B-NHL, 64% of patients presented WM, 11% marginal zone lymphoma (MZL), 7% chronic lymphocytic leukemia (CLL), 4% small lymphocytic lymphoma (SLL) and mantle cell lymphoma (MCL) and less or equal than 2% follicular lymphoma (FL), diffuse large B-cell lymphoma (DLBCL) and other B-NHL.[1]

IgM-Secreting Lymphoma's Biologic Overlap

B-cell development and Malignant Transformation of the Lymphocyte

Precursor B lymphocytes develop into virginal small B-lymphocytes that circulate in the blood, migrate to the mantle zones of lymph nodes (LN) and spleen (S), and then, possibly enter into a germinal center (GC). During B-cell developmental evolution, the rearrangement of Ig heavy (IgH) and light chain (IgL) genes (V_H-N-D_H-J_H on chromosome 14 and V_L-N-J_L on chromosomes 2 and 22 for κ and λ light chain respectively), take place. Thus, one of about fifty functional V_H, another of thirty D, and one of six J_H genes and, in the same way, one of thirty V_L and one of four J_L genes will be used. The process starts in the early pre-B-cell and leads to a unique IgH and IgL rearrangement. If the B-cell enters the GC, it will undergo somatic hypermutation (SHM) and isotype class switch recombination (CSR), thus being selected to recognize a given antigen. Leaving the germinal center, this cell will become a memory cell or an immunoglobulin (Ig) producing plasma cell. In case that one or more oncogenic events take place in a random step of this process, doting the cell with a survival advantage and rendering it prone to proliferation, the resulting daughter lymphoma cell will be identical and, if it has the ability to differentiate into an Ig producing cell, it will secrete a monoclonal component. Consequently, all B-cell mature neoplasms[2] share a somewhat common origin as well as the inherent ability to produce a monoclonal Ig (Figure 1).

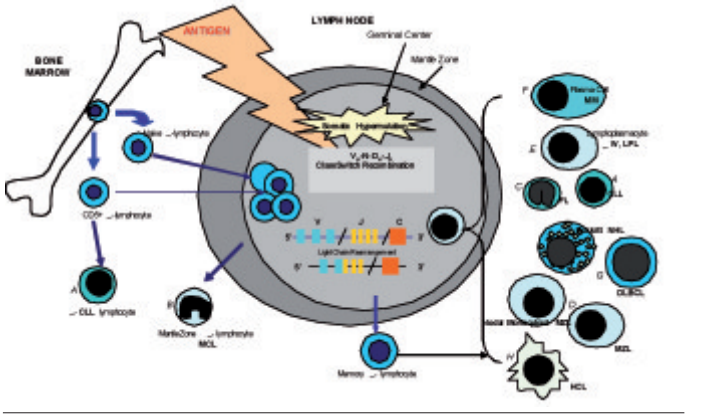

Figure 1. B-Cell Evolution Leading to Mature B-Cell Neoplasms. Modified from Küppers *et al.*, N Engl J Med 1999; 341:1520-1529. A: B-CLL/SLL B-cell [CD5$^+$, CD23$^+$] may arise from a pre-GC B-lymphocyte or from a post-GC B-cell which has undergone SHM. B: The majority of MCL cells [CD5$^+$, CD23, bcl-1$^+$, cyclin D1$^+$] possibly originate from a naïve B-cell of the mantle zone although a subset is of post-GC origin. C: FL lymphocyte [CD5-, CD23-, CD10$^+$, bcl2$^+$] is thought to derive from the GC. D: MALT lymphoma and MZL cells [CD5$^-$, CD23$^-$] are believed to develop under chronic antigenic stimulation. E: LPL/WM lymphoplasmacyte [CD5-, CD23-] is thought to be of post-GC origin. F,G,H: The same is believed for MM plasma cell [CD38$^+$, CD138$^+$], DLBCL and hairy cell.

VH Usage, Somatic Mutations and Antigen Selection in B-NHL

A biased V_H usage has been observed in B-NHL cells, which differs from the one seen in normal B-lymphocytes. It was suggested that a preferential V_H usage may lead to a particular cell behavior or association with autoimmune phenomena. The rate of SHM found in NHL B-lymphocytes gives some clues regarding the pre or post GC nature of the cell. Furthermore, the mutational status constitutes a prognostic factor in some entities, as in CLL. Evidence of antigen selection has been found in some B-NHL, such as MZL and mucosa associated lymphoid tissue (MALT) lymphoma, in some DLBCL, and in multiple myeloma (MM). A tentative report of the observed prevalence of V_H/V_L usage, presence of SHM and CSR in various studies, is shown in table 1. It was recently shown that the malignant WM B-cell lack intraclonal heterogeneity and CSR, while, on the contrary, the mutations of switch regions essential for CSR were present in IgM-MGUS, suggesting that a minority of IgM-MGUS could progress to WM3. A biased V_H3/J_H4 usage was also observed in WM cells by the same group, in keeping with previous studies (see table 1). Results from our group in 11 WM patients showed SHM in 10 of 11 (91%). A V_H3 usage was observed in 6 of 11 (56%) and a J_H4 in 7 of 11 (64%).

Genetic Events

Some B-NHL, such as MCL and FL, display genetic hallmarks that help in diagnosis as well as in the understanding of their pathophysiology. In MCL, the chromosomal translocation t(11;14) leads to overexpression of cyclin D1 and deregulation of cell cycle control; in FL, the t(14;18) prevents the normal switching off of the bcl-2 protein, inhibiting apoptosis of GC cells. However, numerous additional oncogenic genetic events have been described in both entities. In the other B-NHL, unspecific genetic events have been observed, some of which with an increased prevalence and/or a prognostic impact. For example, in CLL, deletion 13q14 is found in 60% of cases and is associated with a favourable prognosis while, on the contrary, the 10-15% of patients with del 17p13 or 11q23 have shorter survival. Genetic alterations at 9p13 involving the PAX-5 gene have been observed in half of LPL cases, but this was not confirmed. Recent studies suggested that in WM deletions of 6q are frequently present, they may confer a worse prognosis and their presence may help in differentiating WM from IgM-MGUS in which they were not found.[4] The absence of 6q deletions in IgM-MGUS possibly indicate that this abnormality represent a secondary event. It was suggested that a region of the chromosome 6q harbours a tumor suppressor gene of pathogenetic significance in WM. The possible role of BLIMP-1, a tumor suppressor gene located in the 6q21 locus and regulating B-cell proliferation and differentiation, is under investigation. However, 6q deletions are encountered in about 30% of B-cell NHL by conventional cytogenetics[5] or fluorescence *in situ* hybridization (FISH). It is found as a secondary event to other chromosomal abnormalities. Different regions of the long arm of the chromosome 6 are found to be missed in various B-NHL, as shown in table 2. A recent gene-expression profiling study showed that WM clustered with CLL and normal B-cell on unsupervised clustering and had a phenotype similar to CLL and very different to MM and normal plasma cells; of note, the most significantly upregulated gene in WM was interleukin-6 (IL-6).[6]

Table 1. Reported Prevalence of VH Usage, Somatic Mutations and Antigen Selection in B-NHL.

Disease	*V_H Usage*	*SHM*	*Isotype CSR*	*Study*
IgM-MGUS		Yes	yes	Kriangkum *et al.*, 2006
WM		Yes	No	Aoki *et al.*, 1995
	V_H3 / J_H4	Yes	No	Sahota *et al.*, 2002
	V_H3 / J_H4	Yes	No	Kriangkum *et al.*, 2004
		Yes*	Yes*	Martin-Jimenez *et al.*, 2004
CLL		Yes (50-70%)		Fais *et al.* 1998
		Yes (50-70%)		Vasconselos *et al.* 2003
			Subset of CLL	Oppezzo *et al.* 2003
			Subset of CLL	Fais *et al.* 1996
	VH1-69 UM			Widhopf *et al.* 2001 and Potter *et al.* 2003
	VH3-21 independent of M** or UM***			Tobin *et al.* 2002
	V_H1-69, V_H3-23, V_H5, V_H6, V_H4-34			Fais *et al.* 1998 Rosenquist *et al.* 1999 Stevenson *et al.* 1995 Kipps *et al.* 1998 Johnson *et al.* 1997 Stewart *et al.* 1993 Tobin *et al.* 2002 Tobin *et al.* 2003
MCL	V_H4-34, V_H3-21 V_H5-51			Thorselius *et al.* 2002
	V_H3-21, V_H3-23, V_H4-34 and V_H4-59	No (80% UM***)	yes	Camacho *et al.* 2003 and Bertoni *et al.* 2004 Babbage *et al.* 2004
FL	no	Yes		Rosenquist *et al.* 1999 and Nope *et al.* 1999
SMZL	V_H1-2 SMZL / V_H4-34 NMZL	Yes		Traverse-Giehen *et al.* 2005
DLBCL	no	Yes		Rosenquist *et al.* 1999
MM	V_H1-69, V_H3-9, V_H3-23			Rettig *et al.* 1996
	V_H3-30, V_H3-15	Yes		Gonzales *et al.* 2005

**In a single patient, ** M: mutated, ***UM: unmutated.*

Table 2. Prevalence of 6q deletions in lymphoproliferative disorders.

Disease	*Karyotype*	*FISH*	*region of 6q deleted area*						*Study*
			6q15	6q21~22	6q23	6q24.1	6q25.1	6q27	
IgM-MGUS	0/12	0/12							Fonseca *et al.*, 2006
WM	0 4/55* (7%)	21/38 (55%) 40/102 (39%)			6q23~q24.3 6q21				Fonseca *et al.*, 2006 Fonseca *et al.*,2006
LPL *BM* *nodal*	 1/4 (25%) 0/10	 1/4 (25%) 0/10***			 6q23~24				 Zhang *et al.*, 1997 Cook *et al.*, 2005
CLL	 4/9 (44%)	2/2 5/9 (55%)	nd	x1	x2 6q23~24	x0	x2	x2	Taborelli *et al.*, 2006 Zhang *et al.*, 1997
MCL	2/3 (66%)	2/3 (66%)			6q23~24				Zhang *et al.*, 1997
FL	 6/14 (43%) 30% (n=336)	7/7 10/14 (70%)	x0	x2	x2 6q23~24 6q11-27	x3	x1	x3	Taborelli *et al.*, 2006 Zhang *et al.*, 1997 Höglund *et al.*, 2004
SMZL		2**/14			6q21				Cuneo *et al.*, 2001
DLBCL	10/71 (14%) 2/7 (28%)	 7/13 4/7 (56%)	x1	x5	not defined x6 6q23~24	x3	x5	x7	Vitolo *et al.*, 1998 Taborelli *et al.*, 2006 Zhang *et al.*, 1997
MM		4/14 (28%)			6q27				Amiel *et al.*, 1999

*nd: not determined, *55/102 had available cytogenetics information, ** both were transformed into high grade histology, ***using a 6q21 probe*

Table 3A. Prognostic Systems in Untreated WM Patients.

Study	*#*	*PF used in the system*	*Survival*	*P*
Gobbi *et al.*, Blood 83; 2939, 1994	144 90 EG + 54 VG	1. Age ≥ 70 y 2. Hb < 9 g/dL 3. weight loss 4. cryoglobulinemia	0-1 point, mS 48 mo 2-4 points, mS 80 mo	0.008
Morel *et al.*, Blood 96; 852, 2000	485 232 EG + 253 VG	1. Age ≥ 65 y 2. Albumin < 4g.dL 3. at least 1 cytopenia 4. 2 or 3 cytopenias	0-1 point, 5y OS, 92% 2 points, 5y OS, 63% 3-4 points, 5y OS, 27%	<0.0001
Dimopoulos *et al.*, Ann Oncol 14; 1299, 2003	122	1. Age ≥ 65 years 2. Hb < 10 g/dL	0 point, mS 172 mo 1 point, mS 107 mo 2 points, mS 46 mo	<0.0001
Kyrtsonis *et al.*, Blood 102; S1, abstr 4814, 2003	220 108 EG + 122 VG	1. Age ≥ 65 years 2. Hb < 11 g/dL 3. Lymphadenopathy	0 point, 10y OS, 60% 1 point, 10y OS, 87% 2 points, 10y OS, 5% 3 points, 10y OS, 0%	0.001
Merlini *et al.*, Semin Oncol 30; 211, 2003	215	1. Age < 60y + Hb ≥ 10 g/dL 60 years + alb ≥ 3.5 g/dL 2. Otherwise 3. Age ≥ 60y + Hb < 10 g/dL 60 years + alb < 3.5 g/dL	Group 1, 10y OS, 54% Group 2, 10y OS, 32% Group 3, 10y OS, 4%	<0.0001
Ghobrial *et al.*, BJH 133; 158, 2006	410	1.Age > 65 years 2.organomegaly 3. PLT < 150 x 109/L	0 point, 10y OS, 57% 1 point, 10y OS, 16% 2 or 3 points, 10y OS, 5%	<0.0001

PF: Prognostic Factor, EG: Exploratory Group, VG: Validation Group, mS: Median Survival, OS: Overall Survival, alb: serum albumin.

The Role of the Malignant Lymphocyte's Microenvironment

The participation of the bone marrow (BM), LN or other extranodal (EN) milieu is needed for the survival and proliferation of most malignant B-cell types. Microenvironmental cells secrete cytokines that constitute growth factors for the neoplastic B-cell, and proangiogenic factors to promote neoangiogenesis that, in turn, contributes to malignant proliferation and spread. They also secrete or express adhesion molecules and chemokines, essential for lymphocyte recruitment, circulation and homing. Research in this field is extensive and ongoing. Very little is known on the microenvironmental role in WM. The contribution of mast cells has been suggested by *in vitro* experiments in which co-culture of autologous BM mast cells with lymphoplasmacytes from WM patients led to mast cell dose-dependent tumor colony formation and/or proliferation, through constitutive CD154-CD40 signaling.[7] Serum levels of interleukin-6 (IL-6) and of its soluble receptor (sIL-6R) have been reported increased in WM. B-cell–stimulating factor (BLyS), that modulates normal B-cell development, stained positively in BM sections of WM patients; in addition, serum BLyS levels were found increased.[8] Results from our group in 35 WM and 19 LPL patients at diagnosis showed elevated serum soluble syndecan-1 levels in both groups compared to healthy individuals; serum VEGF and IL-6 levels were higher in WM than in LPL patients (p=0.014 and 0.042 respectively) while serum Blys levels were higher in LPL patients as compared to both WM patients and healthy individuals (p=0.001 and 0.018 respectively).

IgM-SECTING LYMPHOMAS' CLINICAL OVERLAP

Clinical Manifestations

Patients with B-NHL, secreting IgM or not, will present with lymphoma-associated symptomatology, i.e LN or S enlargement, EN involvement or BM disease with or without a leukemic picture. These findings vary among the different NHL subtypes. For example, MCL is more frequently associated with generalized lymphadenopathy, SMZL with splenomegaly; FL or DLBCL with lymphadenopathy, etc. Fatigue, disease related fever, autoimmune phenomena or symptoms related to BM failure, may also be present. In patients with a serum monoclonal IgM, the IgM-syndrome related symptomatology includes hyperviscosity symptoms (headache, blurred vision, cardiac failure), and in a minority of cases peripheral neuropathy, bleeding tendency, nephrotic syndrome, cryoglobulinemia-associated skin lesions, etc. Nevertheless, in IgM-secreting NHL, a considerable overlap in clinical manifestations is observed and diagnosis should be further based on morphological, histopathological, immunophenotypic and genetic findings.[9,10]

Disease Course and Prognostic Factors

WM is usually an indolent disease with a prolonged survival; however, some patients present a more aggressive course. Routine prognostic factors of survival in WM are age, anemia, the presence of cytopenias, serum IgM levels, serum albumin levels, β2-microglobulin, performance status and the presence of lymphadenopathy-organomegaly. Prognostic systems based on the aforementioned factors have been developed (Table 3A).*Classical* prognostic systems used in NHL are the international prognostic index for aggressive lymphomas (IPI), the follicular lymphoma international prognostic index (FLIPI) for FL and the international staging system (ISS) for MM. These systems have been validated in other subtypes of NHL including WM (Table 3 B). Recently, a prognostic system based on serum monoclonal IgM component, sex, and haemoglobin was proposed for the prognostication of both IgM-MGUS and indolent WM evolution to WM.[11] Almost the same system has been applied for WM patients requirering treatment (see Table 3A). *Conclusions*. New findings enlightening many aspects of WM disease biology are appearing. However, for the time being, none of these data is specific enough to enable the accurate discrimination of WM from other IgM-secreting B-NHL with overlapping features.

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Table 3B. Classical NHL Prognostic Systems Tested in WM.

System	IPI[1]	FLIPI[2]	ISS[3]
PF used in the system	1. Age ≤60 vs ≤ 6 y. 2. AAS III&IV vs I&II 3. No of involved extranodal areas ≤1 vs ≤ 1 4. LDH ≤ N vs ≤ N 5. PS ≥2 vs 0-1	1. Age ≥ 60 y vs <60 2. Hb < 12 vs ≥12 g/dL 3. AAS III&IV vs I&II 4. No of involved nodal areas ≤4 vs ≤ 4 5. LDH ≤ N vs ≤ N	1. β_2-M <3,5 mg/L + alb >3,5 g/dL 2. β_2-M <3,5 mg/L + alb <3,5 g/dL or β_2-M 3,5-5,5 mg/L 3. β_2-M > 5,5 mg/L
Applied Primary in	Aggressive NHL	FL	MM
Tested in WM by	Owen et al, Am J Clin Pathol 116; 420, 2001 Kyrtsonis et al, Ann Hematol 80; 722, 2001	Tzenou et al, submitted at EHA 2007	Dimopoulos et al, Leuk Lymphoma 45; 1807, 2003

[1]NEJM 329; 987, 1993. [2]Solal-Celigny et al, Blood 2003. [3]Greipp et al, JCO 23; 3412, 2005.
PF: Prognostic Factor, AAS: Ann-Arbor Staging, PS: Performance Status, β_2-M: Beta$_2$-Microglobulin, alb: serum albumin.

WM1.2

TRACKING THE MALIGNANT CELL OF ORIGIN IN WALDENSTROM'S MACROGLOBULINEMIA

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Background. Waldenstrom's macroglobulinemia (WM) is characterized by an IgM-expressing lymphoplasmacytoid lymphoma that infiltrates the bone marrow (BM). Typically, WM displays a morphological spectrum, from small B-lymphocytes to maturing cells with CD138 expression. Disease outcome in WM varies, with survival spanning 5-10 years. Factors determining outcome are as yet not known, but characterizing tumour biology in WM will be of value in understanding these. A central question in WM biology has been aimed at defining the cell of origin giving rise to disease and its clonal history. Most studies here have relied on the analysis of immunoglobulin (Ig) variable (V) genes in tumour cells, providing important insights. This arises from the role that V gene encoded determinants play in normal B-cell survival, where they allow antigen recognition via the B-cell surface receptor (BCR). Cognate antigen can trigger somatic mutation (SM) in the germinal center (GC) in secondary lymphoid organs, during normal B-cell development, for which the enzyme activation induced cytidine deaminase (AID) is a critical requirement.[1] A further modification of the BCR can also occur in the GC, when effector function is altered by isotype class switch recombination (CSR), again dependent on AID activity.[1] This involves double strand DNA breaks and recombination of isotype specific genes in the IgH locus on chromosome 14q32. Normal B-cells that exit the GC with mutated V genes circulate as memory B-cells, and generally express CD27.[2] sIgM^{+ve} memory B-cells can also home to the BM.

Mutational status V genes

In WM, tumour-derived V genes revealed SM in early small cohort studies, with no intraclonal variation in sequences between tumour clones, consistent with neoplastic origins from a post-follicular B-cell.[3,4,5] Furthermore, analysis of isotype switch variants appeared to indicate that arrest occurs prior to switch events,[3] apparently substantiated by findings that switch events in WM are impaired *in-vivo*.[5] However, as the numbers of cases examined has increased, some WM tumours have emerged which display unmutated (UM) V_H genes,[4] indicating origins from a naïve, pre-GC cell of origin. Although the extent of UM WM is as yet not known, it clearly indicates that disease origins in WM are heterogeneous. To probe disease presentation further in WM, we evaluated V_H gene features by contrasting cohorts where cDNA had been extensively amplified (amp-cDNA) to increase sensitivity with cases where cDNA was prepared conventionally.[6] Overall, 16/16 cases revealed mutated (MUT) VH genes, suggesting that the UM subset may be a minor component in WM. Mutational status in WM, however, could potentially be an important predictor of outcome, as defined in IgM-expressing chronic lymphocytic leukemia (CLL), where the UM subset has a profoundly poorer outcome.[7]

CSR

Surprisingly, we observed tumour-derived isotype switch variant transcripts in WM using amp-cDNA. Tumour V(D)J-Cγ co-existed with Cα (6/7 cases), and with Cγ (3/7 cases) transcripts, with assays indicating a low frequency.[6] Sterile germline transcripts and switch circle transcripts, generated from excised switch circle DNA, and the hallmark of deletional CSR, could also be identified. *AID* transcripts were also present, indicating a low level of CSR events *in vivo* in WM. These observations were substantiated in WM cases in which cDNA had not been amplified (3/9 cases), with data also pointing to limitations of small WM cohort studies that can clearly overlook tumour-associated events occurring at subclonal levels.[3,4,5] Some WM cells however, have also been shown to undergo isotype switch *in vitro* following appropriate stimuli.[5] Deletional CSR with *AID* expressed poses a potential risk to the genome, and may underlie recurrent abnormal chromosomal translocations in the IgH switch region. In multiple myeloma (MM), IgH switch region (S_H) translocations are frequent clonal events, revealing isotype switch as an important stage in disease origins.[8] In contrast, this does not appear to be the case in MUT WM. Although CSR events occur in some WM cells, they are subclonal and appear not to trigger abnormal 14q32 genomic events, as these are not found in the vast majority of cases analyzed.[9] In this regard, parallels exist with CLL,[7] where although UM cases express both switch transcripts and AID, they display little evidence for any switched Ig expression or S_H chromosome 14q32 abnormalities, negating their role in pathogenesis, as in WM.

CD27 expression

Another feature that impacts on deciphering origins of WM is the expression of the post-GC marker, CD27,[2] also found on normal IgM-expressing memory B-cells. Variable CD27 expression in MUT WM has been proposed as indicative of origins of disease from an unusual memory B-cell that bypasses the GC.[5] To assess this, WM tumour cells were analyzed at a sub-population level.[6] In 2/2 cases, mutated tumour cells were identifiable in both CD27^{+ive} and CD27^{-ve} fractions, confirming heterogeneous CD27 expression within an otherwise monoclonal tumour. The question arises whether this is due to origins from a cell lacking CD27 and undergoing ectopic SM, with tumour cells then acquiring CD27, or whether MUT WM derives from a CD27^{+} post-GC memory B-cell which progresses to loss of CD27 expression. On-going, possibly ectopic, mutations with AID expressed can certainly occur in CD27^{-ve} tumour cells, as we have shown in hairy cell leukemia,10 whereas intratumoural loss of CD27 in MM associates with advancing disease, possibly related to escape from CD27-CD70 apoptotic signals.[11] Furthermore, the existence of CD27^{-ve} memory B-cells remains controversial, and data from our current work to address this in relation to WM will be presented at the IVth IWWM meeting, 2007.

Intratumoral diversification in WM

Isotype switched tumour cells could be tracked further in some WM single cells from both CD27^{+ve} and CD27^{-ve} fractions, at a low frequency (3/45 cells).[6] Switch activity therefore remain a feature of the evolving clone as it losses (or gains) CD27 expression. In single WM cells, none were found to co-express V(D)J-Cμ and V(D)J-Cα/γ, confirming deletional CSR events. The expression of *AID* in some pre- and post-switch cells also raises the possibility that it could catalyze abnormal gene modifications. Excised DNA during CSR, as can occur in WM, has a potential for destabilizing gene expression via re-insertion as transposons. Interestingly, when the pattern of SM was compared in single WM cells, there was unexpected evidence for on-going somatic mutation in V(D)J-Cμ sequences, in both CD27^{+ve} and CD27^{-ve} fractions.[6] AID was also identifiable in CD27$^{\pm ve}$ cells, suggesting on-going ectopic mutational events, occurring at a low frequency. Initiation of SM in normal B-cells requires antigen signaling via sIg, and although most WM tumour cells will express sIgM, it is at present not known whether this plays a role in mediating signals to initiate SM, or indeed what the nature of the antigen might be. It is also as yet not defined whether on-going mutations in WM are associated with a blast phase, as is seen in normal GC B-cells, and what role this may have in feeding the tumour population. It does however indicate that the BM is conducive to mutational activity. A striking feature of lymphoma cells that undergo continual SM, and remain in the GC site, is their ability to generate novel N-glycosylation motifs via mutated nucleotides in V genes. These are functional and in follicular lymphoma appear mandatory, suggesting a role in tumor-stroma interactions.[12] Given that localised mutations can be identified in MUT WM, we examined a series of 14 cases for such sites, using paired V_H and V_L analysis. In these, and in a further 7 WM V_H genes, the incidence of glycosylation sites was at a low, background level. SM in these WM therefore appears not to lead to acquisition of glycosylation sites. Such modifications are also absent in MUT CLL. These findings indicate no relationship between WM and GC lymphoma tumors. Instead, location in BM and heterogeneity in SM activity further point to a closer similarity to CLL of tumour behaviour at this site. Of note, proliferating centres in CLL are localised to the BM.[7] It is most likely that a low level of switching in some WM cells occurs as post-transformation events, since a cell of origin that is continuously doing so would invariably generate a marked IgG/A component in this disease on a frequent basis, and this is uncommon. As these and mutational events are on-going in some WM cells, they do raise the possibility that a degree of clonal expansion could occur on a few occasions, but that in general either disease associated or disease-stroma associated factors appear to advantage the IgM-expressing clone. However, this could be compromised on rare occasions, and may in fact underlie recent observations in a case study of WM, where although the patient presented with a typical IgM only spike, switched tumour-derived progeny comprising 41% of tumour cells together with 57% of the persisting IgM clone could be detected 4 years later.[13] Interestingly, in that study rituximab therapy ablated the IgM^{+} clone, but the IgG^{+} subclone persisted, raising the scenario that CSR events in this case may also have generated further genetic hits to potentiate survival. *Concluding comments*. In summary, these observations are consistent with the concept of neoplastic arrest in most WM as occurring in a mutated IgM-expressing memory B-cell, which retains the capacity to undergo a low level of SM and isotype switching in some cells following transformation.

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WM1.3

IMMUNOGLOBULIN GENE REARRANGEMENTS IN WALDENSTRÖM'S MACROGLOBULINEMIA

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The characterization of VDJH rearrangements as well as related processes such as somatic hypermutation (SHM) and class switch recombination (CSR) have largely contributed to gain insights in the pathogenetical development of B-cell Lymphoproliferative disorders (LPD), because the differentiation process follows a strict hierarchical order in generating the Ig repertoire.[1,2] Gene segment usage, CDR3 composition and somatic hypermutation rates have been described for some B-cell malignancies and B-cell subtypes, where usage of particular VH and DH families and gene segments is often biased.[3,4] In B cell chronic lymphocytic leukemia (B-CLL) the presence of somatic hypermutation (SHM) in the IgH genes is correlated with a more favorable prognosis compared to unmutated cases.[4] In addition, the pattern of SHM and CDR3 composition has been associated with particular VH families and gene segments in B-CLL[4] and multiple myeloma (MM)[5] and incomplete rearrangements are frequently found in precursor B-cell acute lymphoblastic leukemia, MM, and Hairy Cell Leukemia. These characteristics are not completely known in Waldenström Macroglobulinemia (WM) because the low frequency of the disease hampers such analyses. We have recently characterized complete VDJH and incomplete DJH rearrangements in 81 IgM monoclonal gammopathies: 44 symptomatic WM, 27 asymptomatic WM and 10 IgM Monoclonal Gammopathies of Uncertain Significance (IgM-MGUS). Monoclonal VDJH rearrangements could be amplified in 90% of patients, with no differences between the three sub-entities. VH selection was biased in WM, since the most frequently used family and single segment were VH3 and VH3-23 (74% and 25%, respectively), which markedly differs from the repertoire in normal B-cells and MM. Interestingly the VH3-23 segment was never selected in Ig-MGUS (0% vs. 28%, p=0.05). In addition, the VH4-34, which is frequently selected normal circulating B cells,[5] B-CLL,[4] and others such as B-ALL and diffuse large B-cell lymphoma, was never selected in our WM cases and only in one IgM-MGUS. This concurs with the hypothesis that VH4-34, a gene segment frequently associated with autoimmune diseases, is prevented from the normal PC repertoire and neoplastic functional B-LPD such as MM.[4] In the same line, the VH1-69, V3-07 and VH3-21, which are overrepresented in B-CLL, were selected by 0%, 0% and 4% of our IgM monoclonal gammopathies. In addition, the highly frequent selection of the VH3-23 seems to be specific of WM. This differential repertoire selection respect to CLL is on the other side very similar to that observed in MM. Accordingly, these findings reinforced the similarities between WM and MM in a moment in which mRNA expression studies were emphasizing the closeness between WM and B-CLL.[6] This suggests that the origin WM remains between both diseases in the B-cell differentiation. As far as the DH and JH distribution of the VDJH segments are concerned, they did not differ from B-lymphocytes in healthy individuals or other B-cell neoplasias. In addition, monoclonal incomplete DJH rearrangements were detected in 48% of our IgM monoclonal disorders. Interestingly, only one case of IgM-MGUS displayed an incomplete DJH rearrangement, in opposition to the symptomatic or asymptomatic WM cases (10% vs. 54%, p=0.01). Somatic hypermutation with >2% deviation from the germline was seen in 89% of all cases, without significant differences between different symptomatic, asymptomatic and MGUS cases. However, using the number of mutations as continuous variable, symptomatic cases demonstrated a higher grade of somatic hypermutation (SHM), since the median percentage of changes was 6.61%, 76.40% and 9.38%, although differences did not achieve statistical significance (K-W, p=0,277). Such differences were mainly attributed to the VH segment usage, since VH3-23 segments, which were never used in MGUS, displayed a higher grade of SHM than the remaining segments (10.9±2.9 vs. 6.8±3.7, p<0.001). These findings did not relate with any specific clinical characteristics, since all clinical parameters at diagnosis were similar between patients with high or low SHM rate. The only exception was the time to the therapeutic requirement, which was shorter in unmutated patients, although differences were not statistically significant and this unfavorable parameter did not have any impact on the overall survival when the study was closed. The lack of clinical relevance of the unmutated cases in this series reinforces again the dissimilarities between B-CLL and WM, since the presence of unmutated VDJH rearrangements could be the most important prognostic parameter in B-CLL. Regarding DJH rearrangements, all were unmutated, which would make them an eventual attractive target for minimal residual disease investigation. In our study, mRNA transcripts could be evaluated in 21 WM and 7 IgM-MGUS patients. IgM clonotypic transcripts were observed in all cases, while IgD was observed in 83%. Interestingly, non-clinical isotypes (IgA and/or IgG) were seen in three WM (14%) and one IgM-MGUS (14%) patients. This requires a recombination that has been assumed to be impossible in WM.[7,8] However, clonotypic transcripts encoding post-switch isotypes have been observed *in vitro* in WM and IgM-MGUS cells cultured with CD40L/IL-40.[2] In our series we show that this process is possible *in vivo*; in addition, one of the three cases was able to produce a fully monoclonal IgG protein together with the original IgM.[9] Very recently, this phenomenon has been shown to be possible in selected cells of all cases of WM.[10] The clinical relevance of this finding remains to be explored since in our series it did not associate with any specific clinical characteristic. In conclusion, characterization of IgH rearrangements in an extensive series of IgM related disorders allow documenting some WM and IgM-MGUS dissimilarities which could suggest a distinct differentiation process between them. In addition, this characterization allows the identification of differences and similarities with other B-cell Lymphoproliferative disorders that can help to more precisely arrange the tumor target cell of WM along the B-cell differentiation process.

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WM1.4

INHERITED AND ACQUIRED MUTATIONS IN WALDENSTROM'S MACROGLOBULINEMIA (WM): WM AND MM HAVE CLOSE GENETIC RELATIONSHIPS NOT SHARED WITH B-CLL

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Hyaluronan synthase 1 (HAS1) synthesizes, a highly polymeric sugar molecule that is important in cell motility, signaling and mitosis.[1] We identified three aberrant splice variants of HAS1 transcripts, two of which are the result of partial intron retention (intronic splicing), that were found only in patients with WM or multiple myeloma (MM).[2,3] Intronic splicing has been reported only in cancer cells, and is absent from cells of healthy individuals. For 146 MM patients, expression of the intronic HAS1Vb splice variant was strongly correlated with reduced survival (p=0.005)[3] (unpublished). HAS1Vb results from *splicing out* of exon 4 and retention of a segment of intron 4, thereby causing an inframe shift and a new stop codon in exon 5, leading to a truncated protein. HAS1Vc also results from aberrant splicing causing an inframe shift and a new stop codon; HAS1Va which results from deletion of exon 4, an inframe shift and a new stop codon also correlates with reduced survival though at a lower level of significance than is seen for HAS1Vb. Our evidence supports the idea that the truncated HAS1 splice variants are translated and functional in WM and MM. Although the mechanism whereby aberrant HAS1 splicing influences survival is as yet unclear, our analysis of HAS1Vb transfectants shows that HAS1Vb has an intracellular localization to cytoskeletal elements. Although most HA is extra-cellular, HAS1Vb appears to be the only member of the HAS1 family that synthesizes intracellular HA,[3] which may alter mitosis.[4] Aberrant splicing arises from either (a) sequence variations of the DNA localized in splicing elements, or (b) abnormalities in the splicing factors whose assembly is directed by the specific sequences of DNA i.e. splicing elements, to carry out alternative splicing of nuclear pre-mRNA. Based on the classical dogma of splicing, we predicted that mutational events in splicing regions of the HAS1 gene itself were responsible for altered spliceosome assembly, with consequent aberrant splicing of HAS1 pre-mRNA and significantly reduced patient survival. To determine whether inherited germline origin polymorphisms and/or genetic variations, or acquired HAS1 mutations had the potential to alter pre-mRNA splicing of HAS1 to generate HAS1Vb and the associated reduction in patient survival, we sequenced the regions of genomic HAS1 predicted to control generation of the observed splice variants. Because aberrant splicing was occurring in the region of HAS1 exon 3 to exon 5, we carried out extensive sequencing of this region of genomic HAS1 from 7 MM, 6 WM, 5 B-CLL, 3 MGUS and two healthy donors. To gain an appreciation of the mutation pattern of genomic HAS1, we sequenced exon 3 to exon 5 from buccal cells as well as from sorted subpopulations of hematopoietic cells, including malignant B and plasma cells and non-malignant T cells. For some patients, we also sequenced HAS1 from sorted CD34[+] hematopoietic progenitor cells. We identified a series of 182 recurrent and unique mutations in HAS1, some of which were germline in origin and hence inherited, and others which were acquired in somatic cells (defined as being present in hematopoietic and/or malignant cells, but absent from buccal cells). Somatic mutations included those found in all populations of hematopioietic cells tested (including CD34[+] progenitor fractions, but not in buccal cells) and tumor-specific mutations found only in malignant B and plasma cells. Minor alleles for some inherited HAS1 single nucleotide polymorphisms reported by NCBI were found to be significantly over represented in WM and MM, as compared to healthy donors[5,6] (unpublished)(Adamia *et al.* this volume). Unexpectedly, we identified numerous recurrent somatic and germline origin mutations in the sequenced *HAS1* exons and introns - that is the same somatic mutations were found in many to most of the patients analyzed, even though all were unrelated and their cancers were not familial in nature. These were not found in *HAS1* of B-CLL, MGUS or healthy donors analyzed so far. A substantial number of recurrent *HAS1* mutations were shared among MM and WM patients. Most of the recurrent somatic mutations are non-randomly clustered in the vicinity of predicted splicing elements on *HAS1* exons and introns. Furthermore, analyzing the impact of these recurrent mutations on spliceosome assembly using bioinformatic tools predicted a profound impact of mutational clusters on splicing patterns. Provocatively, one cluster of recurrent mutations precisely predicted the splicing pattern required to generate HAS1Vb. We speculate that inherited *HAS1* polymorphisms predispose to WM and MM, but not to B-CLL, followed by progressive acquisition of recurrent *HAS1* mutations in hematopoietic progenitors which pass these mutations to all cells of the hematopoietic lineage as normal differentiation proceeds. These somatic mutations increase the potential for aberrant splicing and hence the risk of developing the disease. Finally acquisition of somatic *HAS1* mutations, both recurrent and unique, occurs in the tumor progenitors, providing a marker for the earliest stages of disease. In combination with the accumulated preceding mutations, cancer -specific mutations, may be the final stage in promoting aberrant HAS1 splicing, thus setting in motion abnormalities that culminate in overt malignancy. The presence of the same somatic mutations independently arising in a group of unrelated patients having either of two otherwise unrelated diseases implies the influence of a strong and consistent selection during oncogenesis for specific *HAS1* mutations during the originating events leading to MM and WM. The presence of shared somatic mutations among MM and WM patients implies that the common early stages of oncogenesis are shared between these two cancers. The cell type in which the culminating genetic events occur determines whether an individual develops MM or WM. Thus, WM and MM have a shared genetic history. In contrast, none of the B-CLL patients analyzed to date have *HAS1* mutations, indicating that WM and B-CLL are unrelated at the early genetic level, despite work showing them to have similar gene expression profiles.[7] It seems likely that for WM and B-CLL, shared gene expression profiles reflect common B lineage differentiation stages rather than mechanistically related transformation events or disease-related characteristics. We have found that inherited predispositions and progressively acquired somatic mutations in the *HAS1* gene of individuals at risk of WM and MM correlate with abnormal intronic splicing of *HAS1*, which in turn has a strong correlation with reduced survival. Our preliminary work suggests that this progressive accumulation of recurrent somatic mutations in the hematopoietic lineage and then in the tumor cells themselves accompanies development of WM and MM, but does not characterize B-CLL. This suggests that *HAS1* may play a central role in a shared oncogenic process contributing to transforming events that underlie both MM and WM. Furthermore, clinical monitoring of patients to determine the mutational status of their HAS1 genes may provide a powerful predictive test for assessing the risk of transformation to overt malignancy. This approach has considerable potential to predict risk for individuals with pre-malignant conditions as well as for monitoring the transition from premalignant to malignant disease. It holds considerable promise for the development of risk assessment strategies, for early detection and monitoring of malignancy before, during and after therapy, and to assess response and predict relapse. As well, the pattern of tumor-specific recurrent somatic mutations is likely to provide a common clonal marker for all such patients. This may enable regular monitoring strategies for every patient to unequivocally identify clinically cryptic tumor cells in the early stages of emerging malignancy, as well as precise molecular monitoring of the response to treatment and stratification of monoclonal gammopathies with the greatest risk of transformation to WM.

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WM1.5

GENETICS AND GENOMICS OF WALDENSTRÖM MACROGLOBULINEMIA

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Waldenström's macroglobulinemia (WM) is an incurable B-lymphoproliferative disorder characterized by lymphoplasmacytic differentiation and associated with monoclonal immunoglobulin M (IgM) secretion.[1,2] This disease is characterized by the proliferation of the tumour clone into the bone marrow. A precise identification of the normal counterpart of the WM clonal cell has not been established, although evidences support that they resemble post-germinal centre memory B-cells that have undergone somatic hypermutation, but transform before isotype switching.[3-8] Class switch rearrangements involving the m switch region were investigated, with no rearrangements identified.[9] The etiology of this syndrome is unknown. WM is believed to be predominantly a sporadic disease, however several reports have identified a familial component, suggesting the existence of a germ-line WM susceptibility gene(s).[10-11]

Chromosomal abnormalities in Waldenström macroglobulinemia

Karyotype abnormalities

The genetic basis of the disease is poorly understood. Limited cytogenetic studies have been performed in WM, and identification of recurrent chromosome abnormalities associated with the pathogenesis has not been very successful because normal metaphases are usually prevalent in karyotypic analysis[8,12-14] The incorporation of the cIgM-FISH technique has allowed analyzing interphase nuclei, overcoming the problems associated with low tumour cell division rate. Despite the clinical difficulty to differentiate this syndrome from other B-cell lymphoproliferative disorders with monoclonal IgM, recent cyto-molecular studies suggested that WM seems to show a unique genomic profile.

Chromosome 6 deletions

The most frequently identified abnormality in WM is the deletion of the 6q arm. Karyotype studies showed this abnormality in 16% of WM patients, but we reported a rate of >50% by using cIgM-FISH.[9] This deletion usually involves chromosome bands 6q21-q23, being the q23 region the most commonly deleted. Several tumour suppressor genes are localized in that chromosomal region such as *BLIMP1* and *MYB*, but an association between deletion and decrease or loss of function of any of them remains to be documented. The 6q deletion is clonally selected. It is likely a progression event since we have shown that patients with Monoclonal Gammopathy of Unknown Significance and isotype IgM (IgM-MGUS) rarely if ever have 6q deletions.[14] Additionally, this abnormality is uncommon in nodal lymphoplasmacytic lymphoma - LPL.[15] The high prevalence of 6q deletion and its unique presence in WM, compared to nodal LPL or IgM-MGUS, suggest a differentially cytogenetic profile associated with this abnormality. The presence of 6q deletions is not known to have clinical associations, but a recent study of ours suggested that patients with 6q presented significantly higher levels of b2m (p=0.001), anemia (p=0.01) and hypoalbuminemia (p=0.001), all features associated with poor prognosis.[16] Moreover, patients with 6q deletion display a shorter treatment-free survival (median of 55.2 months versus not reached in patients without the abnormality after 100 months of follow-up; p=0.03).

Other chromosome abnormalities

Unlike IgM-MGUS and IgM Multiple Myeloma (IgM-MM), translocations that involve the immunoglobulin heavy chain locus at chromosome 14 are very rare or absents in WM.[9,17] Originally, it was believed that WM contained the t(9;14)(p13;q32), involving PAX5 and IgH locus.[18] The t(9;14)(p13;q32) was reported in LPL with a frequency of 50%, but it seems to be restricted to cases with no detectable IgM monoclonal protein.[19] Additionally, the biological effects of up-regulated PAX5 caused by the t(9;14)(p13;q32) are not in agreement with the WM phenotype, due to PAX5 inhibits the production of the J-chain, which is essential in the IgM pentamer formation. In our cohort of WM patients we failed to detect this translocation.[9] Another report, including 69 patients has confirmed our findings.[20] The t(11;18)(q21;q21) translocation usually associated with a subset of marginal zone lymphomas has been reported in sporadic cases of WM(21). However, using the cIgM-FISH strategy we were unable to detect this translocation in our cohort of patients.[9] Our team was also able to show that deletions of 13q14 and 17p13 are not common at the time of diagnosis, but may be observed in 15% of patients at the time of disease progression.[22] Limited studies are available with regards to the ploidy status, showing that WM clonal cells are likely diploid. Our group supported this assumption in a cohort of 15 patients by using centromere probes for chromosomes 7, 9, 11, 12, 15, 17.[9] These chromosomes are frequently involved in numerical gain/losses in related diseases as MM and B Chronic Lymphocytic Leukaemia – B-CLL. However, we have not identified any numerical abnormality affecting that subset of chromosomes. In a recent study, Terre and colleagues[23] reported partial copy gain and trisomies of chromosome 4 in 8 of 39 patients (20%). With the incorporation of new technologies, as the array-based comparative genomic hybridization (aCGH), is becoming possible to realize a whole genome high-resolution study. Using this approach, we identified previously undetected recurrent chromosomal abnormalities such as 3p21-22 and 8p deletions, as well as 3q13-29 gains. We confirmed that ploidy status changes are not common events in WM, detecting low frequency of chromosomes 4, 7, 9, 18 trisomies and chromosome 21 monosomies. In patients with 6q and 8p deletions, we found high frequencies of 6p and 8q arm gains, respectively. Our data suggested that these gains are secondary events, because we were not able to detect these abnormalities in patients without its respective deletions. Conversely, deletions without associated arm gains were commonly observed (*manuscript in preparation*).

Gene expression profiling of WM

Use of gene expression arrays (GEP) as a marker for genomic abnormalities and subsequently as a tool for disease profiling is a powerful approach to characterize genomic changes that are responsible for disease pathogenesis in WM. We employed GEP for comparison of WM with MM, B-CLL, smouldering myeloma, MGUS and normal B and plasma cells as a means to identify differential gene expression signatures as well as deregulated genes associated with WM.[24] WM was found to cluster with CLL and normal B cells following unsupervised hierarchic clustering and only a small set of genes was found to be specific of the disease. We did not identify differences in GEP of patients with and without 6q deletions. The most significantly up-regulated genes were IL-6 and genes involved in the MAPK pathway. In relation with the D-type cyclins, WM and CLL only expressed *D3* whereas MM expressed all 3 cyclin D genes.[25] Further, Gutierrez and colleagues separated WM cells into those with B- (WM-BL) and plasma cell (WM-PC) morphology for gene expression comparison to CLL, MM and normal individuals.[26] Following unsupervised hierarchic clustering, WM-BL samples clustered with CLL while WM-PC samples were segregated with MM. Overall, the authors concluded that BL and PC from WM patients displayed differing patterns of gene expression when compared to BL and PC from CLL and MM. This study also identified up-regulation of *IL-6* in WM samples. This cytokine is currently being considered as possible therapeutic target, and also could explains the clinical observation of elevated C-reactive protein serum levels in many patients with WM. Additionally, this may be one of the many possible factors explaining anaemia in patients with WM. Finally, proteomic analysis of signalling pathways were performed in samples obtained from WM and MM patients.[27] Samples from both diseases were compared before and after treatment with a proteasome inhibitor.[27] While several overlaps in signalling were

observed between WM and MM, the authors believed this to be due to similar pathways utilized in cell signalling for B-cell differentiation. However, after clustering analysis were performed, the authors identified groups of proteins that were expressed by either WM or MM, but not both; which indicates differences in cellular response induced by proteasome inhibitor treatment.

Conclusion

WM is an incurable late B-cell malignancy that secretes high levels of immunoglobulin M. This disorder can typically be differentiated from MM based on heavy chain isotype of antibody produced (IgM) and the lack of lytic bone lesions. While clinically similar, the cytogenetics and genomics underlying MM and WM are quite different. Gene expression profile studies have suggested that WM is more similar to CLL and normal B-cells than to MM. In contrast to MM, the genetic of WM appears to be much simpler, with less observed aneuploidy and fewer structural abnormalities, being 6q deletion the most frequently observed. Although cyto-molecular data recognized a unique genomic signature in WM, our knowledge about molecular pathways involved in the pathogenesis and disease progression is still very fragmented. Tools such as array-based comparative genomic hybridization and gene expression profile allow us to realize a high-resolution whole genome screening for abnormalities as well as to gain insights into the consequences of genomic alterations found in WM. In the future, therapeutic decisions may be based solely on data transformed from these high throughput genomic studies into practical clinical tools used for patients with WM.

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WM1.6

IGM MGUS AND ASYMPTOMATIC WALDENSTRÖM'S MACROGLOBULINEMIA: PROGNOSTIC FACTORS AND EVOLUTION

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IgM monoclonal components (MCs) without evidence of either overt Waldenström's macroglobulinemia (WM) or other malignant lymphoproliferative disease (MLD) are known as IgM asymptomatic monoclonal gammopathies (aMGs), and can be further distinguished into IgM MG of undetermined significance (MGUS) and smouldering WM (SWM). Since variable diagnostic criteria were previously used to separate IgM MGUS from SWM, a reliable distinction of asymptomatic populations with different transformation risk into active disease was lacking until recently. The two entities were definitively divided from the clinico-pathological point of view during the 2[nd] International Workshop on WM (September, 2002).[1] Indeed, the unequivocal histopathological evidence of lymphoplasmacytic (LP) non Hodgkin's lymphoma (NHL) with an intertrabecular bone marrow (BM) infiltration pattern was recognized as the only parameter distinguishing SWM from IgM MGUS, characterized instead by the absence of BM infiltrates, or equivocal evidence of BM infiltrates without confirmatory phenotypic studies. In order to detect whether this definition allowed to identify two patient populations also differing in prognosis, a few studies have been performed so far. By evaluating retrospectively data from 207 IgM MGUS and 217 SWM defined according to the new criteria, Gobbi *et al.* demonstrated that IgM MGUS patients have a slight but significant overall survival (OS) advantage, and SWM patients have an equivalent mortality rate with respect to the general population.[2] In another study, we found that OS did not differ significantly between 138 IgM MGUS and 34 SWM (p=0.76)(3). However, the event-free survival (EFS) at 5 and 10 years was 95% (95%CI, 87-98%) and 83% (95%CI, 71-90%), respectively, in IgM MGUS, and 77% (95%CI, 56-89%) and 42% (95%CI, 19-64%), respectively, in SWM (p=0.0001). These data suggested BM evidence of LP-NHL to identify a subgroup of IgM aMGs with high probability of evolution to overt MLD, needing strict monitoring in view of an early treatment of their disease. As far as risk factors for evolution to overt MLD are concerned, the lack of unequivocal criteria for differentially diagnosing IgM MGUS from SWM as well as the evaluation of MGUS taken together irrespective of the MC isotype, make data before

2002 not homogeneous. In 1,014 MGUS patients,[4] Cesana *et al.* found Ig A and IgM isotype, serum MC levels > 1.92 g/dL, detectable Bence Jones (BJ) proteinuria, the reduction of one or two serum polyclonal immunoglobulins (Ig), the erythrocyte sedimentation rate (ESR) and BM plasma cell (PC) or LP cell levels to be associated with an increased probability of evolution. At multivariate analysis, BM PC or LP infiltration, the presence of BJ proteinuria, polyclonal serum Ig reduction and ESR were independently associated with MGUS malignant transformation. On the basis of predictive variables, a prognostic index (PI) was determined allowing to identify four different risk groups. In other studies an independent prognostic value for evolution was confirmed for detectable BJ proteinuria, reduction of normal Ig and BM PC or LP cell levels, and was also found for the serum paraprotein size.[5] More recently, Rakjumar *et al.*[6] tested the prognostic significance of an abnormal serum free light chain ratio in 1,384 MGUS patients, and showed this parameter to predict malignant transformation independently of the size and type of serum MC. Given a scoring system, IgM MGUS patients with a MC > 1.5 g% and an abnormal serum free light chain ratio would have a 58% 20-year-probability of evolution. Prognostic factors for SWM transformation to active disease were analyzed in few patients, in the contest of large WM series mostly requiring treatment at presentation.[7] In 27 patients diagnosed as having SWM on the basis of IgM MC size greater than 3 g/dL, and/or BM LP infiltration 30% or greater, and/or a diffuse infiltration pattern on BM biopsy, high MC size and low haemoglobin (Hb) level were found to independently predict the risk of transformation.[8] Similarly, Alexanian *et al.* observed Hb levels < 11.5 g/dL, IgM MC greater than 3 g/dL and high β2-microglobulin levels to correlate with the risk of evolution.[9] The finding of high MC levels as a prognostic factor in SWM disagreed with previous data,[7] probably due to different patient selection criteria (in the majority of studies paraprotein levels greater than 5 g/dL had been chosen for SWM diagnosis). Given the assumption that the only parameter defining an overt WM is treatment requirement, the analysis of risk factors for evolution in IgM aMGs on a whole retains an important theoretical value, and allows to analyze large series of cases by evaluating also patients without BM findings. By analyzing 213 IgM aMGs during long-term follow-up, Kyle *et al.* found only the IgM MC size and the albumin level to independently predict evolution to MLD.[10] After the 2[nd] International Workshop on WM, we analyzed 384 patients with IgM aMG defined according to the new criteria (i.e., those patients with any size of serum IgM MC, any degree of BM LP infiltration, any LP infiltration pattern except for the paratrabecular pattern on BM biopsy, no symptoms attributable to either IgM MC or tumour infiltration, and no evolution to overt WM or other MLD for at least 12 months from diagnosis).[3] At univariate analysis MC level (p=0.0001), Hb level (p=0.0002), absolute lymphocyte counts (ALC) >4 $\times 10^9$/L (p=0.0015), ESR level ≥40 mm/h (p=0.0035) and degree of BM LP-NHL infiltration (p<0.0001) were significantly associated with the evolution probability, while BJ proteinuria (p=0.067) and a diffuse BM infiltration pattern (p=0.081) were associated with a trend for increased transformation risk. Absolute neutrophil counts < 1.8×10^6/L, serum β2-microglobulin levels and reduced normal Ig levels were not associated with evolution probability. At multivariate analysis, IgM size (p=0.005) and lymphocytosis (p=0.0001) independently predicted malignant evolution, while Hb level was associated with a trend for a higher progression risk (p=0.076). Assuming a label (x) for each variable [x1=MC in mg/dL (log transformed), x_2=Hb in g/dL, x_3=1 if ALC >4×10^9/L, x_3=0 if ALC 4×10^6/L, x_4=1 if detectable BJ proteinuria and x_4=0 if undetectable BJ proteinuria, and x_5=ESR in mm/h], we calculated a PI (=$1.2636x1-0.2684x_2+2.4165x_3-0.1190x_4+0.4071x_5$) for each patient and identified 3 risk groups on the basis of PI distribution tertiles. The low-risk subgroup (1[st] tertile, PI ″8.97) had EFS rates at 5 and 10 years of 100% and 89% (95%CI, 60%-97%), respectively; the intermediate-risk subgroup (2[nd] tertile, 8.97 < PI ″ 10.06) had EFS rates at 5 and 10 years of 95% (95%CI, 85%-98%) and 83% (95%CI, 64%-93%), respectively; the high-risk group (3[rd] tertile, PI >10.06) had EFS rates at 5 and 10 years of 85% (95%CI, 72%-92%) and 44% (95%CI, 24%-63%), respectively. EFS of patients corresponding to the 3[rd] tertile significantly differed (p<0.0001) from that of patients corresponding to the first two tertiles pooled together [EFS rates at 5 and 10 years of 97% (95%CI, 92%-99%) and 86% (95%CI, 72%-93%), respectively]. Whether previously found risk factors for evolution to symptomatic WM are confirmed for SWM patients, defined according to the Consensus Panel Recommendations of the 2[nd] International Workshop on WM, is still unknown. However, in our series 79.4% of SWM presented a PI by our prognostic model greater than 10.06 (3[rd] tertile),[3] confirming that a large proportion of the population of IgM aMG at high risk of evolution is represented by patients with clear BM evidence of lymphoma. By evaluating IgM-MGUS apart, preliminary unpublished data from our series, re-defined retrospectively according to the new criteria,[1] show that prognostic factors for evolution overlap not only those found in IgG/IgA MGUS, but also those found in IgM aMGs on a whole, suggesting that IgM MGUS could be considered as the first step of an indolent lymphoproliferative disease.

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WM1.7

INTERNATIONAL PROGNOSTIC SCORING SYSTEM FOR WALDENSTRÖM'S MACROGLOBULINEMIA

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Waldenström macroglobulinemia (WM) patients (pts) may require treatment in order to control symptoms caused by anemia, organomegaly or hyperviscosity. Median survival has ranged between 60 and 120 mo. In retrospective analyses, several characteristics were constantly associated with a poor clinical outcome such as an advanced age, a low hemoglobin concentration (Hb), a low platelet count, a low albumin concentration and an elevated serum β2-microglobulin (B2M). Few prognostic indices have been proposed, but none of them have been widely accepted and used. Therefore, 9 cooperative groups or institutions decided to join their records in order to design a prognostically meaningful staging system for symptomatic WM patients, requiring therapy. We performed multivariate analyses with bootstrap validation in a series of 587 pts (median age 67, range: 28 to 95, M/F ratio: 1.7) diagnosed between September 1979 and December 2001. Diagnostic and treatment criteria fulfilled recommendations of the 2[nd] international WM workshop. Front-line treatment was initiated at diagnosis in 69% and 4 to 164 mo later in the remaining pts. Criteria for initiation of therapy included cytopenia, constitutional symptoms, organomegaly, hyperviscosity, IgM-related disorders, which pertained to 51%, 44%, 35%, 31% and 13%, respectively. Treatment regimens comprised alkylating agents, fludarabine and rituximab in 369, 195 and 23 subjects, respectively. Baseline parameters included age>65 yr in 57%, Hb≤11.5 g/dL in 65%, platelet count ≤100×10[9]/L in 9%, granulocyte count ≤1.5×10[9]/L in 9%, B2M >3 mg/L in 56%, albumin ≤3.5 g/dL in 36% and monoclonal protein >7.0 g/dL in 7%. With a median follow-up of 64 mo (range, 6-182 mo), the median survival after treatment initiation was 87 mo (95%CI 79-103) regardless of the type of therapy applied (p=0.3). Using results of univariate survival analyses, recursive partitioning and martingale residuals analyses, 7 adverse characteristics for inclusion in multivariate analyses were identified during an expert meeting: age >65 yr, platelet count ≤100×10[9]/L, B2M >3 mg/L, M-protein >7.0 g/dL, granulocytes ≤1.5 ×10[9]/L, Hb ≤11.5 g/dL and albumin ≤3.5 g/dL. The Cox proportional hazard model with stepwise selection selected the first 6 covariates. Bootstrap resampling (500 replicates) validated the selection of the first 4 covariates in at least 80% of the replicates. Selection of at least one of the last 2 covariates in more than 80% of the replicates indicated a correlation between these 2 covariates, and validated the inclusion of Hb only. Using the combination of age, Hb, platelet count, B2M and M-protein, low risk was defined by the presence of ≤1 adverse characteristic except age, high risk by the presence of >2 adverse characteristics; the remaining patients with 2 adverse characteristics or age >65yr had intermediate risk, comprising 27%, 35% and 38% of patients with 5-yr survival rates of 87%, 36% and 68% (p<0.0001), independent of treatment and age. IPSS split each subgroup identified by previous scoring systems (Morel, Merlini, Ghobrial and Dhodapkar). Conversely, log-rank test was significant only when the latter prognostic system was assessed in low IPSS-risk pts. Thus the combination of age, B2M, M-protein and blood counts provides simple prognostic model for survival in WM, hopefully serving as an objective basis for initiation of therapy and comparison of treatment results.

WM2: Frontline treatment of Waldenström's Macroglobulinemia

WM2.1

INDICATIONS FOR TREATMENT AND THE ROLE OF ALKYLATING AGENTS IN WALDENSTROM'S MACROGLOBULINEMIA (WM)

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The presence of constitutional symptoms such as weakness, fatigue, fever, night sweats, or weight loss are indications for beginning therapy for WM. The presence of progressive symptomatic lymphadenopathy, hepatomegaly, and/or splenomegaly is also indication for treatment. The occurrence of anemia (hemoglobin < 10 g/dL) or thrombocytopenia (platelets < 100×10[9] due to marrow infiltration) are also indications for therapy. Development of the hyperviscosity syndrome, sensorimotor peripheral neuropathy, autoimmune hemolytic anemia, the presence of amyloidosis or symptomatic cryoglobulinemia also require the institution of therapy. Initiation of therapy should not be based on the IgM level *per se* since this may not correlate with the clinical manifestations of WM (Kyle, *et al.*, 2003). Treatment for systemic complications of WM is evolving as newer chemotherapeutic agents are utilized. Therapeutic options include rituximab, fludarabine, cladribine (2-chlorodeoxyadenosine), 2-CdA and alkylating agents as well as autologous stem cell transplantation (Dimopoulos, *et al.*, 2005, Gertz 2005, Dimopoulos, *et al.*, 2005). This discussion will be limited to the use of alkylating agents. Chlorambucil continuously has been a standard therapy for WM for more than four decades. It is usually given in an initial oral dosage of 6-8 mg/day. The dose is then reduced usually to 2-4 mg/day depending upon the leukocyte and platelet counts and patient response. Chlorambucil with or without prednisone may be given in an intermittent schedule, at a dosage of 0.3 mg/kg/day for 7 days every 4-6 weeks. Therapy should be continued until the patient reaches a plateau state defined as the resolution of constitutional symptoms and serum IgM protein reaching a stable state. Facon *et al.* described 167 patients with WM oral chlorambucil over a 19-year period. The median survival was 60 months. Age, gender, and hemoglobin value had no impact on the outcome. In addition, the presence of organomegaly and the percentage of marrow lymphoid cells did not predict a shorter survival (Facon, *et al.*, 1993). There has been one prospective randomized study comparing continuous oral with intermittent administration of chlorambucil. Forty-six patients with WM requiring therapy because of anemia or other laboratory abnormalities, hepatosplenomegaly, lymphadenopathy, or constitutional symptoms were randomized to chlorambucil 0.1 mg/kg/day orally (continuous) or chlorambucil 0.3 mg/kg orally for 7 days repeated every 6 weeks (intermittent). The two patient groups were not different according to age, symptom of fatigue, weight loss, bleeding or purpura, hepatosplenomegaly or lymphadenopathy, performance score, hemoglobin, leukocyte or platelet values, serum creatinine, calcium, serum albumin, serum viscosity, bone marrow plasma cells, size, light chain type of serum monoclonal protein, the amount and type of urine monoclonal light chain. Twenty-two percent had either monoclonal gammopathy of undetermined significance (MGUS) or smoldering macroglobulinemia before protocol entry. Of the 46 patients, 39 (85%) had an M-spike ≥ 3 g/dL at study entry. Of the 7 patients with an M-spike < 3 g/dL, 4 had anemia, 3 had a malignant proliferative process with hemoglobin of 11.7 g/dL and an M-spike of 2.8 g/dL (1 patient), systemic amyloidosis (1), and a massive pleural effusion from plasmacytoid lymphocytes involving the pleura (1). Forty-one of the 46 patients (89%) had a hemoglobin value of ≤ 12 g/dL; 57% had a hemoglobin level of ≤ 10 g/dL. The median age was 63 years and 70% were males. Fatigue was present initially in 52% and weight loss was noted in 22%. Bleeding was present in 17%. The liver was palpable in 24%, and the spleen was palpable in 20%, while lymphadenopathy was noted in 15% at study entry. The median hemoglobin value was 9.9 g/dL (range 5.4-15.4 g/dL). Platelets were less than 100×10[9]/L in 9%, while the serum albumin was < 3 g/dL in 20%. Viscosity was more than 1.9 cp in 8 of the 31 patients in whom viscosity was measured (median 3.5 cp); 39% had a serum viscosity > 4 cp. Size of the monoclonal protein ranged from 1.7-9.2 g/dL (median 4.2 g/dL). IgA and IgG immunoglobulins were reduced in 94% of patients. Seventy-eight percent had IgM kappa. Monoclonal light chains were found in the urine in 72%, but only 14% had an M-protein value of more than 1 g/24h. Criteria for response included > 50% reduction in the serum monoclonal protein, an increase in the hemo-

globin level of ≥ 2 g/dL without transfusion, ≥ 50% decrease of urine monoclonal protein, reduction in the size of the liver or spleen of ≥ 2 cm, or a reduction of ≥ 2 cm in the size of the lymph nodes(Kyle, *et al.*, 2000). Of the 24 patients receiving continuous chlorambucil, 75% had a reduction in serum monoclonal protein of ≥ 50% unrelated to plasmapheresis. Ten other patients had an objective response of the monoclonal protein but had progression of their macroglobulinemia and were considered nonresponders. The median duration from randomization to the time of objective response was 18 months, with a median duration of response of 26 months. Hemoglobin increased by more than 2.0 g/dL without transfusion in 53%. The duration of the hemoglobin response was 17 months. Nineteen of the 24 patients (79%) had objective improvement measured by either a reduction of the serum monoclonal protein or an increase in hemoglobin. Of these 19 patients, only 3 had a reduction of serum monoclonal protein and no improvement in the hemoglobin value. The urine monoclonal protein decreased by ≥ 50% in 5 of the 7 patients with a measurable monoclonal protein. In addition to the seven patients, two others had a reduction in the serum monoclonal protein, but their disease progressed. Of the 22 patients receiving intermittent chlorambucil, 64% had a serum monoclonal protein decrease of ≥ 50% unrelated to plasmapheresis. Median duration from randomization to response was 21 months, while the median duration of response was 46 months. Fifty-nine percent (13 of 22 patients) had an increase in hemoglobin of ≥ 2 g/dL without benefit of transfusion. Median duration from randomization to an increase in the hemoglobin level of ≥ 2 g/dL was 7 months. Median duration of the hemoglobin response was 5 months. Fifteen (68%) had an objective reduction of the monoclonal protein or an increase in the hemoglobin value. Five of seven patients with an initial value of >50 mg/24h. had a reduction in urine monoclonal protein of ≥ 50%. With either continuous or intermittent chlorambucil, the liver decreased by ≥ 2 cm in 6 of 11(55%), while the spleen decreased by ≥ 2 cm in 6 of 9 patients (67%). Lymphadenopathy decreased in 71% of 7 patients. Two patients with pleural effusion requiring repeated thoracenteses had resolution with chlorambucil. The median survival was 5.4 years with no survival difference between continuous and intermittent chlorambucil. Eighty-nine percent have died from *macroglobulinemia* (13 patients), infection,[5] cardiac (4), myelodysplasia/leukemia,[3] gastrointestinal bleeding ,[3] cerebrovascular accident,[2] injury from a fall,[2] other malignancy,[2] and miscellaneous causes.[7] In summary, 79% had an objective response to continuous chlorambucil, and 68% had an objective response to intermittent chlorambucil. The addition of corticosteroids does not seem to increase response rate or survival although they may be useful in patients who have autoimmune hemolytic anemia. Optimal duration of chlorambucil administration has not been defined. In some studies, treatment is continued until a maximum reduction of monoclonal protein is reached (plateau state), and then patients are followed without treatment until there is evidence of disease progression. In other studies, chlorambucil has been administered for 1 to 2 years and then discontinued. There is no evidence that maintenance therapy prolongs survival. Prolonged treatment with alkylating agents increases the possibility of myelodysplasia or acute leukemia(Dimopoulos, *et al.*, 2005). Combinations of alkylating agents may also be of benefit, such as the M2 protocol (BCNU, cyclophosphamide, vincristine, melphalan, and prednisone) at 4- to 5-week intervals. Twenty-seven of 33 patients (82%) showed response (Case, *et al.*, 1991). In another report, 72 patients were treated with melphalan (6 mg/m^2), cyclophosphamide (125 mg/m^2), and prednisone (40 mg/m^2) daily on days 1 through 7 every 4 to 6 weeks for a maximum of 12 courses. Patients with responsive or stable disease were then given chlorambucil (3 mg/m^2) orally each day and prednisone (6 mg/m^2) daily until progression. Fifty-five of 71 (77%) of evaluable patients obtained a response. No grade III or IV toxicities were seen. The major side effects consisted of transient nausea, vomiting, and mild neutropenia(Annibali, *et al.*, 2005). An interesting prospective study is comparing oral chlorambucil 8 mg/m^2 for 10 days every 28 days for a maximum of 12 cycles with oral fludarabine at a dosage of 40 mg/m^2 or IV fludarabine 25 mg/m^2. The results of this study are eagerly awaited (Johnson, *et al.*, 2005).

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WM2.2

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

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Waldenstrom's macroglobulinemia (WM), a rare B-cell malignancy, is incurable. Therapy is currently reserved for symptomatic patients. Conventional treatment consists of alkylating agents (especially chlorambucil), with or without steroids.[1] This treatment gives response rates of about 60% and a median survival time of about 60 months. There is increasing evidence that fludarabine (a fluorinated nucleotide analog of the antiviral agent vidarabine) and cladribine (2-chlorodeoxyadenosine, 2-CdA), purine analogs active in low-grade lymphoid malignancies such as chronic lymphocytic leukemia and low-grade lymphomas, are also active in WM resistant to alkylating agents.[2,3] There is also evidence that purine analogs may yield higher response rates when used as first-line therapy. Most clinical trials in WM are small phase II studies with widely differing inclusion criteria and response criteria. At the 3rd workshop on WM, held in 2004, it was agreed that alkylating agents, purine analogs and rituximab were reasonable choices for first-line therapy, that there were no data from prospective studies to prefer one agent over another, and that cladribine and fludarabine have the same efficacy. Combinations of alkylating agents, purine analogs and rituximab should now be tested in prospective randomized trials for their efficacy and toxicity relative to single-agent therapy .[3]

Purine analog monotherapy

Reported response rates to first-line fludarabine therapy range from 38% to 100% (Table 1). In a phase II trial involving 118 untreated patients with WM, the overall response rate to fludarabine monotherapy was 38% (with complete remissions in 3% of patients) after four cycles of 30 mg/m^2 IV daily for 5 consecutive days, followed by a further four cycles in patients who responded.[4] Most responses occurred within 3 to 6 months of treatment initiation, but 17% and 5% of responses occurred after more than 6 and 12 months, respectively. The 5-year rates of overall survival (OS) and progression-free survival (PFS) were 62% and 49%, respectively. A serum IgM level below 40 g/L and a β-2 microglobulin level of 3 mg/L or more were the only significant predictors of OS. Only the beta-2 microglobulin level was a significant predictor of PFS. The difference in the response rates between the SWOG study and the other cited studies could be due to the small size of the latter and to differences in patient characteristics and response criteria. In smaller series testing cladribine and pentostatin (Table 2) given as a continuous infusion, bolus injection or subcutaneously, the response rate ranged from 55% to 100% and the responses lasted a median of 13 to 41 months.

Purine analog combinations

Purine analogs have yielded higher response rates when combined with cyclophosphamide in small series, with or without rituximab.

Table 1. Use of fludarabine alone or in combination in untreated WM patients.

Regimen	*Duration of treatment*	*Median age*	*Number of patients*	*Overall response (CR+PR)*	*Median survival/ response duration (months)*	*reference*
F: 30 mg/m^2 for 5d	4 cycles+ 4 additional cycles for responders	66	118	38 (C=:3)	5-yr OS: 62%	Dhodapkhar (Blood 2001)
F: 20-30 mg/m^2 IV for 5 d or 30mgIV for 3d	Until maximum response (median 3 cycles)	60	2	100	MRD: 38	Dimopoulos (Am J Med 1995)
F: 25 mg/m^2 for 5d	Maximum response + 2 cycles	64	19	79 (CR=5)	MRD=41	Foran (J Clin Oncol 1999)
F : 25 mg/m^2 for 5d	6 cycles	58	7	85	MRD=21+	Thalhammer-Scherrer (Ann Hematol 2000)
F 25 mg/m^2 for 5d Cyclo 250 mg/m^2 3d	4 cycles	73	2	55	MRD=24	Dimopoulos (Leuk Lymphoma 2003)
F:30 mg/m^2 iv3d Cyclo 300 mg/m^2 iv 3d	Median 4 cycles	64	14	85%(CR=0)	MRD =27	Tamburini (Leukemia 2005)
F 25 mg/m^2 3d Cyclo 250 mg/m^2 3d	Median 4 cycles	58	1	89%(CR=0)	MRD = 38	Tam (Clin Lymphoma Myeloma 2005)
F 25 mg/m^2 3d Cyclo 250 mg/m^2 3d Rituximab 375 mg/m^2 1d	Median 4 cycles	59	4	75% (CR=0)	NA	Tam (Cancer 2006)

F: Fludarabine, Cyclo: Cyclophosphamide, CR complete response;PR partial response, OS: overall survival, MRD: median response duration, N/A, not applicable, N/S: not stated.

Table 2, Use of cladribine and pentostatin, alone or in combination, in untreated WM.

Regimen	*Duration of treatment*	*Median age*	*Number of patients*	*Overall response (CR+PR)*	*Median survival/ response duration (months)*	*reference*
C: 0.1 mg/kg/ d ci for 7 d	2 cycles	65	9	100	N/S	Dimopoulos (Ann Intern Med 1993)
C: 0.1 mg/kg/ d ci for 7 d	2 cycles	65	26	85 (CR=12)	MRD: 13+	Dimopoulos (J Clin Oncol 1994)
C: 0.12 mg/kg/ d ci for 5 d	4 cycles	65	10	90	N/S	Fridrick (Ann Hematol 1997)
C: 4 mg/m^2/ d ci for 7 d or5.6 mg/m^2/d 2h infusion for 5d	2 cycles	69	11	73	18% relapse at 3 years	Delannoy (Br J Hematol 1997)
C: 0.12 mg/kg/ d ci for 5 d	Median 3 cycles	66	7	57	93% OS at 24 months	Liu (Br J Hematol 1999)
C: 0.14 mg/kg/ d ci for 5 d	Median 4 cycles	63	11	55	MRD=17	Lewandowski (Med Sci Monit 2000)
C: 0.1 mg/kg/ d ci for 7 d m^2 iv 3d	2 cycles	67	16	94%	MRD =23	Weber (Sem Oncol 2003)
C: 0.1 mg/kg/ d ci for 7 d m^2 iv 3d = Prednisone	2 cycles	61	20	60	MRD = 9	Weber (Sem Oncol 2003)
C: 1.5 mg/m^2/ d scx3 daily + cyclo 40 mg/m^2 poX2 daily both for 7d	2 cycles	62	37	84	MRD 36	Weber (Sem Oncol 2003)
C: 1.5 mg/m^2/ d scx3 daily + cyclo 40 mg/m^2 oX2 daily both for 7d + rituximab 375 mg/m^2 weeklyX 4	2 cycles	65	27	93	MRD= 60	Weber (Sem Oncol 2003)
Pentostatatin 4 mg/m^2 +cyclo 600 mg/m^2± Rituximab 375 mg/m^2	Median 4 cycles	62	9	76	N/S	Hensel (Clin Lymphoma Myeloma 2005)

C: Cladribine, Cyclo: Cyclophosphamide, Pred: prednisolone, Ci: continuous infusion, CR complete response, PR partial response, OS: overall survival, MRD: median response duration, N/S: not stated

Toxicity of purine analogs

The principal adverse effect of purine analogs is bone marrow suppression, with 30% of patients developing grade 3 neutropenia.[6] Nucleoside analogs must be used with care in patients being considered for high-dose chemotherapy and autologous stem cell transplantation. Several reports show that peripheral blood stem cell (PBSC) collection at steady state can fail in patients with a history of fludarabine exposure.[7] In contrast, PBSC collection can succeed after intermediate-dose Ara-C.[8] The use of agents that damage stem cells is questionable when high-dose chemotherapy and autologous stem cell transplantation are being considered. Purine analogs lead to a sustained reduction in monocyte and T cell counts (both $CD4^+$ and $CD8^+$), thereby impairing cell-mediated immunity and substantially increasing the risk of opportunistic infections.[6] Myelodysplasia has been reported to occur with a crude incidence rate of 3.5-8% after fludarabine-containing therapy. Bowcock *et al.* reported a crude incidence rate of 20% in elderly patients, which could be related to fludarabine dose, and adding cyclophosphamide might enhance this risk.[9] Long-term follow-up of WM patients treated with these agents is needed to assess this risk more precisely. In conclusion, purine analogs are active in both treated and untreated patients with Waldenstrom's macroglobulinemia. However, there is no consensus on the optimal duration of treatment, and the response rate to first-line purine analog therapy is controversial. Nucleoside analogs, which induce rapid cytoreduction, may be the treatment of choice for patients with serious complications such as hyperviscosity, pancytopenia, and severe peripheral neuropathy. Fludarabine is currently being compared with chlorambucil as primary treatment for WM in a prospective randomized trial.[10]

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WM2.3

RITUXIMAB ALONE OR IN COMBINATION IN THE FRONTLINE TREATMENT OF WALDENSTROM'S MACROGLOBULINEMIA

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Waldenstrom's macroglobulinemia (WM) is a low grade lymphoplasmacytic lymphoma that is characterized by the production of a monoclonal immunoglobulin (IgM). The malignant lymphoplasmacytic cells are of B cell origin and while expression of some cell surface antigens is variable, CD20 expression on the malignant clone is nearly ubiquitous, making this surface marker an ideal candidate for targeted monoclonal antibody therapy. Rituximab is a chimeric monoclonal antibody against CD20 that is known to be effective in B cell lymphomas. Based on encouraging preliminary reports documenting clinical activity of rituximab in patients with refractory or relapsing WM, Dimopoulos *et al.* published the first prospective trial of single agent intravenous rituximab 375 mg/m^2 given weekly for 4 weeks (in patients without progressive disease at 3 months this was also followed by 4 additional weekly doses of rituximab) to newly diagnosed patients with macroglobulinemia.[1] Among 27 symptomatic patients with WM, 15 were previously untreated. Forty percent of these newly diagnosed patients achieved partial response (PR) characterized by ≥ 50% reduction in both monoclonal protein/tumor infiltration at involved sites. The median time to response for all patients (newly diagnosed+previously treated) was 3.3 months and only 3 patients had progressed at publication. The authors also described a transient, rapid increase of M-protein in some responding and non-responding patients that occurred 15-30 days after initial treatment, followed by a gradual decrease in this level. This phenomenon, since referred to as an *IgM flare* is not necessarily indicative of treatment failure, and has also been reported after treatment with cladribine.2 One of three patients with neuropathy had improvement with regression of lymphoma (2 stabilized) and 1/1 patient with cold agglutinin anemia responded to therapy. Several prognostic factors were analyzed; patients with M protein < 40 g/L were more likely to respond (p.03) and there was a trend towards improved response in patients with a kappa light chain (p.19). Toxicity was mild, with immediate infusional reactions noted most frequently, and 4 infectious episodes noted at some point prior to progressive disease (zoster, erysipelas, bronchitis, and UTI). Simillar results were noted in a trial of extended rituximab therapy (375 mg/m^2 IV weekly during weeks 1-4 and 12-16).[3] Twelve previously untreated patients were included in a study of 29 patients that resulted in an overall PR rate of 48.3%, overall MR rate of 17.2% (response was not reported separately for previously untreated patients) and median TTP for untreated patients of 17 months. For both untreated and previously treated patients, response was only noted in 20% of 5 patients with IgM > 6000 mg/dL. A correlative study performed in these patients also evaluated whether expression of complement resistance antigens CD46, CD55, and CD 59 differed at baseline and post-treatment with rituximab. The only significant finding was on analysis of mean fluorescence Intensity (MFI) of CD55 expression after treatment with rituximab. Baseline MFI of CD55 revealed no significant differences between responding and nonresponding patients, however, after treatment, a significant increase in CD55 MFI was noted in 4/4 non-responding pts compared with no difference in 7 responding patients (p.0006). If confirmed, these results suggest that targeted therapy, aimed at CD55 antigen expression, is worthy of study. In an attempt to clarify prognostic factors for successful rituximab therapy of WM, a retrospective review combined data from 23 previously untreated patients who received standard dose rituximab in 2 prospective trials from the University of Athens and MD Anderson Cancer Center.4 The response rate of 35% was similar to that in the aforementioned study (Dimopoulos *et al.*) and the median TTP was 12.6 months. Multivariate analysis identified a monoclonal protein of ≥ 40 g/L and serum albumin < 35 g/L as significant predictors of TTP. Thus, 3 risk groups were developed based on these results; patients with both of these risk factors had the shortest median TTP (3.6 months), patients with neither risk factor had a long median TTP of >40 months, and, as expected, patients with one risk factor had an intermediate risk of progression of 11.1 months. More recently some of the same authors reported a study of 35 previously untreated patients with WM who received extended rituximab (as described above). 5 An overall response (OR) of 43% was reported, and after a median follow-up of 43 months, 19 patients progressed (5/15 responders) with a median progression free survival (PFS) of 23.6 months for all patients (not reached for responding patients). While patients with a higher albumin and lower M protein and those with hemoglobin > 10 g/dL and a kappa light chain were noted to have a higher frequency of response, univariate analysis indicated that hemoglobin > 10g/dL was the single most important factor predicting a long PFS (median 57.5 months Vs 5.4 months). Other factors associated with longer PFS are consistent with previous reports and included albumin > 3.5 g/L (median 25.5 vs. 5.3 months), M protein < 4g/L (48mos vs. 6.2 months), absence of hyperviscosity (23 months vs. 3.5 months), and kappa light chain type (25.4 vs. 5.1 months). In a multicenter Phase II trial of standard dose rituximab.6 Twelve of 34 (35.3%) evaluable untreated patients had ≥PR, another 17.6% achieved MR and only 17.6% had disease progression. The median duration of response was 27 months and only 1 patient had died by the time of publication. In contrast to the aforementioned studies, pretreatment hemoglobin and monoclonal IgM level were not predictive of response (no similar analysis was performed for PFS). Grade 3-4 toxicities were infrequent, but included

metabolic/electrolyte disturbances (glucose, calcium, potassium, magnesium and sodium) and known infusion related toxicities. Based on the significant single agent activity of rituximab in untreated WM, several investigators have reported significant activity of the antibody in combination with nucleoside analogues, alkylating and novel agents. We previously reported a program of 2-chlorodeoxyadenosine (2-CdA) 1.5 mg/m^2 sc tid x 7d, cyclophosphamide (Cy) 40 mg/m^2 po bid x 7d and rituximab (Rit) 375mg/m^2 iv q wk x 4 wk (repeated at 6wks)(18 patients).[7] Updated results (median follow-up, 68.5 months) reveal an overall response (>PR) of 94% (17% achieved CR), median time to remission was 2.4 months (2-CdA-Cy-Rit), and duration of first remission was 58.6mos, which appears better than the 25..6 months historically noted after treatment with the identical program without rituximab. We also evaluated time to retreatment (TTRT) since many patients remain asymptomatic and do not require retreatment at the time of relapse. Median TTRT has not yet been reached (only 1 patient required retreatment), but appears improved compared with the same program without rituximab (56.3 months w/2-CdA-Cy, p=0.02). Similar results have been noted with cyclophosphamide-rituximab and other nucleoside analogs like pentostatin and fludarabine, but inclusion of previously untreated patients with WM remains limited (< 6 pts each). One concern has been the difficulty with stem cell collection in patients after treatment with 2-CdA and thus the role of rituximab in other combinations is of particular interest. Dimopoulos *et al.* reported the primary treatment of 60 patients with WM given cyclophosphamide 100mg/m^2 po bid on d1-5 and dexamethasone 20 mg IV followed by ,rituximab 375 mg/m^2 IV on d1, repeated q21d x 6.8 Seventy percent of patients (63%PR, 7%CR) responded and PD was noted in only 10%. .At a median follow-up of 24 months, 60% of patients remain progression free. Twenty percent of patients experienced g3-4 neutropenia. Since stem cell collection was possible in all patients in whom it was attempted, this regimen shows particular promise for primary treatment of WM. Preliminary results of a program of bortezomib 1.3 mg/m^2 IV and dexamethasone 40 mg IV on days 1,4,8,11 and rituximab 375 mg/m^2 on d11 x 4 cycles (repeated after 3 months) in 10 evaluable patients have recently been presented.[9] Response was rapid (median 1.1 months) and all patients achieved at least a minor response and 50% achieved at least PR. The program was well tolerated (no neuopathy has occurred), but because 40% of patients developed herpes zoster, valacyclovir prophylaxis has been added. The same author recently reported results combining the novel agent lenalidomide (25 mg/d po x 21d repeated q 28d x a total of 48 weeks) with rituximab.(375 mg/m^2/wk, wks 2-5, 13-16) in 10 untreated patients (12pts total).[10] Eighty-five percent of patients had an acute hematocrit decrease (median 4.2%) and toxicities of myelosuppression and IgM flare (requiring pheresis, 2 pts) were also noted. Although 3/8 evaluable patients achieved PR and 4/8 patients achieved MR, 8/12 patients discontinued treatment. While the results are promising, the ideal dose and schedule for this regimen remain unclear. These trials demonstrate significant activity of rituximab for primary therapy of WM. While this drug should be used judiciously as a single agent in patients with high levels of circulating IgM (to avoid flare-related hyperviscosity), its greatest use may be in patients with marrow hypocellularity or for those in whom stem cell collection is warranted. Preliminary results, however, indicate that the addition of rituximab to combinations of chemotherapeutics/novel agents results in high response rates and durable remissions even after limited therapy, indicating this agent is likely to continue to play a significant role in primary therapy for Waldenstrom's macroglobulinemia.

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WM2.4

OVERVIEW IN SALVAGE TREATMENT IN WALDENSTROM'S MACROGLOBULINEMIA

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Waldenstrom's macroglobulinemia is characterized by the infiltration of lymphoplasmacytic cells in the bone marrow associated with an IgM monoclonal protein of any size. Only patients with symptoms manifest by anemia, thrombocytopenia, lymphadenopathy, organomegaly, hyperviscosity, neuropathy, or an IgM-associated clinical disorder should be treated. At the Second International Workshop on Waldenstrom's Macroglobulinemia, the options for first-line therapy formulated by a consensus panel included single-agent therapy with alkylating agents, nucleoside analogs, and rituximab. It was not possible at that time to recommend the use of one first-line agent over another, although it was noted that exposure to agents that would deplete stem cells should be avoided in patients who would be candidates for high-dose therapy and autologous stem cell transplantation. The option for the treatment of relapsed disease included the re-use of a front-line agent if a prior response has been obtained that was deemed durable. Alternatively, another front-line agent could be used as a single agent. Other options included combination chemotherapy, thalidomide with or without steroids, autologous transplantation, and alemtuzumab.[1] A number of clinical trials exploring the use of rituximab as part of combination therapy have been performed. Rituximab as a single agent produced an objective response rate of 35%.[2] A number of publications in the past 36 months have indicated the ability of rituximab in combination to produce higher response rates. Weber *et al.* reported 90 consecutive untreated patients using cladribine alone or in combination with prednisone, cyclophosphamide, and rituximab. The overall response was 94% for cladribine alone, 60% for cladribine and prednisone, 84% for cladribine and cyclophosphamide, and 94% for cladribine, cyclophosphamide, and rituximab. The only prognostic factor predicting shorter survival was hemoglobin <9 g/dL. This supports the potential role of cladribine in both untreated and treated Waldenstrom's macroglobulinemia. The use of rituximab combined with cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) was reported in 13 patients, ten of whom had previously been treated. Eight and six, respectively, had had prior fludarabine and rituximab. Three patients received maintenance rituximab. There were three complete responses unconfirmed, eight partial responses, and a minor response with 10 of the 11 patients who had a major response in continuous remission with a median follow-up of nine months. This is a highly active regimen for the management of refractory/relapsed Waldenstrom's macroglobulinemia, and it is currently the subject of an Eastern Cooperative Oncology Group trial.[3] Other attempts to combine rituximab, a steroid, and an alkylating agent have been reported by Dimopoulos and colleagues. Patients received rituximab on day 1, cyclophosphamide orally 100 mg/m^2 p.o. b.i.d. for five days, and dexamethasone 20 mg IV every 21 days for a total of six courses. Over a period of four years, 70 patients have been treated with 60 having completed the intervention. Their median age was 71 years and 70% of patients achieved a 50% reduction of serum monoclonal protein and 7% complete responses. Progressive disease was only seen in 10% of patients. The median time to response was four months, and at 24 months median, 60% of patients are progression free. There was only one treatment-related death.[4] Substituting fludarabine for dexametha-

sone, Vargaftig *et al.* had previously shown that fludarabine and cyclophosphamide produced a 78% response rate and subsequently added rituximab and reported on 21 patients with a median age of 65 and a median IgM level of 4090. Nineteen of the 21 had previously been treated with a median of two lines of therapy including three patients receiving autologous stem cell transplantation. Fifteen were relapsed and four were refractory. The regimen was given every four weeks and included rituximab 375 mg/m^2 on day 1, fludarabine 40 mg/m^2 orally days 1 through day 3, and cyclophosphamide 250 mg/m^2 orally days 1 through day 3. Twenty-one patients were treated with a single cycle and 19 received two or more for a median of 4.5 cycles, maximum 6. The overall response rate was 76% with 48% partial responses, 24% minor responses, and 5% complete responses. There were five patients who had stable disease (24%), and none had progressive disease. All four patients previously treated with fludarabine responded, and two of three with previous stem cell transplant responded. Grade 3-4 neutropenia was the most common toxicity (48%). With a median follow-up of five months, 21 were alive and two had relapse. The authors concluded that rituximab and fludarabine orally and cyclophosphamide produced a response rate of 76% with acceptable toxicity.[5] Tam *et al.* reported on their experience with fludarabine combination therapy used with cyclophosphamide in nine patients and with cyclophosphamide and rituximab in five patients. In the 14 patients previously treated, partial responses were obtained in 76% of patients and did not differ based on regimen. The median remission duration was 38 months. The actuarial five-year survival was 55% for previously treated patients.[6] Tamburini administered the combination of fludarabine, cyclophosphamide, and rituximab to 49 patients (35 previously treated) with a 78% response rate, and only two patients had progressive disease. The median time to treatment failure was 27 months. Prognosis was influenced by age >65 and IgM <4 g/dL.[7] The novel agents, thalidomide and bortezomib, both appear to have activity in the management of Waldenstrom's. Thalidomide 200 mg escalated to 400 mg each evening with rituximab weeks 2 through 5 and 13 through 16 were administered to 25 patients, five previously treated. There was one complete response, 15 partial responses, and two minor responses, for a major response rate of 70% and an overall response rate of 78%. The median duration of response is 19.6+ months.[8] Dimopoulos *et al.* reported on the use of bortezomib with relapsed or refractory Waldenstrom's macroglobulinemia. Ten previously treated patients, eight of whom who had received three or more prior regimens, were treated with standard-dose bortezomib with six partial responses occurring at a median of one month. The toxicities were typical-thrombocytopenia, fever, and fatigue.[9] The use of nonmyeloablative stem cell transplant for patients with refractory Waldenstrom's were reported in an effort to determine if there was a graft-versus-tumor effect. Twelve patients with refractory Waldenstrom's in the Seattle consortium were transplanted using an HLA-matched related donor in seven and an unrelated donor in five. Conditioning was low-dose total body radiation therapy with or without fludarabine. Eleven of the 12 received peripheral blood stem cells. Median time from diagnosis to allogeneic transplant was 6.6 years. Patients had received a median of 4.5 prior regimens. All patients but one achieved stable engraftment with >95% chimerism. Grades 2 through 4, acute graft-versus-host disease occurred in 58%, and extensive chronic graft-versus-host disease in 58%. The treatment-related mortality was 17%. Responses were seen in 91%. Four patients achieved a complete response and only one has progressed, although one patient died of transformed large cell lymphoma. All seven sibling transplants responded. Three of the five matched unrelated donor transplants responded. The Kaplan-Meier progression-free survival at five years is estimated to be 61%. Graft-versus-tumor effects were observed in the majority of patients.[10] Agents that appear promising in the management of Waldenstrom's macroglobulinemia include ^{131}I-tositumomab which produced a response in a single patient. This agent is limited by the extensive marrow infiltration in most patients and the potential of this agent to produce long-term myelosuppression. Imatinib has been used in the treatment of Waldenstrom's on the principle that it blocks stem cell factor signaling and induces apoptosis in Waldenstrom's mast cells thought to be important in the pathogenesis of the disease. Thirteen patients, all of whom had relapsed and refractory disease, received a median of three months of imatinib therapy. Six of 13 attained a >25% decrease in serum IgM at a median of 2.5 months. A phase I-II study of Atacicept (TACI-Ig) to neutralize APRIL and BLyS has also been reported. Four Waldenstrom's patients entered this trial, and no dose-limiting toxicity was seen. Three Waldenstrom's patients had stable disease after the first cycle. One Waldenstrom's patient remains stable, and one had a minimal response. The treatment was well tolerated without dose-limiting toxicity. Perifosine is an oral Akt inhibitor which induced apoptosis in Waldenstrom's cells demonstrated by flow and did not produce cytotoxicity in healthy donor, peripheral blood mononuclear cells. Perifosine induced significant reduction in Waldenstrom's tumor growth in a mouse model suggesting it will be an active agent. There appears to be *in vitro* synergy of perifosine with rituximab and bortezomib. *Summary*. The use of combination therapy with nucleoside analogs and alkylating agents, rituximab with a nucleoside analog, and nucleoside analogs plus alkylating agents are all considered appropriate for the management of newly diagnosed and relapsed Waldenstrom's macroglobulinemia. The activity for these combinations is at least as good if not better than single-agent therapy. Alkylators plus purine nucleoside analogs, nucleoside analogs with rituximab, thalidomide and stem cell transplantation are all options to be considered in the complex management of patients with Waldenstrom's macroglobulinemia.

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WM2.5

THE ROLE OF AUTOLOGOUS TRANSPLANTATION IN WALDENSTROM'S MACROGLOBULINEMIA

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The published experience on high-dose treatment (HDT) supported by autologous stem cell transplantation (ASCT) for Waldenstrom's Macroglobulinemia (WM) consists mostly of retrospective single Institution reports[1-6] with small number of patients and 3 registry analysis from US,[7] France[8, 9] and EBMT;[10] the last 2 are presented (or updated) in this Workshop. Table 1 summarizes available data. The limited experience with HDT in WM is mostly due to the advanced age at the time of disease diagnosis and the relatively indolent nature of the disease, which makes such an aggressive treatment option less attractive. However, all studies confirm that HDT with ASCT is feasible in WM patients even in heavily pretreated with a non relapse mortality rate at 6% in the largest series.[10] Harvest of auto SC should be done prior to nucleoside analogue exposure – since these agents may impair SC collectability[11] and before extensive bone marrow infiltration occurs. The occurrence of 2nd cancers in WM patients after autografting (at least 5 cases reported) should be taken into consideration.[7, 8] A small series of 7 patients received HDT as part of their induction regimen with promising results.[2] Most patients have been transplanted in advanced phase of their disease, beyond 1st relapse. A variety of preparative regimens have been used such as BEAM, high

dose melphalan or cyclophosphamide with or without total body irradiation. A high response rate and prolonged remissions have been observed in patients who were clearly resistant to conventional chemotherapy. With the available data we can argue that ASCT as upfront treatment for WM should be still considered only within the context of clinical trials, in younger patients with adverse prognostic factors. Moreover ASCT as salvage regimen should be considered for all fit patients with advanced disease where conventional treatment options have failed. The possibility of stem cell collection in patients pretreated with nucleoside analogues makes the option of SC harvest and storage early in the course of the disease reasonable for certain patients. Moreover, the use of novel agents like rituximab and bortezomib, with confirmed activity against WM, who spare patients' stem cell pool warranties the planning of further studies with HDT based on those treatment options.

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Table 1.

Ref	*Patient No*	*Age median (range)*	*Time to HDT (years)*	*Disease status*	*Preparative regimen*	*Response*	*FU in months (range)*	*Outcome*
1	6	51 (45-69)	4 (3-9)	2 in 1st remission 4 in ≥1st relapse	5 Mel 200 1 Mel 140+TBI	3 PR	12 (5-52)	4 AW 2 progressed,died
2	7	49 (39-61)	< 1	3 in 1st remission 4 1ry refractory	7 Cy TBI	5 PR 2 CR	19 (3-30)	7 AW
3	5	Unknown	Unknown	5 in 1st remission	5 Mel 200	1 Pr 4 CR	Unknown	Unknown
4	1	71	Unknown	1 Refractory	1 Bu Mel 200	CR	11	AW
5	1	50	1.1	1 Refractory	1 Mel 140	CR	12	AW
6	4	49 (30-58)	0.9 (0.8-10)	1 1ry refractory 3 in ≥1st relapse	1 Thiotep Bu Cy 3 Cy TBI ± VP16	3 PR 1 ED	32 (1-123)	1 ED 2 AW 1 2ry AML, died
7	10	56 (44-62)	3 (0.8-11)	10 in ≥1st relapse 3 TBI± other 5 CyAracVP16	2 BuCy	Unknown	65	6 AW 1 progressed, died 1 2nd cancer, died 2 no progression, died
8,9	32	56 (34-68)	3.2 (0.5-23)	32 ≥1st relapse	13 BEAM 16 TBI + other	Unknown	45	18 alive 10 progressed, died 4 no progression, died
10	201	53 (22-73)	53 (22-73)	20% in CR1 12% in PR1 68% in ≥1st relapse	44% BEAM 28% TBI + other 14% Mel 14% Other	Unknown	26	149 alive (112 AW) 36 progression, died 16 toxicity, died

WM2.6

NOVEL AGENTS IN THE TREATMENT OF WALDENSTROM'S MACROGLOBULINEMIA: UPDATE OF WMCTG CLINICAL TRIALS

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Despite advances in therapy, Waldenström's macroglobulinemia (WM) remains an incurable B-cell disorder with elusive complete remissions (8-10%). As such, novel therapeutic agents and combination strategies are needed, particularly if curative efforts are to be pursued. As such, we have prioritized the development of novel as stem cell sparing agents in the treatment of WM.

Proteosome inhibition (WMCTG 03-248, 05-180)

Bortezomib, a stem cell sparing agent, is a proteosome inhibitor which induces apoptosis of primary WM lymphoplasmacytic cells, as well as the WM-WSU WM cell line at pharmacologically achievable levels. Moreover, bortezomib may also impact on bone marrow microenvironmental support for lymphoplasmacytic cells. In a multi-center study of the Waldenstrom's Macroglobulinemia Clinical Trials Group (WMCTG), 27 patients received up to 8 cycles of bortezomib at 1.3 mg/m^2 on days 1, 4, 8, and 11. All but one patient had relapsed/or refractory disease. Following therapy, median serum IgM levels declined from 4,660 mg/dL to 2,092 mg/dL (p<0.0001). The overall response rate was 85%, with 10 and 13 patients achieving a minor (<25% decrease in IgM) and major (<50% decrease in IgM) response. Responses were prompt, and occurred at median of 1.4 months. The median time to progression for all responding patients in this study was 7.9 (range 3-21.4$^+$) months, and the most common grade III/IV toxicities occurring in > 5% of patients were sensory neuropathies (22.2%); leukopenia (18.5%); neutropenia (14.8%); dizziness (11.1%); and thrombocytopenia (7.4%). Importantly, sensory neuropathies resolved or improved in nearly all patients following cessation of therapy. In an ongoing trial by the WMCTG, bortezomib has been combined with dexamethasone and rituximab (BDR) for the primary therapy of patients with WM. As part of this study, patients are receiving intravenous bortezomib at 1.3 mg/m^2 and dexamethasone at 40 mg on days 1, 4, 8, 11, along with rituximab (at 375 mg/m^2) on day 11. Among 23 evaluable patients completing the first 4 cycles of induction therapy, median serum IgM levels declined from 4,830 to 1,450 mg/dL (p<0.0001) and median Hct rose from 29.8 to 37.1% (p=0.0006). The overall response rate was 91% with categorical responses as follows: CR (n=2); PR (n=13); MR (n=6). Responses were prompt, and occurred at median of 1.5 months. With a median follow-up of 8.1$^+$ (range 2.8-18.6$^+$) months, no responding patients have progressed. The most common grade III/IV toxicities occurring in > 5% of patients were sensory neuropathies (48%); leukopenia (13%); neutropenia (9%); and thrombocytopenia (9%). Notably, 4 of the first 7 patients receiving BDR in this study developed herpes zoster reactivation necessitating prophylaxis with daily valcyclovir (1 gm).

Monoclonal antibody therapy (WMCTG 02-079)

Alemtuzumab is a humanized monoclonal antibody which targets CD52, an antigen widely expressed on bone marrow LPC in WM patients, as well as on mast cells which are increased in the BM of patients with WM and provide growth and survival signals to WM LPC through several TNF family ligands (CD40L, APRIL, BLYS). As part of a WMCTG effort, 28 subjects with the REAL/WHO clinicopathological diagnosis of LPL, including 27 patients with IgM (WM) and one with IgA monoclonal gammopathy were enrolled in this prospective, multicenter study. Five patients were untreated and 23 were previously treated, all of whom had previously received rituximab. Patients received 3 daily test doses of alemtuzumab (3, 10, and 30 mg IV) followed by 30 mg alemtuzumab IV three times a week for up to 12 weeks. All patients received acyclovir and bactrim or equivalent prophylaxis for the duration of therapy plus 8 week following the last infusion of alemtuzumab. All patients tolerated test dosing, and completed a median of 33 infusions post test-dosing. Among 27 patients evaluable for response, median Ig levels decreased from 3,665 to 1,495 mg/dL (p<0.0001). The overall response rate was 81%, which included 1 CR (4%), 10 PR (37%), and 11 MR (41%). Hematological toxicities were common among previously treated (but not untreated) patients and included grade 3/4 neutropenia 39%; thrombocytopenia 18%; anemia 7%. Grade 3/4 non-hematological toxicity for all patients included dermatitis 11%; fatigue 7%; and infection 7%. CMV reactivation and infection was commonly seen among previously treated patients and may have been etiological for one death on study. Two other patients also succumbed on study, one related in part to drug therapy for CMV infection and another due to complications of alemtuzumab induced thrombocytopenia and Von Willebrand deficiency.

Signal inhibitors

Inhibitors of Stem Cell Factor Signaling (WMCTG 05-140)

Characteristic of WM is an increased number of mast cells (MC) which are found in association with LPC, and stimulate LPC growth through several TNF-family members including CD40L, APRIL and BLYS. As such, the direct targeting of MC in WM may yield therapeutic results. One important growth and survival factor for MC is stem cell factor (SCF), which signals through CD117. Imatinib mesylate blocks SCF signaling through CD117, and induces apoptosis of WM BM MC and LPC, both of which highly express CD117. As such, we performed a Phase II study of imatinib mesylate in patients with relapsed and refractory WM. Intended therapy consisted of imatinib mesylate which was initiated at 400 mg daily over the first month, and subsequently dose escalated to 600 mg daily for up to 2 years. Dose de-escalation to 300 mg daily was permitted for toxicity. 28 patients with a median of 2 prior therapies have been enrolled on this study. With a median follow-up of 9 months, serum IgM levels for 27 evaluable patients declined from 3,110 to 2,530 at best response (p<0.0001). The overall response rate was 26%, with 2 PR and 5 MR. Responses were prompt, and occurred at a median of 2.1 months. Major treatment related toxicities included anemia, thrombocytopenia, leukopenia and edema and lead to treatment cessation in 9 patients. Importantly, tryptase levels which measure mast cell burden declined from 6.6 to 2.0 ng/mL (at 1 month), and were at 2.9 ng/mL (at 3 months) for 7 evaluable patients. The interim results of this study demonstrate that imatinib mesylate is an active salvage therapy, and may impact on mast cell burden in WM.

Phosphodiesterase inhibitors (WMCTG 05-087)

Inhibition of phosphodiesterase 4 (PDE-4) leads to apoptosis of malignant lymphoma cells. The mechanism by which PDE-4 inhibition leads to apoptosis remains to be defined but may involve dysregulation of cyclic AMP. Sildenafil citrate is a potent phosphodiesterase-5 inhibitor, which also exhibits weak PDE-4 inhibition and is used to treat erectile dysfunction. Interestingly, we and others have observed responses to sildenafil citrate among patients with WM and chronic lymphocytic leukemia (CLL). Moreover, sildenafil citrate induces apoptosis of primary tumor cells from patients with WM and chronic lymphocytic leukemia. In view of these data, the WMCTG conducted a prospective phase II study of sildenafil citrate in patients with slowly progressing WM who did not meet consensus eligibility for active therapy. Patients on this study were initiated at the dose of 25 mg daily, then dose escalated weekly by 25 mg until they reached the final dose of 100 mg daily. Thirty patients were enrolled, 18 of whom were previously untreated. All patients demonstrated progressing disease prior to enrollment. Patients were evaluable for response after 3 months of therapy. At best response, serum IgM levels declined from 3,550 to 2,965 mg/dL (p=0.007), with 22/30 patients demonstrating a decrease in serum IgM levels (range -4 to -45%). Overall, 5 (17%) patients demonstrated at least a MR, with a median TTP for responders of 6.1 (range 2.5-9.8) months. Therapy was well tolerated, and there were no grade 3/4 toxicities. Future efforts aimed at developing more potent phophodiesterase-4 inhibitors are contemplated. In summary, advances in the biological understanding of WM are yielding newer and more targeted therapies for the treatment of this malignancy and have led to development of several novel agents. Clinical trials further establishing the optimal use of these agents, as monotherapy or in combined therapy are warranted.

WM3: Novel treatment approaches, treatment of advannced disease

WM3.1

PROTEOMIC ANALYSIS IN WALDENSTROM MACROGLOBULINEMIA

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To better understand the molecular changes that occur in Waldenstrom's Macroglobulinemia (WM), we employed antibody-based protein microarrays to compare patterns of protein expression between untreated WM and normal bone marrow controls. The Antibody Microarray (BD Clontech, Palo Alto, CA) detects a wide variety of proteins (both cytosolic and membrane-bound) representing a broad range of biological functions, including signal transduction, cell-cycle regulation, gene transcription, and apoptosis. The microarray contains 512 highly specific and sensitive monoclonal antibodies against human polypeptides. To determine differences between symptomatic and asymptomatic WM, we selected 10 cases of WM, 5 with symptomatic WM and 5 asymptomatic. The asymptomatic WM /MGUS samples were selected by the presence of an IgM monoclonal protein with an M-spike less than 1.0 gm/dL, and the presence of lymphoplasmacytic cells of less than 10% in the bone marrow and the absence of symptoms related to WM. In addition, follow up of these patients demonstrated no progression to symptomatic WM to the date these studies were performed. After $CD19^+$ and $CD138^+$ selection from the samples and another 3 bone marrow normal control samples, protein extraction was performed and the cell extracts were labeled with Cy3 and Cy5 dyes as per the manufacturer's protocol. The mean of the ratios of Cy5/Cy3 of both slides were analyzed using Clontech Excel software developed specifically for each microarray lot by the manufacturers. In addition, to confirm the expression patterns obtained in the protein microarray, we used WM and IgM secreting lymphoma cell lines along with primary $CD19^+$ or $CD138^+$ cells or concomitant $CD19^+$/$CD138^+$ cells from another group of patients with WM (N=10) and normal donors (N=5). Unsupervised clustering of the WM samples demonstrated a homogenous pattern of expression in all the samples. We analyzed polypeptides that were up- or down-regulated by > 1.3 fold or >2 fold as compared to normal control. Using the >2 fold cutoff, the microarrays identified 6 dysregulated polypeptides in at least 60% samples of WM. All polypeptides were overexpressed in the WM cells as compared to control cells. These polypeptides were signal transduction regulators such as Ras related proteins including Rab4 and p62DOK; Rho related proteins including CDC42GAP, and ROKα; and other proteins such as SNX-1, Roaz and FAS. Using the > 1.3 cutoff, 105 polypeptides were upregulated and 74 downregulated in at least 60% samples of WM. These included polypeptides involved in cell cycle regulation such as CDK2 and RCC-1, histone deacetylases such as HDAC3, and modulators of apoptosis, such as the proteins in the PI3K pathway and proteosome/ubiquitin pathway. We then determined whether there was a difference in protein expression in patients with asymptomatic disease/MGUS as compared to those with symptomatic WM who required therapy. Unsupervised clustering showed no difference in protein expression between samples of patients with symptomatic versus asymptomatic disease. However, there were 3 proteins identified as upregulated in symptomatic WM as compared to asymptomatic WM//MGUS by >2 fold expression level. These included the heat shock protein HSP90, the Ras family protein CDC25C and the chemotaxis protein p43/EMAPII. To validate the results of the protein microarray, immunohistochemistry on paraffin embedded tissue from the same biopsies used for the protein array analysis was performed and immunoblotting from another 10 samples of newly diagnosed symptomatic WM and 5 different controls that were not included in the protein array analysis was performed. The proteins p62DOK, Rab4 and HSP90 were overexpressed in WM samples (N=3) compared to normal control cells. Similarly, the WM cells lines and IgM secreting lymphoma cells lines (BCWM1, RL and WM-WSU) had a high expression of all 3 proteins. To confirm the functional significance of protein elevation, we used the HDAC inhibitor Trichostatin and HSP90 inhibitor 17-AAG. Trichostatin and 17-AAG inhibited WM cells survival and induced apoptosis at 24 and 48h in a dose- dependent fashion. There is an urgent need to elucidate the molecular pathways that mediate proliferation and resistance to apoptosis in WM in order to provide targets for novel therapies. Transcriptional profiling in WM has identified some pathways that are upregulated in WM.[1] Proteomic analysis represents a technique that yields more information at the functional protein level. The antibody array technology represents a high-throughput new technology to identify novel proteins and rapidly screen multiple samples yielding molecular signatures and profiles. Some of the polypeptides identified in this analysis might contribute to the pathogenesis of WM including those in the Ras and Rho families of kinases. Ras proteins included included Rab4 and p62DOK. Oncogenic Ras expression occurs in up to 40% of multiple myeloma cases and correlates with aggressive disease.[2,3] This study, therefore, identifies a role of Ras signaling pathway in WM. Rab4 is a Ras-like small GTPase that coordinates protein transport from the endosome to the plasma membrane. It is associated with prolonged activation of MAPKinase in some malignancies. P62DOK or RasGAP- associated docking protein was originally defined as a tyrosine-phosphorylated 62-kDa protein that coimmunoprecipitated with p21Ras GTPase-activating protein (RasGAP). RasGAP is an essential component of Ras-activated signaling pathways.[4,5] RasGAP down-regulates Ras activity and plays a role in cell growth and differentiation.[4,5] Similarly, proteins in the Rho pathway were upregulated in WM as compared to normal controls. The GTPase RhoA has been implicated in various cellular activities, including the formation of stress fibers, motility, and cytokinesis.[6,7] Cdc42 belongs to the Rho family of small GTP binding proteins along with Rac, and Rho. It is involved in regulating a variety of cellular functions including actin cytoskeleton organization, cell growth control and development, transcriptional activation, membrane trafficking, and cell transformation.[8] ROKα is a p150 serine/threonine kinase binding RhoA only in its active GTP-bound state promoting formation of stress fibers and focal adhesion complexes.

Other polypeptides that were upregulated by 1.3 fold include HDAC3. Histone acetyltransferases (HATs) can stimulate gene transcription by acetylating histones, facilitating an open chromatin state. Alteration in the chromatin structure allows access of transcription factors to the promoter regions and results in activation of gene transcription. Histone deacetylases (HDACs) play a critical role on the pathogenesis of B-cell malignancies such as in large B-cell lymphoma and multiple myeloma. In addition, we demonstrated that the HDAC inhibitor Trichostatin inhibited growth and survival of primary WM cells and WM cells lines confirming that HDACs are important regulators of survival in WM. We further demonstrated that the molecular changes occured early in the disease in cases with asymptomatic WM/MGUS analogous to results in patients with multiple myeloma where the molecular abnormalities identified in MGUS are similar to those identified in symptomatic Multiple Myeloma.[9] HSP90 was upregulated in symptomatic WM as compared to asymptomatic WM/MGUS indicating that this protein is upregulated with progression of disease. HSP90 has been implicated in the pathogenesis and resistance of many malignancies including multiple myeloma, another plasma cell dyscrasia.[10] We further confirmed the functional signifcance of this protein in survival of WM cells by demonstrating that the HSP90 inhibitor 17-AAG induced significant apoptosis and inhibition of growth in WM cell lines and primary patient samples. Previous studies of gene expression profiling in 23 patients diagnosed with WM identified a homogenous expression profile of WM cells that was similar to that of CLL. The most significantly up-regulated gene was IL-6 and the most significantly associated pathway for this set of genes was MAPK signaling. Although changes in mRNA levels do not always translate into changes at the protein level, we have identified multiple members of the Ras/MAPK pathway upregulated in this protein array analysis reflecting consistency between gene and protein expression profiling. In summary, our studies have identified for the first time novel proteins that are differentially dysregulated in WM, which, both enhances our understanding of disease pathogenesis and represent targets for novel specific inhibitors.

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WM3.2

THE GENE EXPRESSION SIGNATURE OF CLONAL CELLS FROM WALDENSTROM'S MACROGLOBULINEMIA: DIFFERENCES AND COMMONALITIES WITH THE NORMAL CELL COUNTERPART AND OTHER RELATED LYMPHOPROLIFERATIVE DISORDERS

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Aims. We have used genome-wide expression profiling (GEP) to investigate the transcriptomic signature of WM related to normal status, as well as to explore the differences and similarities in expression patterns of clonal cell populations from WM and from those of chronic lymphocytic leukemia (CLL) and multiple myeloma (MM). *Material and Methods*. Bone marrow samples from 10 patients with WM, 12 with MM and 11 with CLL, along with 8 normal B lymphocytes samples (NBL) obtained from peripheral blood and 5 normal plasma cells (NPC) samples obtained from the bone marrow of healthy donors, were used for the analysis. The isolation of clonal B lymphocyte and plasma cell populations was carried out by multiparameter flow cytometry sorting with the appropriate monoclonal antibodies. Total RNA (100 ng) was amplified and labeled according to Affymetrix technology and hybridized to *Human Genome U133A* microarray. *Results*. The comparative analyses of the gene expression profile of WM-BL vs NBL identified a set of 171 genes expressed differentially between the two groups. Three members of the activator protein 1 (AP-1) group, JUN, FOSB and BATF, and genes involved in B cell development, such as BTK, CD69, CD83, IRF8 and ITPR1 were deregulated in WM-BL. When comparing the expression profile of WM-PC with that of NPC, a total of 498 genes (mostly included in RNA post-transcriptional modification, DNA replication and cellular assembly and organization categories) were up-regulated in WM-PC group. Interestingly, a set of 4 genes (LEF1, MARCKS, ATXN1 and FMOD) was able to discriminate clonal BL from WM and CLL. The most important genes that discriminate PC from WM and MM were those involved in plasma cell differentiation such as PAX5 (overexpressed in WM-PC), and IRF4 and BLIMP1 (underexpressed in WM-PC). We also investigated the relationship between the three B lymphoproliferative disorders. One of the most significant overexpressed genes, both in WM and CLL, was the IL10 receptor (IL10RA). *Conclusions*. These results delineate a distinct transcription signature of clonal cells from WM, which is genetically different from the MM and CLL cell-counterpart. The differentially expressed genes have important functions in the B-cell differentiation and oncogenesis.

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WM3.3

GENE EXPRESSION PROFILING OF WALDENSTROM'S MACROGLOBULINEMIA REVEALS GENES THAT MAY BE RELATED TO DISEASE PATHOGENESIS

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Introduction. To determine the molecular events linked to the pathogenesis, progression and clinical outcome of Waldenstrom's Macroglobulinemia (WM), we performed gene expression profiling (GEP) of the bone marrow (BM) WM tumor compartment. This tumor compartment includes two immunophenotypically distinguishable tumor populations: B-lymphocytes ($CD19^+$) and plasma cells ($CD138^+$). *Material and Methods.* BM aspirates were obtained from untreated patients with the consensus panel diagnosis of WM (Semin Oncol. 2003; 196-200). BM WM B-cells (WMBCs) and plasma cells (WMPCs) from 28 WM patients and 9 healthy controls were sequentially isolated using CD19 and CD138 microbeads, respectively. Using Affymetrix microarrays (U133 Plus2.0), we analyzed the GEP of WMBCs and WMPCs, and compared with their normal counterparts. Hierarchical cluster and significant analysis of microarray (SAM) test were used for the data analysis. *Results*. Unsupervised hierarchical cluster analysis demonstrated a distinct gene expression pattern between WMBC and WMPC versus their normal controls. By performing SAM test with 0.1% FDR, a set of 2463 (1533 and 903 up- and down-regulated, respectively) and 693 genes (563 and 130 up- and down-regulated, respectively) were differentially expressed in WMBC and WMPC, compared to their normal counterparts, respectively. A supervised hierarchic cluster analysis clearly demonstrated significant differences in WMBC and WMPC compared to controls. Particular genes of interest to lymphoplasmacytic cells growth and survival included BCL2, a key arbiter of the commitment to programmed cell death at the mitochondria that expressed in many cancers, was upregulated in tumor cells. Among other transcripts overexpressed in WMPCs were genes involved in transcription (ZKSCAN1, ZMYM1, ZNF189, ZNF19, and ZNF559) and interferon response (IFI16 and IFIH1). Of the underexpressed genes, the AP1 family genes JUND and FOSB were the most significant downregulated genes. Of the most significantly dysregulated genes in WMBC, IGLL1, has been shown to be involved in BCL-6 rearrangment and may contribute to dysregulation of BCL-6 in lymphoma. Upregulation of CCR2 expression may contribute to the marrow homing properties of the WM clone in responses to MCP ligands. Data comparing and contrasting WMPC and myeloma PC will be presented. These data begin to provide new molecular insights into the pathogenesis of WM.

WM3.4

NOVEL SPLICE VARIANT TRANSCRIPT OF SIVA IN WALDENSTROM'S MACROGLOBULINEMIA

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Introduction. The transmembrane protein CD27 is a member of the tumor necrosis factor receptor (TNFR) family that binds to its ligand CD70. Recently, we demonstrated that CD27-CD70 interaction supports growth and survival of Waldenstrom's macroglobulinemia (WM) cells. We also detected high levels of soluble CD27 (sCD27) in the sera of patients with WM; however, the mechanisms of soluble CD27 secretion in the sera of WM patients remain unclear. SIVA, a proapoptotic protein, binds to the cytoplasmic tail of CD27. Overexpression of full-length, wild type SIVA has been associated with the induction of apoptosis in several malignancies that suggests an important role for SIVA in the CD27-dependent apoptotic pathway. Two splice variants of SIVA, SIVA1 and SIVA 2, have been identified in humans thus far. *Methods and Results*. To elucidate underlying mechanisms of sCD27 release in the sera of WM patients, we first examined the expression patterns of SIVA transcripts in WM tumor cells (n=8) by RT-PCR. We found that all patients expressed both previously- reported splice variants, SIVA 1 and SIVA 2. Herein, we describe for the first time, a novel splice variant of SIVA detected in 4/8 WM patients. To further characterize the novel splice variant transcripts of SIVA, designated SIVA-Va, we cloned RT-PCR products obtained from the two WM patients into the TOPO TA vector. Positive sub-clones were identified by PCR with SIVA1 gene specific primers. Plasmids isolated from positive clones were sequenced. Sequences were identified through alignment with the published sequence of human SIVA mRNA. Further bioinformatic analysis demonstrated that the novel variant of SIVA is a result of partial retention of an intron. In general, this type of splicing is associated with a malignant phenotype. In conclusion, the aberrant novel variant of SIVA, SIVA-Va, may compromise binding of full-length, wild type SIVA to CD27; thereby facilitating the cleavage of CD27 from the plasma membrane of WM tumor cells leading to the disruption of the CD27-CD70 signaling in WM.

WM3.5

ABERRANT POST-TRANSCRIPTIONAL REGULATION OF TNF FAMILY MEMBERS AND THEIR ADAPTOR MOLECULES ESSENTIAL TO B-CELL GROWTH AND SURVIVAL IN WALDENSTROM'S MACROGLOBULINEMIA

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Introduction. Despite considerable advances to date, the genetic basis for Waldenstrom's macroglobulinemia (WM) remains unclear. We have focused our studies on members of the tumor necrosis factor (TNF) family, given their integral role in neoplastic, as well as normal B-cell growth and survival. TNF receptors use specific but overlapping sets of cytoplasmic adaptor proteins (TRAFs) for signaling. TRAFs are known to play an important role in cell growth and cell survival through the activation of the key transcription factor NF-κB. Activation of this molecule has been noted in many B-cell malignancies. However, the role of these molecules on WM B-cell survival and growth remains to be defined. As part of these studies, we elucidate the critical importance of splicing aberrations of TRAF and some of the TNF family member genes as modulators of WM cell growth and survival. *Methods and Results*. Using RT-PCR and multiplex RT-PCR, we identified expression patterns for TRAF2, TRAF5, CD40 and BLYS in $CD19^+$, $CD34^+$, $CD3^+$, and $CD14^+$ cells obtained from the BM aspirates of 12 WM patients. Differences in TRAF2, TRAF5, CD40 and BLYS splice variant expressions were observed in $CD19^+$ versus other cell populations obtained from the same WM patients. In $CD19^+$, $CD34^+$, $CD3^+$, and $CD14^+$ cells from BM of WM patients we observed distribution patterns of splice variants of CD40 and BLYS similar to those observed for TRAF variants. Most importantly we identified novel splice variant for TRAF2 (TRAF2Va) in $CD19^+$ cells from 8/12 WM patients. Sequence alignment analysis identified TRAF2Va novel variant resulting from a deletion of an exon leading to inframeshift. By bioinformatic analysis, TRAF2Va was predicted to encode a region essential for protein-protein interactions as well as DNA binding, suggesting disrupted TNF signaling pathways and NF-kB activation. *Conclusions*. Taken together, these results suggest that in WM patients, TNF-family members and their adaptor molecules responsible for normal B-cell growth and survival are subjected to aberrant post-transcriptional regulation. As a result, these aberrations most likely alter both canonical and noncanonical NF-kB signaling. The clinical consequences and significance of this finding, as well as frequency of novel variant transcripts in large population of WM is currently the focus of further investigation.

WM3.6

THE FUNCTIONAL ROLE OF CD27-CD70 INTERACTIONS IN WALDENSTROM'S MACROGLOBULINEMIA

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Introduction. Waldenström's macroglobulinemia (WM) is a B-cell malignancy characterized by an IgM monoclonal gammopathy and bone marrow (BM) infiltration with lymphoplasmacytic cells (LPC). In support of these characteristics, mast cells (MC) are commonly present in excess and provide a proliferative advantage to the tumor through several TNF-family ligands (CD40L, APRIL, BLYS). In this study, we investigated the interaction between CD27 and CD70, another pair of TNF-receptor/ligand family members, in the pathogenesis of WM. *Methods and Results*. To this end, we first examined the serum levels of soluble CD27 (sCD27) by ELISA in patients with WM (n=66), Multiple Myeloma (MM)(n=25) and healthy donors (HD) (n=16). We found that sCD27 attains significantly higher levels in WM (median=120 U/mL) versus both MM (median=54U/mL) and HD (median=46U/mL) [$p<0.0001$]. We also demonstrated, via RT-PCR and flow cytometric (FACS) analysis, that CD27 and CD70 are constitutively expressed on WM LPC, MC and the WM cell lines. To then further elucidate the functional role of sCD27-CD70 interactions in WM, we incubated BM MC and LPC with recombinant-sCD27 [0.1-50 μg/mL] for 24 hours and, using FACS analysis, observed an increase in the cell-surface expression of both CD40L and APRIL in MC alone. We next tested the SGN-70 (anti-CD70) humanized antibody to block sCD27-CD70 signaling; BM MC and LPC were incubated with SGN-70 [1 μg/mL], which inhibited the previously-noted, sCD27-induced upregulation of CD40L and APRIL. The ability of SGN-70 [0.01-20 g/mL] to mediate the direct induction of apoptosis, complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC) was also determined; as a result, significant ADCC activity against the BCWM1 cell line was observed. Lastly, SGN-70 [1 mg/kg, i.p., qOD] was tested in a WM SCID-hu mouse model; using ELISA, tumor engraftment and disease progression were monitored by measuring both serum human IgM and sCD27 levels in 12 mice. The results show that SGN-70 inhibited tumor growth in all treated mice. *Conclusion*. The results of these studies demonstrate a functional role for sCD27 in WM pathogenesis, along with its utility as a surrogate marker of disease and a target in the treatment of WM.

WM3.7

SERUM CONCENTRATIONS OF ANGIOGENIC CYTOKINES IN WALDENSTROM'S MACROGLOBULINEMIA: THE RATIO OF ANGIOPOIETIN-1 TO ANGIOPOIETIN-2 AND ANGIOGENIN CORRELATE WITH DISEASE SEVERITY

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Introduction. Angiogenesis represents an essential step of disease progression in several hematological malignancies. Microvessel density is increased in 30% of patients with Waldenstrom s macroglobulinemia (WM), but there is very limited information for the role of angiogenic cytokines in WM. The aim of this study was to evaluate the serum levels of different angiogenic cytokines in WM and explore possible correlations with clinical data. *Patients and Methods.* We studied 78 serum samples from 56 WM patients (38M/18F; median age: 71 years) in different phases of their disease. Twenty-four patients were evaluated prior any kind of treatment: 21 symptomatic before therapy administration and 3 asymptomatic who did not need therapy. Twenty patients were studied during active disease after treatment (refractory/relapsed WM) and 12 patients during remission after response to previous therapy. Furthermore, 11 patients with IgM-MGUS and 30 healthy controls were also studied. Serum concentrations of VEGF, angiogenin, angiopoietin-1 (Ang-1), Ang-2 and basic fibroblast growth factor (bFGF) were measured using an ELISA methodology (R&D Systems, Minneapolis, MN, USA) as well as serum levels of VEGF-A (Diaclone SAS, Besancon, France). *Results*. All patients had elevated values of VEGF, VEGF-A, angiogenin, Ang-2, and bFGF compared with controls ($p<0.001$). The ratio of Ang-1/Ang-2 was reduced in WM ($p<0.001$) but not in IgM-MGUS patients. Angiogenin levels correlated with disease status: continuous elevation from healthy subjects (mean±SD: 239.5±58.4 ng/mL) to IgM-MGUS (312.9±86.8 ng/mL), asymptomatic WM (340.1±52.2 ng/mL) and symptomatic, untreated patients (552.3±268.9 ng/mL, $p<0.001$); then reduced in patients at remission (369.9±219.9 ng/mL, $p=0.03$) and increased again in relapsed/refractory disease (458.7±162 ng/mL, $p=0.04$). Angiogenin correlated with serum albumin (r=-0.392, $p=0.001$). WM patients with lymphadenopathy had reduced levels of Ang-1/Ang-2 ratio compared with WM with no lymphadenopathy (2.5±0.5 vs. 9.4±7.6, $p<0.01$). Furthermore, the ratio of Ang-1 to Ang-2 correlated with beta2-microglobulin (r=-0.572, $p<0.0001$), hemoglobin (r=0.33, $p=0.01$) and albumin (r=0.276, $p=0.049$). Finally, a positive correlation was observed between VEGF-A and beta2-microglobulin (r=0.284, $p=0.03$). *Conclusion*. We showed, for the first time in the literature, that patients with WM have increased serum levels of angiogenin, VEGF, VEGF-A, bFGF and reduced values of Ang-1/Ang-2 ratio, which seems to be implicated in WM severity. The confirmation of our results will give the potential for using angiogenin for the follow-up of WM patients and targeting angiogenic molecules for the development of novel anti-WM agents.

WM3.8

USE OF THE IMMUNOMODULATORS THALIDOMIDE AND LENALIDOMIDE TO AUGMENT RITUXIMAB CLINICAL ACTIVITY IN WALDENSTROM'S MACROGLOBULINEMIA

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Introduction. Rituximab is active in Waldenstrom's macroglobulinemia (WM), producing response rates of 30-40%. Lower response rates are observed among patients with FcγRIIIA-158 FF polymorphism; high B_2M (≥3.0 mg/dL), and high IgM levels (≥6,000 mg/dL). We investigated two immunomodulators: Thalidomide and its analogue Lenalidomide in combination with Rituximab given previous studies demonstrating increased ADCC activity against lymphoplasmacytic cells (BJH 128:192; Cancer Res 65:11712). *Methods.* We conducted 2 phase II clinical trials in symptomatic patients with the clinicopathological diagnosis of WM using consensus panel criteria. Intended treatment and patient characteristics were as follows.

Table 1.

	Thalidomide and Rituximab	*Lenalidomide and Rituximab*
Intended therapy:	Weeks 2-5, 13-16: Rituximab (375 mg/m²/wk); Weeks 1-52: Thalidomide (200 mg po qHS for 2 wks, then 400 mg po qHS)	Weeks 2-5, 13-16: Rituximab (375 mg/m²/wk); Weeks 1-48: Lenalidomide (25 mg po qD for 3 weeks, 1 week off)
Enrolled:	25	16
Previous therapies:	0 (0-1)	0 (0-2)
Age:	62 (44-86 yrs)	65 (49-85 yrs)
Serum IgM:	3,670 (924-8,610 mg/dL)	4,000 (1,180-7,130 mg/dL)
Hct:	34.1 (23.6-42.6%)	32.1 (24-36.6%)
BM Involvement:	40 (5-80%)	37.5 (5-90%)
B_2M:	2.6 (1.4-8.3 mg/L)	3.3 (1.8-6 mg/L)

Results. In the phase II study of Thalidomide and Rituximab in WM, 23/25 patients were evaluable and responses included: CR (n=1); PR (n=15); MR (n=2); SD (n=1) for an overall (ORR) and a major response rate (MRR) of 78% and 70%, respectively. Median serum IgM levels decreased from 3,670 (924-8,610 mg/dL) to 1,590 (36-5,230 mg/dL) (p<0.001), while the median hematocrit rose from 33.0 (23.6-42.6%) to 37.6 (29.3-44.3%) (p=0.004) at best response. With a median follow-up of 42[+] months, the median TTP for evaluable patients on study was 35 months, and 38[+] months for responders. ORR was associated with median cumulative Thalidomide dose: CR/PR/MR (29,275 mg) vs. SD/NR (7,400 mg); p=0.004. ORR were unaffected by FcÁRIIIA-158 polymorphism (81% vs. 71% for VV/FV vs. FF); IgM (78% vs. 80% for <6,000 vs. ≥6,000 mg/dL); and B_2M (71% vs. 89% for <3 vs. ≥3 g/dL); p=NS. Dose reduction of Thalidomide occurred in all patients and led to discontinuation in 11 patients. Among 11 patients experiencing grade ≥2 neuroparesthesias, 10 demonstrated resolution to grade 1 (n=3) or complete resolution (n=7) at a median of 6.7 (range 0.4-22.5 months). In our phase II study of Lenalidomide and Rituximab in WM, 12/16 patients were evaluable and responses included: PR (n=4); MR (n=4); SD (n=3); NR (n=1) for an ORR and MRR of 67% and 33%, respectively, with a median TTP of 15.6 months. In two patients with bulky disease, significant reduction in node/spleen size was observed. Acute decreases in hematocrit were observed during first 2 weeks of Lenalidomide therapy in 13/16 (81%) patients with a median hematocrit decrease of 4.4% (1.7-7.2%), resulting in hospitalization in 4 patients. No evidence of hemolysis or more general myelosuppression was observed in these patients. *Conclusions*. Thalidomide in combination with Rituximab is highly active, produces long-term responses, and may overcome unfavorable prognostic determinants previously reported with Rituximab monotherapy in WM. The use of Thalidomide along with Rituximab appears superior in efficacy, and better with regard to tolerability versus those observed with Lenalidomide and Rituximab in a similar clinical population of patients with WM.

WM3.9

ALLOGENEIC STEM CELL TRANSPLANTATION (ALLO-SCT) IN WALDENSTROM'S MACROGLOBULINAEMIA (WM). AN ANALYSIS OF 106 CASES FROM THE EUROPEAN BONE MARROW REGISTRY (EBMT)

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Lymphoma WP of the EBMT

Introduction. Complete response is infrequent in WM patients and there is no cure. The role of allo-SCT has not been extensively explored and limited data are available. *Patients and Methods*. We studied 106 patients who underwent an allo-SCT for WM up to December 2005, from HLA-identical (75%) or unrelated donors (25%). Median age at transplant was 49 years (21-65; 49% >50 years). Median time from diagnosis to allo-SCT was 34 months (5-310) and median number of previously failed treatment lines of three. Nineteen patients (18%) had failed an autograft. At allo-SCT, 10 patients (10%) were in CR≤2, 35 (33%) in PR1, 29 (27%) in PR≥2 and 32 (30%) had relapsed or refractory disease. Conventional conditioning protocols (CT) were used in 44 (41%) patients and reduced intensity conditioning (RIC) regimens in 62 (59%). Peripheral blood was the stem cell source in 84 cases, with some form of T-cell depletion in 19% of them. *Results*. Forty-eight (45%) patients developed acute GVHD (grades III-IV, n=14) with no statistically significant differences between CT and RIC. After a median follow up of 31 months (3 to 169), 17 (16%) patients have relapsed at a median time of 8 (1-89) months post allo-SCT. The incidence of relapse at 3 years was 18%; 12% after CT and 25% after RIC. Thirty-five (33%) patients died, five (5%) from disease progression and 30 (28%) from non-relapse mortality (NRM). Cumulative incidences of NRM at 1 and 3 years were of 27% and 31%, respectively. The progression free survival rates were 61%, 50% and 48% at 1, 3 and 5 years and the overall survival 69%, 63% and 63%, respectively. In a multivariate analysis, conditioning regimen had no impact either on NRM or on relapse rate. Refractory patients had a higher relapse risk (p=0.03). The use of TBI in the conditioning was associated with a lower relapse risk (p=0.02) and a trend to a better PFS (p=0.1). *Conclusion*. This study suggest that allo-SCT is a feasible and well tolerated procedure even in this rather old population of patients, and it is followed by a low relapse rate and a promising survival.

WM3.10

INCREASED INCIDENCE OF DISEASE TRANSFORMATION AND DEVELOPMENT OF MDS/AML IN WALDENSTROM'S MACROGLOBULINEMIA (WM) PATIENTS TREATED WITH NUCLEOSIDE ANALOGUES

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Background. WM is an indolent B-cell lymphoma. NA are widely used in the treatment of WM, and are considered as appropriate first line agents for the treatment of WM (Gertz *et al.*, Semin Oncol 2003; Treon *et al.*, Blood 2006). Increased incidences of disease transformation and development of MDS/AML have been observed among patients with indolent B-cell malignancies receiving NA. We therefore sought to delineate the incidence for these events in a large population of WM patients treated at our institution. *Methods.* 326 previously treated patients with the consensus panel definition of WM, who received treatment with (n=173) or without (n=153) a NA were included in this analysis. Baseline characteristics between NA and non-NA treated patients were not significantly different and were as follows: median age 59 years; male/female ratio 1.4; median B2M 2.9 mg/L; serum IgM 3,000 mg/dL; BM involvement 40%; Hct 34%; WBC 5,100/uL, and PLT count 243,000/uL. For patients receiving NA, treatment consisted of either fludarabine (n=117; 68%), cladribine (n=48; 27%) or both (n=8; 5%). For non-NA treated patients, therapy included chlorambucil, rituximab, CVP, CHOP, thalidomide, and cyclophosphamide alone or in combination with rituximab, and alemtuzumab. Median follow-up of patients was 64 (range 10-270) months. *Results*. Among NA treated patients, 10 (5.7%) patients had transformation to an aggressive NHL (to DLBCL) (n=7; 4%) or developed MDS/AML (n=3; 1.7%). Disease transformation and development of MDS/AML occurred at a median time of 48 (range 7-114), and 48 (range 38-52) months following NA treatment, respectively. In con-

trast, among non-NA treated patients, only 1 patient demonstrated disease transformation (to DLBCL) at 10 months and no patients developed AML/MDS (p=0.025). *Conclusion*. These data demonstrate an increased incidence of disease transformation and development of MDS/AML among WM patients treated with NA.

WM3.11

CLINICAL PROFILE AND TREATMENT OUTCOME IN 103 PATIENTS WITH AL AMYLOIDOSIS ASSOCIATED WITH IGM PARAPROTEINAEMIA

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Introduction. Most patients with AL amyloidosis (AL) have a subtle underlying plasma cell dyscrasia and underlying IgM paraproteinaemia is rare. The profile of AL amyloidosis associated with IgM paraproteinaemia remains poorly studied . Majority of the IgM paraproteinaemia patients have an underlying lymphoproliferative disorder and the treatment outcome is not well known. *Materials and Methods*. We report the clinical profile and outcome of 103 patients with IgM associated AL amyloidosis seen at the National Amyloidosis Centre, UK. Patients were selected if they had a confirmed diagnosis of AL amyloidosis, presence of an IgM paraprotein and absence of any other monoclonal protein in the serum or urine by electrophoresis or immunofixation. Response was assessed using the worse of either conventional paraprotein response as per with Waldenstrom's (WM) criteria or free light response (FLC) by amyloidosis consensus criteria. *Results*. The M:F ratio was 1.7:1, median age 65 yrs. The bone marrow showed lymphoplasmacytoid infiltration 42 (41%), lymphoid infiltration in 24 (23%), plasma cell infiltrate in 10 (9%) and was normal or non-diagnostic for a clonal population in 27 (26%). The median number of organs involved was 2, including renal 56%, heart 32%, liver 41%, lymph nodes 22%. The median IgM level was 9g/l (range immunofixation positive to 60g/l). A total of 75 patients were assessable for response to initial treatment. Twenty five (32%) of the 77 evaluable patients responded with no conventional complete responses (CR). An FLC response was seen in 12/22 (55%) patients evaluable for a FLC response, with a CR by FLC criteria in 3/22 (14%) and partial response in 9/22 (41%). Combination chemotherapy (VAD, CHOP, CVP, R-CVP) or purine analogues (fludarabine, cladribine, FCR, cladribine rutiximab) appeared to be more effective with 13/22 (59%) responding when compared as a group to the conventional oral agents (chlorambucil, oral melphalan and oral cyclophosphamide) with 7/34 (20%) responders but the numbers are too small for meaningful statistical comparison. Organ responses occurred in only 2 of 25 (12%) hematological responders. On multivariate analysis, the factors independently affecting survival were performance status and liver involvement by consensus criteria. *Discussion*. In summary, the presenting features of IgM associated AL are similar to AL with non-IgM paraproteins, though lymph node involvement was more common. The response to treatment was poor with no complete responses though patients given intermediate dose chemotherapy appeared to have higher responses. Similarly, the improvement in organ function was small. This study highlights the continuing difficulty in effectively treating patients with IgM paraproteinaemia associated AL amyloidosis and studies using the more effective regimes are needed.

POSTER SESSION I

Group 1: Genetics

PO-101

HIGH LEVEL AMPLIFICATIONS AND HOMOZYGOUS DELETIONS ARE DETECTED BY HIGH RESOLUTION ARRAYCGH IN MULTIPLE MYELOMA PRIMARY SAMPLES

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Multiple myeloma is a malignant plasma cell neoplasia characterized by a wide variability in clinical features, responses to treatment, and survival times among patients. FISH and CGH studies have shown that genomic changes may affect almost all chromosomes. For the first time, a high resolution arrayCGH has been used to analyze 26 MM primary samples after the enrichment in CD138-positive plasma cells. We used the Human Genome CGH 44k microarrays (Agilent Technologies, Palo Alto, CA). This platform consists of approximately 44000 60-mer oligonucleotide probes that span the human genome with an average resolution below 50 Kb. The platform has gene focused coverage in order to ensure adequate coverage in most commonly studied genomic regions. FISH and SKY analysis have been applied for validation of the obtained results. This approach identified copy number imbalances in all cases and revealed the presence of several igh level amplifications (HLA) and homozygous deletions (HD). Nine HLA were found in 6q12, 9q22.2, 9q34.2, 14q21.1, 16q22.2, 17p13.2, 17q11 and 19q13. Among the genes contained in all the amplified regions we highlight SYK and CCNE1 which are involved in signal transduction, Six HD were observed in 6q23.2, 11q22.1, 13q12, 22q11.22, Xq21.31 and Xq25.Reinforcing the putative pathogenic role of this finding, these regions also showed up as single copy losses in several additional samples of our series. These changes are being validated by FISH assays. Among the most frequent aberrations, we identify a novel Xq21.33-qter duplication in 5 out 26 cases and one cell line. Spectral Karyotyping studies were carried out in L363 and case #20 in order to validate the results. We also developed a FISH assay for validation of the cases and for the identification of possible recurrence in following series. This analysis demonstrated that duplications were the unbalanced outcome of translocations with breakpoints at Xq21: t(X;14)(q21.33; q31.1) in L363, and t(X;21)(q21.33;q22.3) in case #20. Both translocations do not involve IGH locus. Acknowledgment: Work partially funded by Red Tematica FIS 03/136: Mieloma multiple de la genesis a la terapeutica.

PO-102

FISH, PCR AND CGH, ON FACS SORTED PLASMA CELLS IN MM AND MGUS

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Introduction. Chromosomal abnormalities in MM are complex and some have prognostic significance. Exploration for novel chromosomal and gene aberrations is necessary to gain more insight into the pathogenesis of MM and to identify events responsible for transformation of MGUS towards MM. However, application of standard techniques on bone marrow (BM) is often hampered by the low-level BM infiltration and the low mitotic index of PCs. The use of a PC targeting strategy is mandatory. Here we present different molecular/cytogenetic techniques (FISH, PCR and CGH) performed on immunophenotypically pure, aberrant PC populations selected from the BM by flow cytometry. *Materials and Methods.* BM samples of 20 MM and MGUS patients, with% PC ranging from 1-57%, were available. Immunophenotyping and PC purification was performed on a FACS-Aria(r) (BD). Sort gates were defined by expression of cyIg$^+$ or cyIg$^-$ combined with presence or absence of CD56 expression, within the CD138$^+$/CD38^{++} PC gate. PCs were spotted on glass slides and in culture wells (for interphase and metaphase-FISH) and collected in microtubes (for PCR and CGH). Interphase- and metaphase-FISH was performed with a broad panel of commercial probes. CGH was performed on DOP-PCR amplified DNA from the sorted PCs. *Results*. The results of FISH, PCR, CGH on FACS sorted PCs gave comprehensive information on chromosomal aberrations. All sorted PC suspensions of 20 MM and MGUS patients showed at least one chromosomal aberration, demonstrating that the percentage of PCs is no limiting factor. The different techniques confirmed and supplemented the results. The most recurrent detected aberrations were: deletion of 13q, 17q, 20q and 14q and gains of 4q, 1q, 3q, 5q, 7q, and 8q, as translocation IgH. *Conclusions*. FACS sorting requires only small amounts of sample and allows flexible PC targeting based on specific immunophenotypical characteristics, *e.g.* as in the present study: IgLight chain isotype, CD56 positive or negative. Applying techniques as FISH, PCR and CGH on these immunophenotypically pure PC populations allows the detection of chromosomal aberrations in samples with very low percentages of PCs, such as in MGUS. Further studies on FACS sorted PC will ultimately allow the identification of genetic changes involved in the transformation of MGUS towards MM.

PO-103

MAPPING ARRAYS IDENTIFY IGH ASSOCIATED LOSSES AND GAINS IN MYELOMA

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Introduction. Translocations involving the immunoglobulin heavy chain gene (IgH) are key initiating events in approximately 50% of myeloma cases. There is clinical heterogeneity between cases with the same translocation, suggesting the importance of associated genetic events. We used high resolution SNP based mapping arrays to examine cases with primary IgH translocations to identify copy number changes associated with the translocation breakpoint regions. *Materials and methods*. FISH was performed on CD138 selected bone marrow plasma cells from 48 patients with newly diagnosed myeloma to identify primary IgH translocations. Copy number analysis was performed using Affymetrix GeneChip Human Mapping 500K Arrays and gene expression profiling performed using Affymetrix U133 plus 2.0 expression arrays. *Results*. 20 of 48 cases had primary IgH translocations: 10 t(11;14), 4 t(4;14), 3 t(14;16), 2 t(14;20) and 1 t(6;14). Using 500K mapping arrays, 8 of these 20 cases had either loss or gain involving the derivative chromosomes. Two of 4 t(4;14) cases had loss of expression of FGFR3 associated with deletion of the entire derivative 14, including 4p16.3-pter, the location of FGFR3 and 19 other genes. These results suggest a likely location of tumour suppressor genes that may maintain the malignant clone despite the loss of FGFR3. One of 3 t(14;16) cases had deletion of 16q23.1-qter and the remaining 2 cases had interstitial deletions within potential tumour suppressor gene WWOX at the presumed translocation breakpoint with loss of expression of WWOX in all 3 cases. Gains of the translocated region 11q13-qter (containing CCND1) were identified in 4 of 10 t(11;14) cases and a gain of the translocated 3p21.1-pter (containing CCND3) was identified in the t(6;14) case, with associated gain of the derivative 14q32.2-q32.33. These results demonstrate the molecular mechanisms by which the cyclin genes can first be dysregulated by translocation then by duplication. *Conclusions*. Deletions and gains of translocated regions occur in 40% of cases with primary IgH translocations and are an additional mechanism for gene dysregulation. These regions also provide a focus for identifying key collaborating genes in cases with IgH translocations, may provide an explanation for the clinical differences within translocation groups, and warrant further investigation.

PO-104

GENE EXPRESSION PROFILING ANALYSIS IDENTIFIES NOVEL POTENTIAL CANCER-TESTIS ANTIGENS IN MULTIPLE MYELOMA

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Introduction. The identification of new tumor-associated antigens is critical for the development of effective immunotherapeutic strategies. Cancer-testis (CT) antigens represents attractive targets due to their restricted pattern of expression. CT genes have been previously classified into four categories according to their expression profiles: (i) testis-restricted (expression in testis and tumor samples only), (ii) *tissue restricted* (mRNA detected in 2 or fewer non-gametogenic tissues), (iii) *differentially expressed* (mRNA detected in three to six non-gametogenic tissues), and (iv) *ubiquitously expressed*. We aimed at finding novel putative CT genes expressed in multiple myeloma (MM) using cDNA microarray

analysis. *Methods*. We analysed gene expression profiles of 5 testis samples, 64 primary myeloma cell (MMC) and 24 normal tissue samples including the B cell lineage, using Affymetrix U133AB microarrays. The normal tissue data were available on a public database. We looked for genes simultaneously expressed in more than 10% of the patients, in 3/5 testis samples and in less than 7 normal tissues. *Results*. Among the 54000 probe sets available in Affymetrix U133 Set chip, our method of gene selection based on the Affymetrix detection call resulted in a 180-fold enrichment of 11 known CT genes. According to the previously defined CT gene categories, we found 4 novel *testis-restricted*, 30 *tissue-restricted* and 45 *differentially expressed* CT genes. Expression pattern of 20 genes were confirmed by qRT-PCR. Immunogenicity of one gene has already been demonstrated in other cancers by defining a T-cell epitope. *Conclusions*. Using cDNA microarray analysis, we found several novel CT antigen candidates expressed in MM. Further studies are warranted to determine their immunogenicity.

PO-105

PATHOGENESIS OF RB1 HAPLOINSUFFICIENCY IN ¢13 MYELOMA

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Chromosome 13 deletion (Δ13) is identified in 50% of patients with multiple myeloma (MM) and confers a poor prognosis that suggests the presence of critical tumor suppressor genes. Investigations to date have lacked adequate resolution and the molecular phenotype associated with Δ13 has not been established. Furthermore, the functional impact of single-copy RB1 loss on myeloma cell growth has not been determined. Array comparative genomic hybridization (aCGH) was performed on 79 MM samples and 50 human myeloma cell lines (HMCLs) and gene expression profiling (GEP) was performed on 72 newly diagnosed MM and 50 HMCLs. Patient and HMCL Rb protein levels were assessed by immunoblot analysis. Adherent KMS-11 MM cells were labeled with carboxyfluorescein succinimidyl ester (CFSE) to allow tracking of cellular division by flow cytometry. Validated RB1 siRNA was added to silence 50% of RB1 protein levels and cells were infected with an Rb-expressing adenovirus to restore Rb function. aCGH of patient samples and HMCLs identified a CDR of 8.4 MB, which contained CYSLTR2, CDADC1, ITM2B, PCDH9 and RB1. Immunoblot analysis indicated Rb protein levels were reduced by 50% in Δ13 patients and HMCLs with mono-allelic Rb loss. A validated GEP signature was derived from Δ13 patients and was enriched for E2F, E2F targets and apoptosis genes. Reduction in CFSE fluorescence was observed for Rb knock-down which indicated increased cellular division as compared to the reduction of proliferation observed for Rb-expressing adenovirus. Further, CFSE proliferation fit analysis resulted in an increased proliferation index for 50% Rb loss. In addition, Rb protein levels correlated with mRNA levels and DNA copy number, suggesting mono-allelic loss of RB1 could be tumorigenic through a haploinsufficiency mechanism. This is supported by increased cellular proliferation observed after 50% reduction in Rb by siRNA. Due to inclusion of RB1 within the CDR, a GEP profile enriched for E2F target genes and increased proliferation after reduction in Rb, RB1 is indicated as the critically deleted gene in MM patients with Δ13. Therefore, haploinsufficiency of RB1 is suggested as a contributing mechanism for decreased survival in patients with Δ13.

PO-106

GENE EXPRESSION PROFILE IN BONE MARROW - DERIVED STROMAL CELLS OF MYELOMA PATIENTS

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Introduction. Multiple Myeloma (MM) is an incurable disease with median patients' survival of five years. This dismal prognosis is mostly due to resistance of MM to conventional therapies. Therefore, some of the attention has been redirected toward MM microenvironment. The important role of endothelium, osteoclasts and osteoblasts in MM progression has been documented. Here, we compared the gene expression profile in bone marrow stromal cells (MSC) from healthy subjects (hMSC) and from MM patients (mMSC). We also assessed the influence of myeloma-derived microvesicles (MMV) on hMSC. *Materials and methods*. mRNA was isolated from four hMSC and six mMSC. Expression level of genes involved in angiogenesis, invasion and MSC proliferation and differentiation were evaluated using real-time RT-PCR. MMV were isolated from MM cells' supernatants. *Results*. mRNA expression in mMSC in comparison to hMSC was increased by 14 folds for IL8, 3 folds for VEGF, MMP9 and decreased by 2 folds for HGF. Changes in osteogenic genes expression in mMSC were also observed. RUNX2, collagen1 and osteocalcin were downegulated by 6, 11 and 5 folds respectively. hMSC were exposed to 30 mg/mL of MMV for 8 and 24 hours and changes in gene expression were quantified. Analysis revealed that mRNA expression pattern in MMV-treated hMSC was similar to mRNA pattern in mMSC. We noticed increased level of IL8 expression: 4.5 fold after 8 hours and 2 fold after 24 hours stimulation. Slight upregulation of MMP9 level was seen at 8 and 24 hours. HGF expression was compromised by approximately 2 folds at both time points. 8-hour exposure to MMV resulted in downregulation of RUNX2, collagen1 and osteocalcin mRNA by 1.5, 3 and 2 folds respectively. After 24 hours, level of RUNX2 and collagen1 remained constant and level of osteocalcin decreased to 3.5 folds. *Conclusions*. Based on our results, we postulate that tumor cells alter the expression pattern of several genes responsible for tumor progression and bone integrity. The effect of MM cells on MSC can be recapitulated by MMV. This suggests presence of protumorigenic activators and osteogenic inhibitors in MMV. Currently the biochemical composition of MMV is being assessed.

PO-107

MUTUALLY EXCLUSIVE ACTIVATION OF NFKB OR STAT3 SIGNALING PATHWAYS IN MULTIPLE MYELOMA

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Introduction. Previous work in our laboratory has identified the importance of the NFκB signaling pathway for plasma cell (PC) survival and accumulation in Multiple Myeloma (MM). Although up to 43% of patient PCs appear to have activated NFκB by gene expression profiling (GEP), the remainder must rely on an alternate signaling pathway for their survival. *Methods and Results*. We examined our MM GEP datasets and performed supervised clustering of patients according to an index of NFκB activity. This revealed that the majority of patients not activating NFκB were associated with a distinct signature that includes the protein phosphatase of regenerating liver-3 (PRL-3). We report that elevated PRL-3 expression is observed in 1/15 normal PCs, 4/22 MGUS patients and 47/126 patients with either smoldering or overt MM (6.7%, 18.2% and 45.6% respectively). Less than 10% of patients were seen to have both an elevated NFκB index and high PRL-3 expression. After assigning patients to either a high or low PRL-3 expression level, we re-examined the GEP data using a gene set enrichment algorithm and confirmed that patients with PRL-3 expression also exhibit expression of many genes in the IL-6/Stat3 pathway. Interestingly, analysis of the PRL-3 promoter revealed an overlapping competing binding site for both NFκB and STAT3, suggesting that these play antagonistic roles in the transcriptional regulation of this gene. PRL-3 may therefore act as an indicator of the relative levels of NFκB and STAT3 activation. Patients with higher expression of PRL-3 were found to have statistically significant higher mean values of creatinine, C-reactive protein as well as lower albumin levels. Although plasma cell labeling indices were not elevated in patients with high PRL-3 expression, these patients demonstrate a more aggressive disease course and a statistically significant lower overall survival rate in one of the three datasets we examined. *Conclusion*. Based on these preliminary results, we propose that the majority of MM patients depend on either the NFκB or STAT3 signaling pathways for their survival and that these are mutually exclusive. Experiments are underway to confirm the biological role of PRL-3 in STAT3 mediated MM signaling, growth and survival.

PO-108

RIBOSOMAL PROTEINS ARE OVEREXPRESSED IN HYPERDIPLOID MM

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Background. Multiple myeloma (MM) is proposed to consist of two main pathogenetic groups. While hyperdiploidy (HD) is characterized by multiple trisomies, most nonhyperdiploid myelomas (NHD) harbour a recurrent IgH-translocation. First aim was to compare HD versus NHD by gene expression profiling (GEP). Secondly, this comparison was modified by excluding the cases with t(11;14). *Patients and methods*. CD138-positive bone marrow plasma cells from 74 patients with previously untreated MM (42 training group (TG), 32 validation group (VG)) were purified by MACS-sorting. Sorted cells were analyzed by interphase-FISH with probes for 6q21, 8p12, 9q34, 11q23, 13q14, 15q22, 17p13, 19q13, t(4;14) and t(11;14). HD and NHD were defined by using a copy number score (CS), which was calculated by subtracting the number of probes indicating chromosomal losses from the number of probes detecting additional copies (CS >0: HD; CS ≤0: NHD). GEP was performed with Affymetrix DNA-microarrays. Nearest shrunken centroid classification (NSCC) was used to discriminate the different groups, using GCRMA-normalized gene expression values. The prediction error was estimated by means of nested cross-validation using 10 repetitions of 10-fold cross-validation within the training set and separately calculated by use of the NSCC classifier of the training set to predict the validation set. *Results*. In the TG, both HD and NHD were found in 21 patients. The VG comprised 13 patients with NHD and 19 patients with HD. NSCC resulted in a predictor for HD versus NHD of 81 probe sets with a cross-validated misclassification rate of 14.2% for the TG and 26.5% for the VG. Three of the top ten genes were ribosomal proteins, overexpressed in patients with HD. MRPS27, a mitochondrial ribosomal protein, achieved the highest predictive score. Without t(11;14), the predictor consisted of 21 probe sets with a prediction error of 14,2% for the TG and 13% for the VG. MRPS27 was the gene with the second highest predictive score. *Conclusions*. Overexpression of ribosomal proteins is linked to cell growth, disease progression, and drug resistance. Whether the combined overexpression of ribosomal and mitochondrial ribosomal proteins plays a role in MM pathogenesis, has to be evaluated.

PO-109

GENOMIC ABERRATIONS IN MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE (MGUS) AND SMOLDERING MULTIPLE MYELOMA (SMM) AND THE RISK OF PROGRESSION TO MULTIPLE MYELOMA (MM)

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Introduction. Molecular cytogenetic defined subgroups of patients with MGUS and SMM may differ in their risk of progression to MM; but to what extent is not known. These subgroups have been firmly established in MM as associated with pathogenesis and clinicopathologic features. Similar abnormalities have been observed in MGUS. *Materials and methods*. We studied patients diagnosed with MGUS or SMM defined by accepted criteria. Patients were excluded if they had active MM. Patients provided written informed consent for the collection of a research bone marrow aliquot collected at the time of routine bone marrow procurement. Cytospin slides were made and studied for translocations: t(4;14), t(11;14), t(14;16), IgL-λ-light chain (+22q11) and deletions of chromosome 13q (D13) and 17p. In cases where we did not find a primary IgH translocation we looked for +14q32 NOS using a break-apart strategy. We used interphase fluorescence *in situ* hybridization (FISH) combined with cytoplasmic immunoglobulin (cIg-FISH) as previously described by us. *Results*. We studied the following cohorts of patients: 215 for t(4;14) and abnormal in 20 (9.3%); 200 for t(11;14) and abnormal in 36 (18.0%), 192 for t(14;16), and abnormal in 5 (2.6%), 201 for Δ13 and abnormal in 77 (38.3%), 173 for 17p and abnormal in 5 (2.9%), 180 for IgL-λ-light chain translocations and abnormal in 11 (6.1%) and 149 for +14q32 and abnormal in 41(27.8%). A total of 168 patients were evaluable for all assays. Twenty-seven patients (16%) progressed to active MM at a median of 20.2 months. In our initial set of observations we discern a higher risk of progression for those with t(4;14) (31.3% progressed) and lower in patients with t(11;14) (4,2% progressed). Δ13 or 17p13 (with low power due to few cases) did not seem have a discernible effect on progression. *Conclusion*. Additional follow up time and cases will be needed to better define the role of genetic markers to predict progression to MM. Although translocations of IgH and IgL- Ï are found in similar frequency in MGUS/SMM to that of MM, the subtypes would appear to dictate differential risk of progression. In this larger study we find a lower prevalence of D13 than in MM consistent with it being a progression event (38% versus 54%). Deletions of 17p13 are also less common and also indicative of a progression event (3% versus 11%).

PO-110

CHROMOSOMAL ABNORMALITIES OF PLASMA CELLS AND CORRELATION WITH IMMUNOPHENOTYPE IN MULTIPLE MYELOMA

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Introduction. Multiple Myeloma (MM) is characterised by genomic instability, with numerical and structural chromosomal abnormalities. A classification based on the presence of cytogenetic aberrations detected by FISH has recently been proposed. In this study we investigated the correlation between some specific genetic features of myeloma cells and their immunophenotypic profile. *Materials and methods*. Between August 2002 and December 2006, 821 consecutive MM patients, 624 at diagnosis and 197 at relapse, referred to our Department, were evaluated. FISH analysis was performed on bone marrow plasma cells (BMPC) enriched using anti-CD138-coated magnetic beads (Miltenyi Biotech GmbH, Germany). Nuclei from fixed PC were prepared for interphase FISH using standard methods. DNA probes (Vysis, Downers Grove, IL) were used to detect chromosome 13 and 17 deletion (del13 and delp53), gain of 11q23 and t(11;14)(q13;q32), t(4;14)(p16;q32), t(14;16)(q32;q23) translocations. The identification and quantification of BMPC and their immunophenotypic characteristics were assessed by flow cytometry on whole blood. PC can be easily identified by their strong reactivity to anti-CD38 MoAb and their specific CD138 expression. The phenotype was defined using triple or quadruple MoAbs combinations for the following antigens: CD56, CD45, CD40, CD19, CD20, CD52, CD117, surface and cytoplasmic kappa/lambda. *Results*. Overall, 89.2% of the patients showed at least one chromosomal alteration and del13 was found in 49.7%. No difference in del13 prevalence was observed according to age, serum beta-2 microglobulin, clinical stage and immunoglobulin isotype. A significant correlation between del13 and poor prognosis (p=0.02) was found by analysing suvival in 201 patients. p53 gene deletion was detected in 12.1% of 314 patients; t(11;14) in 20.9% of 211 patients; t(4;14) in 22.5% of 472 patients; t(14;16) in 3.7% of 137 patients and gain of 11q23 in 56% of 100 evaluated patients. PC carrying del13 showed a statistically significant lower CD45 expression than those without (p<0.0001). Moreover, they less frequently expressed CD19 and CD20 (p<0.0001 and p=0.003). We also observed that patients with PC carrying del13 had a significantly higher BMPC infiltration (p=0.001). The frequency of del13 was significantly higher in female patients (p=0.001) and in lambda subtype (p=0.03). PC carrying delp53 were more often associated with del13 (p=0.04) and less frequently expressed CD52 or CD19 (p=0.04). The presence of t(11;14) was correlated with CD20 expression (p<0.0001) and with a lower CD56 and CD117 expression (p<0.0001 and p=0.003). Moreover, the frequency of del13 was significantly lower in patients carrying t(11;14), (p=0.02). A correlation was also observed between t(4;14) and del13 (p=0.002). Del13 was significantly lower in patients carrying gain of 11q23 (p=0.02). Patients with PC carrying almost one IgH translocation represented 45% of the total samples and had a significantly higher frequency of delp53 (p=0.02); they showed more frequently a CD45 negative (p=0.001), CD19 negative (p<0.001), CD117 negative (p<0.0001) and CD20 positive (p<0.001) phenotype. PC carrying both del13 and CD45 negative phenotype were

also associated with the expression of surface monoclonal immunoglobulins (p=0.003). *Conclusions*. The molecular classification of myeloma subtypes and a longer patient follow up are mandatory to establish the real prognostic value of chromosomal abnormalities and accurately enrol patients in prospective trials.

PO-111

ALTERED CHROSOMES IN MULTIPLE MYELOMA WITH NORMAL FISH PROFILE

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Introduction. The difficulty of obtaining dividing cells and complexity of the resulting cytogenetic analysis in multiple myeloma (MM) patients has encouraged the use of fluorescent *in situ* hybridization (FISH). Nevertheless the FISH technique does not actually provide a chromosome model that adequately categorizes these patients. Although FISH is more sensitive and specific than conventional cytogenetic techniques (CG), it does not completely evaluate all of the chromosomes and only analyzes the hybridized part. The prognosis of altered karyotypes is studied in patients with *de novo* MM using FISH and CG. *Patients and Results*. On diagnosis a total of 148 patients with *de novo* MM were enrolled in the study and treated according to the Spanish GEM 2000 protocol. Median patient age was 60 years. All were simultaneously studied with CG and FISH. The FISH analysis employed the probes LSI [Rb-1 (13q14), IgH (14q32] and their most frequent partners. *Results*. Metaphase CG and interphase FISH analyses detected alterations in 69/148 patients (47%) and a normal karyotype in 79/148 patients (53%). 57 patients (39%) presented chromosome alterations in the CG analysis, including 37 (25%) with normal FISH results. The alterations CG showed in chromosome 1 in 24/57 patients were generally associated with complex karyotypes. FISH detected alterations in 32 patients: 19/32 had deletions in 13q14, 3 had t(4;14) and 10/32 had IgH/CCND1 fusion. The survival results were analyzed in different groups: 1) Interphase-FISH; 2) CG-Metaphase; 3) Abnormal Metaphase and Normal Interphase; 4) Normal Metaphase and Normal Interphase. The risk of relapse and death was positively correlated with chromosome alterations observed in the CG (p=0.02). The median global and event-free survivals for patients with a abnormal CG and normal FISH were less than in patients in whom CG and FISH were both normal (p=ns). *Conclusions*. Using CG our study detected chromosomal alterations in (25%) patients in whom FISH had not detected any alteration. These patients unfavorable prognosis justifies the routine use of CG to allow more precise classification and appropriate treatment. We strongly recommend conventional cytogenetic and FISH analyses be performed in the work-up of all patients with *de novo* or relapsed MM.

PO-112

CYTOGENETIC FINDINGS IN PATIENTS WITH MYELOMA IN WESTERN AUSTRALIA

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Introduction. We reviewed cytogenetic abnormalities of 348 consecutive patients with multiple myeloma in Western Australia, the geographically largest state in Australia which covers a land mass of approximately 25% of Europe. Specifically, we investigated the prevalence and prognostic value of cytogenetic changes in patients who proceeded to transplantation. Methods A retrospective analysis of transplant databases over the last 10 years from all of Western Australia tertiary hospitals was performed and compared to findings reported in the literature. Clinical and laboratory results (specific for prognostication according to WHO) were collected, and patients were assigned to the International Staging System stages for myeloma. Cytogenetic analysis was performed, mostly at diagnosis using 24 hour unsynchronized cultures stimulated with IL6. FISH studies were performed on CD138 purified populations or 24 hour unsynchronized cultures using directly labeled VYSIS probes. Conventional cytogenetics was performed on 26 patients until 2000. FISH analysis with a 13q probe was commenced in 1998 with further probes added over time. Results of cytogenetics were then correlated with clinical and laboratory data and evaluated with survival outcome. Results Data was available on 348 patients. Of these, 175 patients proceeded to transplantation. The abnormality rate by conventional cytogenetics was 35% (9/26). By FISH, the most frequent abnormality found was 13q deletion (47%), followed by hyperdiploidy (43%), t(11;14) (18%), p53 deletions (7%) and t(4;14) (6%). Our observations differ from the reported incidence of t(4;14) (15%) with the incidence of the other abnormalities concordant with published data. Among patients with 13q deletions, anaemia, elevated β2 microglobulin and advanced stage appear more prevalent. When we analyzed the impact of these genetic abnormalities, the presence of 13q deletion is associated with inferior survival, frequently with more rapid relapses and treatment refractoriness. *Conclusions*. We confirmed the importance of cytogenetics as a prognostic tool in multiple myeloma, and its role in clinical management. Some difference was noted in the prevalence of cytogenetic abnormalities in Western Australia to those reported in other population groups.

PO-113

IGH GENE REARRANGEMENTS ARE STRONGLY ASSOCIATED WITH HYPERDIPLOID VARIANT MULTIPLE MYELOMA IN KOREA: A FLUORESCENT *IN SITU* HYBRIDIZATION STUDY USING 14 PROBES

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Introduction. Two independent pathways are suggested in the early pathogenesis of multiple myeloma; nonhyperdiploid tumors with a high incidence of IgH translocations and hyperdiploid tumors associated with multiple trisomies. In most of the previous studies, nonhyperploid MM were definded by cytogenetic study or DNA analysis. To define ploidy, we used interphase FISH with 8 different kinds of probes and evaluated the incidence of chromosome aberrations including IgH gene rearrangement and 13q deletion, in relation to ploidy level on Korean patients with MM. *Materials and Methods*. A total of 135 cases diagnosed as MM between 1997 and 2003 from Seoul National University Hospital and the Asan Medical center were enrolled in this study. Conventional cytogenetic studies and FISH studies with different probes specific for the regions containing the genes or chromosomes (13q, 1q, IgH gene, 17p13, 9p21, CEP 7, 11, and 12) were performed. *Results*. Of 135 patients with MM, 21 (15.5%) patients had hyperdiploidy karyotype in the cytogenetics. Of note, 41 (30.4%) patients were detected as hyperdiploidy variants by FISH test. Rearrangements of IgH gene region were observered in 37.4% of Korean patients with MM and were more frequent in hyperdiploidy variants than in nonhyperdiploidy variants. Incidence of deletion 13q in hyperdiploidy variants was 34.7% and also more frequent in hyperdiploid variants. *Conclusion*. According to the results of FISH studies, IgH rearrangements and 13q deletions were not associated with nonhyperdiploidy MM and appeared more frequently in hyperdiploidy variant in Korean patients with MM.

PO-114

EUROPEAN MYELOMA NETWORK RECOMMENDATIONS FOR FISH IN MYELOMA

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Background. Fluorescence *in situ* hybridisation (FISH) testing in multiple myeloma poses difficulties not encountered in most other haematological malignancies due to 1) frequent low level infiltration of the bone marrow with the malignant plasma cells and universal but often underrecognised sampling problems. This means that the sample being tested for FISH often contains fewer than 10% plasma cells and so some means of plasma cell concentration or identification is essential for FISH results to be valid. 2) often poor hybridization efficiency in myeloma thought to be due to the large amounts of immunoglobulin that may be present. This is compounded by the difficulty in obtaining suitable control samples. 3) difficulties in deciding which markers to test for and which probes to use on a routine basis due to the recent and changing understanding of the implications of chromosome abnormalities in myeloma. *Aims.* to achieve consensus recommendations for use throughout Europe. A workshop to debate these problems was held in London on March 11th 2005 and attended by representative of 31 different European laboratories. Achievements: good agreement was obtained and

recommendations formulated for 1) purification versus combined immunostaining and FISH and optimal methods for each, 2) optimal use and storage of cells, 3) cut-off levels for positive results, 4) use of control probes, 5) number of cells required, 6) requirements for checking, 7) probes to use for different abnormalities, 8) basic abnormalities to test for and essential information to include in reports. The recommendations, updated to incorporate further scientific developments, will be presented.

PO-115

DELETION 13Q MARKS DISEASE PROGRESSION IN MYELOMA

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Introduction. Detection of abnormal metaphases in multiple myeloma is associated with a short survival,[1] indicative of advanced disease. Deletion of 13q is associated with poor prognosis when detected by metaphase cytogenetics but not interphase FISH (iFISH).[2,3] Deletion 13q status by iFISH was compared with ISS stage to determine if it has a role in disease progression. MATERIALS: 75 consecutive patients with plasma cell dyscrasia were staged by ISS criteria. Karyotype, ploidy analysis of plasmacytes by image cytometry and iFISH with the RB1 (13q14) locus was performed on bone marrow aspirate. Patient outcome was followed over a median observation period of 74 months and each parameter was correlated against overall survival by Kaplan - Meier analysis. *Results*. Patients with presentation ISS stage I disease demonstrated prolonged median survival (MS) over other stages (MS 74 vs 31 months, p=0.001), confirming it as the indolent disease stage. Deletion of RB1 was significantly associated with ISS stage II and III disease (X2 p=0.037), identifying it as a factor in transition to more aggressive disease. Survival of patients with an RB1 deletion approached significance when all disease stages were considered (MS 25 months vs not reached, p=0.128) but showed no difference among just ISS stage II and III patients (0=0.624). Abnormal karyotype was associated with advanced ISS stages (X2 p=0.002) and closely correlated with survival (MS 12 vs 74 months, p<0.001). Image cytometry identified patients with non-diploid plasmacytes in all disease stages but detection did not correlate with survival. *Conclusions*. Detection of an abnormal karyotype from usually hypoproliferative plasmacytes identifies a group of patients with advanced disease and short survival. The association of poor prognosis with 13q deletion by metaphase cytogenetics but not iFISH likely reflects the more advanced state of the disease when metaphases are detectable. ISS stage I criteria identifies those patients with indolent disease and prolonged survival. RB1 deletion is found to be significantly associated with ISS stages II and III patients and identifies it as a factor in transition from the indolent disease state. This finding requires verification in a larger study as it has implications for prognostic testing algorithms.

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PO-116

IDENTIFICATION OF PRIMARY MAFB TARGET GENES IN MULTIPLE MYELOMA

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In multiple myeloma (MM) five primary recurrent translocations involving the Immunoglobulin Heavy chain (IgH) locus have been identified. One of the five partner loci maps to 20q12 and involves the MAFB gene resulting in its ectopic expression. We aim here to characterize the pathophysiological role of MAFB in MM. In an attempt to identify MAFB target genes in MM we used an inducible system to upregulate MAFB in MM cell lines not carrying the t(14;20). MAFB target genes were identified by micro array analysis at different time points after MAFB induction. Gene expression patterns of the original MM cell line were compared to the modified MM cell line. Our inducible system provides the identification of genes that are directly up regulated by MAFB expression. A total of 284 modulated transcripts were identified and to validate the *in vitro* data, the identified signature was verified in two available independent datasets of MM patients including MAFB positive patients. This approach led to the identification of a core group of 14 putative target genes upregulated *in vitro* as well is *in vivo*. These genes may be responsible for the oncogenic transformation of MAFB expressing myeloma cells. The functional implication of some of these genes (a.o. NOTCH2) is discussed.

PO-117

INTERSTITIAL DEL(14)(Q) INVOLVING IGH ARE RECURRENT IN MM

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Introduction. Chromosomal deletions commonly found in human malignancies are regarded as hallmarks for the localization of tumor suppressor genes. To identify the gene(s) targeted by del(14)(q) frequently observed in B-cell malignancies, particularly in myeloma and CLL, we initially mapped the deletions using a high-resolution arrayCGH. *Materials and methods*. 23 B-cell leukemia/lymphoma cases with del(14) were subjected to chromosome 14-targeted aCGH. *Results*. 14 cases showed interstitial del(14)(q) involving IGH at 14q32.33 (loss of the 3'IGCH region). Similar deletions were further identified by FISH in 22 additional leukemia/lymphoma cases (altogether 36 cases). Seventeen of them were diagnosed as CLL (particularly atypical CLL), 12 as MM and 7 as low grade B-NHL (lgNHL). ArrayCGH and/or FISH showed that the proximal breakpoints of these deletions were clustered in the 14q24.1 one-BAC region in 21 cases (15 CLL, 3 MM, 3 lgNHL) and varied in 15 cases (9 MM, 4 lgNHL, 2 CLL). Further analysis of the recurrent del(14)(q24.1q32.33) covering approximately 36 Mb mapped the 14q24.1 breakpoints within and outside of the ZFP36L1 gene and showed clustering of the 14q32.33 breakpoints in the constant region of IGH, proximal to the 5' (Eμ) enhancer sequences. These findings therefore suggest that the del(14)(q24.1q32.33), and other analogous IGH-involving interstitial del(14)(q), might represent novel chromosomal aberrations leading to activation of unknown oncogenes at 14q by their juxtaposition with regulatory elements of IGH. Extensive expression analysis via quantitative PCR and microarray profiling (Affymetrix), however, failed to identify a chromosome 14 gene uniformly upregulated in cases with del(14)(q24.1q32.33). Therefore, the molecular consequences of this deletion having potential of intrachromosomal t(14q32/IGH) are largely unclear. *Conclusions*. Interstitial del(14)(q) affecting IGH are novel recurrent chromosomal aberrations in B-NHL. These deletions predominant in CLL and myeloma, may operate in a translocation-like manner. The constant involvement of IGCH in the del(14) suggests that they likely happened during an illegitimate Ig class switch recombination. Despite of our extensive studies, the gene targeted by the recurrent del(14)(q24.1q32.33) has remained elusive. Further investigations are needed to unravel the mechanism(s) and role of IGH-involving interstitial del(14)(q) in B-cell malignancies.

PO-118

CYCLIN D1, CD20 AND PAX5 EXPRESSION IN MYELOMA: CORRELATION WITH KARYOTYPE

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Introduction. The t(11;14) which deregulates cyclin D1 as a consequence of its juxtaposition to the IgH locus is the commonest translocation observed in myeloma. Some studies have suggested that t(11;14) myeloma is characterised by specific immunophenotypic features particularly

the expression of the B cell antigens CD20 and PAX5. Nuclear expression of cyclin D1 protein is used in many haematopathology laboratories as an indicator of an underlying t(11;14). Gene expression profiling however suggests that overexpression of cyclin D1 is not confined to t(11;14)+ cases but may also be encountered in hyperdiploid myeloma presumably as a consequence of additional copies of chromosome 11. The purpose of this study was to correlate cyclin D1, CD20 and PAX5 protein expression with karyotype. *Materials and methods*. 99 patients with symptomatic myeloma were included in this analysis. The majority of patients were studied at diagnosis prior to entry into the MRC myeloma IX trial. FISH studies were used to assess cases for the t(11;14) and Cyclin D1/ CCND1 copy number while immunohistochemistry was used to determine the expression of cyclin D1, CD20 and PAX5. *Results*. Cyclin D1 was expressed in all cases with a t(11;14) (n=17) but was also demonstrable in 35% (29/82) of the remaining cases which lacked the translocation. Protein expression correlated with cyclin D1/CCND1 copy number as it was detected in 15% (4/27) of cases with 2 copies, 30% (7/23) with 3 copies and 56% (18/32) with 4 or more copies respectively. Expression of CD20 and/or PAX5 was detected in 53% (9/17) of cases with a t(11;14) and was also detected in 31% (10/32) of cases with 4 or more copies of cyclin D1/CCND1 but was detected in significantly lower proportion, 6% (3/50), of cases with 2 or 3 copies (Fisher's exact test, p=0.003). *Conclusions*. Cyclin D1 protein expression is not confined to t(11;14) myeloma and shows some correlation with cyclin D1/CCND1 copy number. Cyclin D1 protein expression cannot be used in the routine laboratory setting as a marker for the t(11;14). CD20 and PAX5 expression is similarly not confined to t(11;14) myeloma and also appears to correlate with cyclin D1/CCND1 copy number.

PO-119

FLUORESCENT *IN SITU* HYBRIDIZATION (FISH) ANALYSIS OF IGH REARRANGEMENT BY 3 KINDS OF PROBES INVOLVING IGH GENES: IMPROVED SENSITIVITY BY USE OF AN IGH BREAKAPART PROBE DURING FOLLOW-UP OF MULTIPLE MYELOMA

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Introduction. IgH (immunoglobulin heavy chain) gene rearrangement is frequently observed in multiple myeloma and B cell lympoma. Interphase fluorescence *in situ* hybridization (FISH) can be employed to detect IgH gene rearrangement efficiently in myeloma cell with low mitotic activity, independently of multiple translocation partners of IgH gene. Currently available FISH probes for the detection of IgH rearrangement include the IgH/CCND1 dual color, dual fusion probe, IgH/BCL2 dual color, dual fusion probe, and IgH dual color breakapart rearrangement probe. The aim of this study is to evaluate the detection rate of these 3 probes for the IgH gene rearrangement and compare the concordance of the results, thus determining which probe most sensitively detects IgH gene rearrangement. *Materials and Methods*. We applied 3 different probes of IgH FISH, IGH/CCND1 dual color, dual fusion probe (Downers Grove, IL, USA), IGH/BCL2 dual color, dual fusion probe (Downers Grove, IL, USA) and IGH dual color break apart rearrangement probe from Vysis Products (Downers Grove, IL, USA) on 132 Korean patients with multiple myeloma and compared the concordance of the results. *Results*. 45 of 132 patients (34.1%) had the IgH gene rearrangement. 25 of 45 patients (55.6%) showed the positive results to all three probes but 20 of 45 patients (44.4%) showed discrepancy among the probes. IGH dual color break apart rearrangement probe shows most highly detectable rate (55.2%), compared to IGH/CCND1 (39.9%) and IGH/BCL2 (36.1%). Ten of 20 patients (50%) showing discrepant results among 3 probes were only positive for IGH break apart probes. *Conclusions*. These results demonstrate that IGH dual color break apart rearrangement probe are superior to the other two probes in qualitative and quantitative ways. Theoretically, the IgH rearrangement probe therefore can detect IgH gene rearrangement with same efficiency, but we found the discrepancies between the results of FISH using 3 probes. Thus, we recommend IGH dual color break apart rearrangement probe in the diagnosis and follow-up of multiple myeloma.

PO-120

THE T(14;20)(Q32;Q12): A RARE CYTOGENETIC CHANGE IN MM ASSOCIATED WITH POOR OUTCOME

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Introduction. Aberrations involving immunoglobulin genes (Ig) are recurrent in Multiple Myeloma (MM) and carry prognostic significance. One of them, the t(14;20)(q32;q12) leading to overexpression of the transcription factor MAFB has been identified in myeloma cell lines, rarely in MM, and never in MGUS. The clinical significance of this cryptic aberration remains however unknown. The aim of the present study was to assess the frequency and prognostic value of this chromosomal change. *Materials and Methods.* A series of 40 patients with MM or MGUS were assessed by interphase FISH either on immunologically identified kappa/lambda positive plasmacells (I-FISH) or on CD138 purified plasmacells (PCs). Cases selected were consecutive cases sent for routine cytogenetic analysis in which a translocation involving Ig was demonstrated by FISH (split signal with the Breakapart probe, Abbott) and was shown to be distinct from t(11;14), t(4;14), t(14;16) and t(6;14) (using specific probes), respectively. A double color interphase FISH assay was applied for the detection of the t(14;20), using a set of BAC probes located on 20q11-q12: RP4-616B8 and RP3-404H4 (centromeric to MAFB, labelled in Spectrum Orange) and RP4-644L1 and RP1-94E24 (telomeric to MAFB, labelled in Spectrum Green). *Results*. A characteristic split signal, was observed in three patients (7,5%), 2 females and 1 male, aged 71-72 years (median 71). All of them suffered from MM in advanced stage (Durie and Salmon stage II in 1 and stage III in 2 patients) and displayed multiple bone lesions. In all the light chain isotype was kappa, 2/3 showed transient response to standard chemotherapy (MPT in 1 and VAD.

PO-121

IGH REARRANGEMENTS IN PLASMA CELL DYSCRASIAS

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Introduction. up to 50% of myeloma (MM) cases have a primary IgH rearrangement with CCND1, FGFR3/MMSET, cMAF, CCND3 and MAFB being the best known partner upregulated genes. Although the first two of these have been reported in many different series there is little information on the frequencies and significance of the others in myeloma and associated plasma cell dyscrasias or on how many cases may harbour as yet unidentified primary IgH rearrangements (IgHr). *Methods.* FISH for the above translocations, together with t(8;14) to rule out the commonest secondary IgHr, deletions of 13q and 17p, and ploidy assessment, was carried out on CD138-purified plasma cells from 1388 bone marrow samples (25 primary amyloid (AL), 138 MGUS, 93 smouldering MM & 1132 MM, age range 27-93, median 65) sent to the above unit from 121 UK centres. Overall survival was available for 928/964 newly diagnosed MM cases (median follow-up 23 months, maximum 71 months). *Results*. Overall frequencies were t(11;14) 17% with a significant excess in AL (17/25 vs 181/1132 MM p<0.001), t(4;14) 10% with a shortage in MGUS (3/138 vs 129/1132 p<0.001), t(14;16) 3%, t(14;20) 2% with an excess in MGUS (7/138 vs 17/1132, p=0.01), t(6;14) 1% (none in AL), unidentified IgHr 9% with only 25/131 cases showing clear evidence of being secondary abnormalities. Both t(14;16) and t(14;20) showed short survivals (24 & 17 months, cf t(4;14) 22 months, vs 45 months, p=0.032, p=0.007, p<0.001 respectively). IgHr in MGUS were significantly less associated with del(13) than in MM but this was entirely due to t(11;14) cases (p<0.001). Non-hyperdiploidy, particularly near-tetraploidy., was much more strongly associated with t(11;14) and t(14;16) cases that those with t(4;14), t(14;20) or t(6;14). There was an excess of light chain/non-secretory disease with t(11;14) (p<0.001) and t(6;14) (p=0.001) across all diagnoses, whereas the excess of IgA paraprotein with t(4;14) (p<0.001) was confined to MM cases. IgHr were associated with lambda light chains (p=0.027) but this was entirely due to t(11;14) (p=0.001) and t(14;16) (p=0.001). Although there appeared to be less bone disease at presentation for t(4;14), t(14;16) and t(14;20), this was only significant for

t(14;16) (p=0.03). *Conclusions*. Despite common pathways of disease initiation according to the TC classification there are interesting differences between t(4;14), t(14;16) and t(14;20) in their associations with disease features, whereas t(11;14) and t(6;14) appear similar. Up to 7% of plasma cell disorders harbour as yet unidentified potential primary IgHr.

PO-122

TRANSCRIPTIONAL FEATURES OF 1Q GAIN IN MULTIPLE MYELOMA

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Introduction. Abnormalities of chromosome 1 are among the most frequent chromosomal alterations in MM (45% of patients). The long arm of chromosome 1 is associated with amplification (1q/gain) that can occur as isochromosomes, duplications or jumping translocations. 1q/gain MM patients are characterized by complex karyotypes and aggressive disease, and their number increases as the condition goes from smoldering to overt MM, suggesting that these regions contain critical genes for disease progression. These findings along with the limited information concerning specific transcriptional profiles, prompted us to molecularly characterize 1q/gain MMs by FISH and microarray analyses. *Material and method*. Purified plasma cells from 77 MMs at diagnosis were characterized by FISH for the presence of 11 polisomy, the most recurrent IGH translocations, ploidy status, chromosome 13 deletion, and by global gene expression profiling using the Affymetrix U133A arrays. Assessment of 1q/gain by FISH was performed by using three BAC clones specific for the BCL9 (1q21.1), CKS1B (1q22) and ARF1 (1q42.13) loci, and setting the threshold as 10%. *Results*. 1q/gain was identified in 40/77 (51.9%) patients; three (75%) or four (12.5%) signals of all the 1q probes were found in 35 patients and, in the remaining five samples (12.5%), the probes mapping to 1q21 and 1q22 showed more signals than that mapping to 1q42. 1q/gain was observed in the majority of purified plasma cells (median 96%) in all but three patients (range 12-20%). 1q extra copies significantly correlated with chromosome 13 deletion (p<10-4), and the absence of chromosome 11 polisomy (p=0.038), but not with ploidy status (p=0.0971). The differential expression of 61 genes (mainly localized on chromosome 1q12-1q44) distinguishes MM patients with or without 1q/gain. Functional analysis of the identified genes revealed their involvement in energy production pathways, intracellular protein transport, and endoplasmic reticulum-stress induced responses. The transcriptional fingerprint was robustly validated on a publicly available gene expression dataset, with a global classification rate of 85.2% for the independent cohort of MM cases. *Conclusion*. These data improve our knowledge concerning the specific genes/pathways deregulated by 1q abnormalities, and provide a promising focus for further studies aimed at defining new therapeutic strategies in MM.

PO-123

MYELOMA CELL CKS1B EXPRESSION IN 171 NEWLY DIAGNOSED PATIENTS IS NOT ASSOCIATED WITH INFERIOR SURVIVAL

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Introduction. In recent years global gene expression profiling studies introduce several new potential prognostic markers in multiple myeloma (MM). Up-regulation and amplification of the gene CKS1B in myeloma cells has lately been associated with a poor prognosis in MM patients. The CKS1B gene is located on chromosome 1q and is involved in the induction of cell proliferation by inhibiting the cyclin-dependent kinase inhibitor p27(kip1). *Materials and Methods.* We investigated the prognostic value of CKS1B expression by real time quantitative polymerase chain reaction techniques in purified myeloma cells from 171 untreated, newly diagnosed MM patients. 36% of the patients (<65 years) were treated with high dose melphalan (200 mg/m^2) with stem cell support. 56% (>65 years) were treated with standard melphalan and prednisolone, and 8% were untreated. Median follow-up was 53 months. The clonal myeloma cells in bone marrow aspirates were immunophenotyped by 3- or 4-colour flowcytometry, and patients were only included if an aberrant clone was identified. Hereafter, aberrant plasma cells (CD38^{++}/CD19^{-}/CD45^{-}/i/CD56$^{-/+/++}$) were sorted directly into PCR tubes by fluorescence activated cell sorting (FACS) using a FACS Aria (BDIS). In all cases plasma cell purity above 98% was obtained. Purified normal plasma cells from 9 healthy volunteers were collected as controls. A cDNA archive was generated by global reverse transcription. By using a polyadenylating step 5/-oligo(dT)-transcript-poly(A)-3/cDNA were generated and finally amplified by PCR using a sequence independent X-(dT)24 primer. Gene expression of CSK1B and the housekeeping gene β-actin were analysed by RQ-PCR and CKS1B expression levels were normalized to β-actin. Expression of CKS1B was observed in 52% of the patients. *Results and Conclusions*. We investigated the prognostic value of CKS1B expression by quantitative PCR techniques in, flowcytometry sorted, highly purified myeloma cells from 171 untreated MM patients. CKS1B over-expression did not correlate with a poor outcome in the patients. In the high dose melphalan treated patients the overall survival was similar in patients with or without up-regulated CKS1B expression. In patients treated with conventional chemotherapy expression of CKS1B showed a trend towards a survival benefit (p=0.05). So, our data do not confirm that myeloma cell over-expression of CKS1B is associated with a poor prognosis.

PO-124

CHROMOSOME 1 ABNORMALITIES IN MYELOMA AND MGUS

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Introduction. chromosome 1 is frequently abnormal in myeloma (MM) with both gain of 1q and loss of 1p seen. Gain of 1q has been suggested to be a possible marker of the transition from MGUS to MM. In MM the presence of extra copies of CKS1B over other 1q markers has been suggested to be a strong poor prognostic indicator. *Methods.* FISH with probes in 1q12-21(PDZK1), 1q22 (CKS1B), 1q31.3 and 1p12, 1p32.3 (CKN2C) was carried out on 341 patients (58 MGUS, 33 SMM and 250 MM, age range 32-93, median 65) where additional FISH results for deletions 13q and 17p, the major IgH translocations and ploidy assessment had been performed. Overall survival was available for 321 patients (median follow-up 34 months, maximum 63 months). *Results*. 67/334 (20%) patients showed loss of at least one marker on 1p (22 1p12 only, 11 1p31.3 only, 34 both). The frequency of 1p loss was 10% in MGUS (6/57) and 22% in SMM (7/32) and MM (54/243) (MGUS vs MM p=0.04). 21/332(36%) patients showed gain of at least one marker on 1q. Amplification was rare with most cases showing equal gain (1 or 2 copies) of all three probes, but there was an excess of CKS1B in 4 cases all of which appeared otherwise normal on 1q. The frequency of 1q gain was 16% in MGUS (9/55), 24% in SMM (8/33), and 43% in MM (104/242) (MGUS vs MM p<0.001, SMM vs MM p=0.03). This resulted in cases with a ratio of 1q/1p>1 being 11/54 MGUS (20%), 12/32 SMM (38%) and 109/235 MM (46%) (MGUS vs MM p<0.001). There was a strong association between 1q gain and del(13) (p=0.0002) and t(4;14)(p=0.012), and between 1p loss and p53 deletion (p=0.004). Univariate analysis showed shorter survival for del(13) (29 vs 49 mo, p=0.022), p53 deletion (16 vs 49 mo, p=0.004), 1q gain (25 vs 49, p=0.002), 1p loss (24 vs 49, p=0.011) and the ratio 1q/1p>1 (25 vs 49 mo, p=0.011). *Conclusions*. there was a significantly higher frequency of both 1q gain and 1p loss in MM compared to MGUS consistent with both being quite late changes, but at least two patients have had stable MGUS for several years in the presence of 1q gain. There was little suggestion that specific gain of CKS1B is likely to be a useful prognostic indicator.

PO-125

CLONAL EVOLUTION OF CHROMOSOME 1 ABERRATIONS IN MYELOMA

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Introduction. Molecular profiling techniques have identified copy number alterations (CNAs) involving the loss of 1p and gain of 1q as associ-

ated with tumor progression and poor prognosis in multiple myeloma (MM). In an effort to better understand the mechanisms underlying the origin and the relationship of 1p and 1q aberrations we analyzed metaphase cells of patients with complex aberrations identified by routine cytogenetic analysis. *Materials and Methods*. Fifty patients with complex 1q aberrations were reexamined in detail by high-resolution G-banding, fluorescence *in situ* hybridization with locus specific probes for regions of both the 1p and 1q, and spectral karyotyping (SKY). *Results*. Evidence for the segmental deletion of 1p was found in five patients with the deletions beginning in 1p13 in the proximal short arm and becoming progressively larger distally to band 1p31. SKY identified an additional five patients with cryptic deleted segments of 1p either translocated to non-homologous chromosomes or misclassified and designated as marker chromosomes. The smallest region of overlap for duplications involved 1q12~23, which showed at least 3 copies in all patients. Secondary clonal evolution of the duplicated and/or deleted segments of both 1p and 1q was mediated by the decondensation and breakage within the 1q12 pericentromeric region. Up to three different breakpoints were identified within the same 1q12 pericentromeric region of some patients resulting in multiple subclones with different deleted segments of 1p or duplications of 1q12~23. The 1q12 pericentromeric heterochromatin reduplicates itself and the adjacent 1q21~23 region either by direct or inverted duplications. These segmental duplications can subsequently become jumping segmental duplications (JSDs) of the 1q12~23 region. JSDs of 1q12~23 identified in eight patients are known to encompass a region with a large number of genes associated with tumor progression and poor prognosis in MM including CKS1B, BCL9, and MCL1 among others. A model for the clonal evolution of chromosome 1 aberrations is presented characterizing both primary and secondary events in the progression of interstitial deletions of 1p and duplications of 1q. *Conclusions*. CNAs of both 1p and 1q and tumor heterogeneity in MM appear to be driven in part by the disruption and duplication of pericentromeric heterochromatin.

PO-126

THE INFLUENCE OF POLYMORPHISM IN THE INFLAMMATORY GENES IL-1,β IL-6, IL-10, PPARγ2 AND COX-2 IN PATIENTS WITH MULTIPLE MYELOMA UNDERGOING AUTOLOGOUS BONE MARROW TRANSPLANTATION

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Inflammatory cytokines are suspected to play a role in the pathogenesis of multiple myeloma. It is therefore possible that genetic variations leading to a modified inflammatory response will influence the outcome of these patients. We investigated the influence of single nucleotide polymorphism in genes involved in the inflammatory response in 348 patients undergoing high dose treatment followed by autologous stem cell transplantation. We found that the polymorphism in IL-1β T-31C significantly influence overall survival (p=0.02). Homozygous carriers of the variant C-allele had a significantly longer survival as compared to the homozygous wild type allele TT carriers (relative risk=2.1; p=0.008). There was no statistically significant difference between men and woman. The polymorphism in IL-6 G-174C, IL-10 C592A, PPARγ2 Pro12Ala, COX-2 A-1195G, and COX-2 T8473C did not influence overall survival in this group of patients.

PO-127

HAS1 GENE POLYMORPHISMS MAY CONTRIBUTE TO MYELOMAGENESIS

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Introduction. In MM, we have identified aberrant intronic splice variants of hyaluronan synthase1 (HAS1) that correlate strongly with poor survival. We investigated the genetic basis for this aberrant splicing and intron retention. *Methods.* Extensive cloning and sequencing of HAS1 gDNA was performed for multiple cell populations (purified B, PC, T, and CD34[+] progenitors) from MM patients. We identified a higher frequency of genetic variations in HAS1 exons and introns than were found in B-CLL or healthy donors (HD). *Results*. Inherited HAS1 variations may be significant predisposing factors that increase the risk of developing MM. Based on sequencing data we detected reported HAS1 polymorphisms that appeared to be present at higher frequency in MM patients. As a first step in determining the importance of mutation events in HAS1, we evaluated the distribution of 7 reported SNPs, identified in our sequencing analysis, in populations of 379 MM patients and 100 HD using a MassARRAYTM System. The genotyping analysis demonstrated that in MM population, the majority of patients were homozygous for the minor alleles in 3 out of 7 SNPs, while in HD populations, the number of individuals homozygous or heterozygous for these minor alleles were equal. For an additional HAS1 polymorphism, Taqman allelic discrimination assay performed for 270 MM patients and 124 HDs showed that this SNP (HAS1 833 A/G SNP) was significantly more likely to be homozygous in MM patients (p=0.0002). Additionally, the three SNPs genotyped by MassARRAYTM System and HAS1 833 A/G SNP genotyped by allelic discrimination assay are located in relatively close proximity, suggesting they be signature markers to help identify individuals at risk of MM. *Conclusion*. This work suggests that polymorphisms in the HAS1 gene may be associated with MM. Additionally, increased homozygosity for HAS1 SNPs may provide an informative genetic marker for risk assessment of those individuals with the greatest risk of developing MM. In the context of our previous work, we speculate that HAS1 polymorphisms may contribute to transformation events in MM by influencing HAS1 splicing. Such inherited genetic changes may be among the earliest events in the cascade that leads to overt MM.

PO-128

THE PROTECTIVE ROLE OF NQO1*2/*2 GENOTYPE AGAINST THE DEVELOPMENT OF MULTIPLE MYELOMA IN KOREA

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Introduction. Incidence of multiple myeloma (MM) shows interethnic differences, which may be explained by am individual's susceptibility to carcinogen. NAD(P)H:quinone oxidoreductase 1 (NQO1), phase I enzyme, reduces quinones to hydroquinone derivatives in two electron step. It may act as either detoxification or activation enzyme depending on reduced hydroquinones. A single nucleotide polymorphism NQO1*2 (C609T) cause an amino acid substitution from proline to serine and decrease NQO1 enzyme activity.8 The aim of this study is to investigate the association between NQO1 polymorphism and multiple myeloma risk, stage and other prognostic factors. *Materials and Methods.* We conducted case-control study using bone marrow samples and peripheral blood sample from 117 patients (male-female ratio, 79:38; median age, 60 years; range, 30-81 years) and peripheral blood sample from 166 healthy donors (male-female ratio, 89:77; median age, 49 years; range, 23-81 years). NQO1 genetic polymorphism was determined by TaqMan allelic discrimination assay. *Results*. NQO1 *1/*1, *1/*2, *2/*2 genotype frequencies were 31.6%, 63.2%, 5.1% in cases 31.9%, 48.3%, 19.9% in controls respectively. The frequency of NQO1 *2/*2 in patients was significantly low (p=0.001) and the OR was 0.24(95% CI: 0.01-0.68, p=0.006) to NQO1 *1/*1 genotype, which show decreased multiple myeloma risk. The frequency of NQO1 *1/*2 in patients was significantly high. There were no significant differences in multiple myeloma stages, hemoglobin, calcium, β2-microglobuin, M-protein, creatinine among NQO1 genotypes. *Conclusion*. In conclusion, decreased risk for multiple myeloma in NQO1 *2/*2 genotype in this study suggests the protective role of NQO1 *2/*2 genotype in the pathogenesis of multiple myeloma. Increased frequency of NQO1 *1/*2 genotype in cases could also predict that NQO1 probably result in activation of potential etiologic agent for multiple myeloma. No significant differences in multiple myeloma stages and prognostic factors among NQO1 genotypes suggest that NQO1 might be only involved in initiation of multiple myeloma pathogenesis, not in proliferation of multiple myeloma.

PO-129

RELATION OF IL-10-592 POLYMORPHISM TO THE SUSCEPTIBILITY AND CLINICAL OUTCOME OF MULTIPLE MYELOMA IN JAPANESE PATIENTS

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Introduction. Interleukin 10 (IL-10), a cytokine that regulates inflammation, is reported to be involved in multiple myeloma (MM) cell proliferation and survival. The polymorphism of position-592 in the promoter of IL-10 gene is a strong determinant of IL-10 expression. However, it is unclear whether IL-10 -592 polymorphisms alter the susceptibility and clinical outcome of MM. We examined the single nucleotide polymorphism located within the promoter region of IL-10 genes in Japanese patients with MM. *Methods.* Seventy nine patients with MM [age range, 40-83 years; stage I (n=9), stage II (n=20), stage III (n=50); IgA(n=12), IgG(n=47), IgD(n=1), non-secretory (n=3), Bence Jones(n=16)], 46 patients with MGUS (age range, 44-86 years), and 200 healthy controls were included. Fifteen patients with transformation of MGUS to MM were included in MM group. Genotyping was determined by the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) technique. Genotype and allele frequencies were compared between the study groups by χ^2 test. The Kaplan-Meier method was used in the calculation of overall survival. Overall survival curves were compared with the log-rank test. *Results*. IL-10-592 AA, AC and CC genotype frequencies were not significantly different between MM patients (51%, 34%, 15%) and controls (42%, 43%, 15%). The IL-10-592A/C genotype was detected in 10 of 16 patients (63%) with transformation of MGUS to MM and 20 of 46 patients (43%) with MGUS who did not progress to MM (odds ratio [OR], 2.2; 95% confidence interval [95% CI], 0.7-7.0; p=0.15). The prognosis of MM patients undergoing stem cell transplantation was similar among 3 genotype groups. However, MM patients not undergoing stem cell transplantation with IL-10-592AC genotype had a shorter overall survival compared with patients with IL-10-592AA genotype (median survival , 47 months vs. not reached), although there was no significant difference. In the clinical characteristics at diagnosis including sex, Ig type, Durie-Salmon staging system, and international staging system, there was no difference between patients with IL-10-592AC genotype and IL-10-592 non AC genotype. *Conclusion*. These results suggested that the IL-10-592 A/C genotype was not associated with the susceptibility to MM, however may contribute to transformation of MGUS to MM and prognosis in Japanese patients.

PO-130

ANALYSIS OF DNA REPAIR GENES VARIANTS IN MULTIPLE MYELOMA PATIENTS

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Introduction and Aim. A malfunction of the network responsible for genome stability *e.g.* DNA repair and high accuracy of DNA synthesis during DNA replication may be related to the pathogenesis of Multiple Myeloma (MM), whose tumoral clone is characterized by a remarkable high genetic instability. Among all the chromosomal alterations so far described, translocations involving chromosome 14 and several chromosomal partners are the most frequent and the most importantly correlated to MM prognosis. In particular, it has been shown that the presence of t(4;14)(p16;q32) at diagnosis may have a unfavourable impact on prognosis. In this study, a group of 82 MM patients and a group of 259 healthy donors were genotyped for common SNPs in DNA repair genes. The aim was to evaluate the overall frequency of these variants in MM patients and in particular in those patients carrying t(4;14)(p16q32). *Methods*. PCR-RFLP assays were used to detect five polymorphisms in the DNA repair genes APE1, XRCC1, NBS1, XRCC3, and XPD. An RT-PCR assay was adopted to identify the IgH/MMSET fusion gene, as t(4;14)(p16q32) surrogate. *Results*. Allele frequencies in XRCC1, XRCC3, XPD23 and NBS1 SNPs genes were similar in MM patients and healthy donors. On the contrary, the APE1 variant genotype was significantly associated to a MM increased risk (p=0,04). Moreover, genotype combination of polymorphic-XRCC3 and normal-NBS1 had a marginally significant lower frequency in MM patients, when compared to healthy donors (2,4% vs. 8,5%, p=0,05). Overall, in this cohort of patients the t(4;14)(p16q32) frequency was 26%. Considering MM patients with NBS1 variant allele, the incidence of t(4;14)(p16q32) positive patients was higher, compared to those t(4;14)(p16q32) negative (19,1% vs. 9,8%); however this difference was not statistically significant. *Conclusions*. The preliminary results presented here support the hypothesis that common polymorphisms in DNA repair genes may be an important modifier of individual susceptibility to MM. In particular, APE1 polymorphisms may have a role in MM onset. Moreover, the contribution of the NBS1 variant allele to MM onset or to t(4;14)(p16q32) insurgence cannot be excluded. Further studies are ongoing in a larger sample-size population.

PO-131

INVESTIGATION OF HTSNPS IN LIG4, XRCC4&5 AND RISK TO MYELOMA

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Introduction. Multiple myeloma is an incurable B cell neoplasm accounting for nearly 1% of all cancer related deaths worldwide. Though associated with occupational exposure in the farming and petrochemical industries, the epidemiology remains largely conjectural. Chromosomal translocations involving the immunoglobulin heavy chain locus at 14q32 and a number of recurrent partner loci are detected in most patients at diagnosis. These primary events are felt to represent aberrant class switch recombination (CSR), a process that normally functions to alter immunoglobulin isotype along with immune response maturation. The genetic basis of CSR, from initiation of the DNA double-strand break (DSB) through to detection and repair, has been elucidated. We hypothesize that germ line polymorphisms in genes implicated in the non-homologous end joining (NHEJ) pathway which mediates DSB repair during CSR may contribute to susceptibility to myeloma. *Materials and Methods*. 25 haplotype tagging (ht) SNPs in 3 genes central to the NHEJ pathway were genotyped in myeloma patients and controls from the EpiLymph study, a European study of the epidemiology of lymphoid neoplasms, and from an Irish hospital registry (306 cases, 263 controls).The genes examined were Ligase 4, XRCC4 and XRCC5. Genotyping was performed using Taqman based assays on the ABI 7900 HT platform. *Results*. For ligase 4, htSNP rs 1805386, a significant difference was observed in allele frequencies between cases and controls (p=0.032). For the htSNP rs963248 in XRCC4, Allele A was significantly more frequent in cases than in controls (p=0.0105249), as was the AA genotype (p=0.026). Haplotype analysis was performed using Unphased for rs963248 in combination with additional SNPs in XRCC4. The strongest evidence of association came from the A-T haplotype from rs963248-rs2891980 (p=0.008). For XRCC5, the genotype GG from rs1051685 was detected in 10 cases from different European nationalities but in only 1 control (p=0.015). Interestingly, this SNP is located in the 3' UTR of XRCC5.*Conclusions*. These findings provide support for the hypothesis that allelic variance in the DNA DSB repair genes involved in the NHEJ pathway contributes to susceptibility to myeloma. However these results need to be confirmed in an independent study.

PO-132

RETINOBLASTOMA PHOSPHORYLATION CORRELATES WITH RESPONSE BUT NOT DEL(13Q)

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Introduction. Inactivation of the retinoblastoma 1 protein (Rb) by phosphorylation allows G1 to S phase cell cycle progression. Since the Rb

gene resides on chromosome 13q, the poor prognosis associated with del(13q) in multiple myeloma (MM) is often assumed to be due to decreased Rb function. Likewise, data in other cancers suggests that a high degree of cell cycle progression correlates with response in chemotherapy regimens directed at cycling cells and correlates inversely with regimens that do not target cycling cells. The mechanism of Dexamethasone (D) and Revlimid (R) combination therapy in MM is not known. Given this background, we devised a study to determine whether Rb phosphorylation analysis could elucidate the mechanism of D/R and whether the poor prognosis of del(13q) is due to loss of Rb function. *Materials and Methods*. Myeloma cells in core biopsies from newly diagnosed, treatment naive, stage II or III patients enrolled in the BiRD (Biaxin, Revlimid, Dexamethasone) trial (n=72) were analyzed for Rb protein expression as well as phosphorylation at serine 807/811 (pSRb), an assessment of progression beyond the mid-G1 cell cycle checkpoint, by quantitative image analysis of dual immunohistochemistry (IHC) reported as the percentage of positive MM cells and average nuclear Rb and pSRb pixel density. FISH was performed for del(13q) on the accompanying aspirates. Data were compared to clinical response (International Myeloma Working Group Uniform Response Criteria). *Results.* sCR/CR correlated with Rb phosphorylation (Chi-square p=0.03). However, there were no associations between del(13q) and the level of Rb protein expression or phosphorylation. 12/16 (75%) patients with del(13q) showed Rb protein expression at levels comparable to MM with no del(13q). *Conclusions*. The correlation between Rb phosphorylation and response suggests that the D&R regimen preferentially targets myeloma cells that have advanced beyond the mid-G1 cell cycle checkpoint. These data also suggest that image analysis of dual IHC might be used to predict response or as an aid in deciding which drug regimen to use for a given patient. Lastly, analysis of Rb protein expression shows that the poor prognosis associated with del(13q) is not likely due to loss of Rb function.

PO-133

PHOSPHORYLATED RETINOBLASTOMA EXPRESSION BY PLASMA CELLS IS ASSOCIATED WITH A TREND FOR ADVANCED STAGES, REFRACTORINESS AND SHORTER SURVIVAL FOLLOWING CHEMOTHERAPY IN PATIENTS WITH NEWLY DIAGNOSED MYELOMA

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Aim. Events involved in the regulation of cell cycle regulation act in a complex fashion and form the basis for a new classification (Hideshima *et al.* 2004). We had previously analyzed the expression of upstream regulatory elements: cyclin D(CycD), A and their inhibitors p16 and p21 by immunocytochemistry on bone marrow myeloma cells(ASH 2005). Here we are reporting the expression of a further downstream protein that plays a critical role in the transsion from G1 to S phase: phosphorylated retinoblastoma (pRb). *Patients and methods.* Bone marrow biopsy samples were obtained from 76 newly diagnosed myeloma (ISS stage I/II/III: 13/30/15, age: 59 (32-75)). Patients were treated with VAD,Thal-Dex, MP or other (44/8/7/4) and received transplantation (single / double/ allo: 23/5/3). Antibodies (Neomarkers: Cyc D1, Cyc D3,Cyc A, p16, p21, p27, Cell Signaling: pRb, Santa Cruz: Cyc D2) were used to stain with Zymed ABC Px Kit or Ventana stainer. SPSS version 13.0 was used for Kaplan-Meier univariate overall survival analysis. *Results*. Although 40/76 of the cases expressed at least one of the CycDs, CycA was observed less frequently (15/76). Loss of CDKI (p16/p21/p27) was a frequent finding and was more frequent (84%) among patients those CycD1$^+$. This suggests a G1/S transsion resulting from CycD1 activation caused by the loss of p16 inhibition. However the downstream regulator pRb is not produced in most of these patients (17/24). Overall, plasma cells expressed pRb in only 24% of the patients. None of these patients were in ISS stage I or expressed p16. Parameters ie LDH, Beta2microglobulin were similar between responders and refractory patients. Only 7/24 (29%)of the CycD1$^+$patients co-expressed pRb, and was associated with refractoriness, compared to CycD1$^+$pRb$^{(-)}$ patients (Response Rate: 3/11 vs 17/28, p=0.06). All pRb$^+$ patients responded less: 33,3 .% vs 56,6% (p<0.0,03). Kaplan-Meier analysis showed a trend for longer OS in pRb negative patients(44% vs 80% at 5 years, p=0.39). *Conclusion*. A trend towards advanced stages and refractoriness to therapy, independent of the presence of other cell regulatory proteins and clinical parameters, favours the poor prognostic role of pRb and support the findings of S.Chen-Kiang (Cancer Res, 2005).

PO-134

TRAF3 IS A NOVEL TUMOR SUPPRESSOR THAT IS FREQUENTLY INACTIVATED IN MYELOMA

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Introduction. To identify novel genetic factors contributing to the pathogenesis and prognosis of myeloma we have initiated a comprehensive genomic screen using array-based comparative genomic hybridization (aCGH). To simplify the initial analysis we focused on bi-allelic deletion events, which are intrinsically limited to small genomic regions but are likely to harbor tumor suppressor genes. *Materials and Methods.* Genomic copy number changes were assessed in 68 MM and 46 human myeloma cell lines (HMCLs) samples using the Agilent Human Genome 44B microarray (Agilent Technologies). *Results*. The most common bi-allelic deletion identified in the 114 samples occurred at 14q32. This microdeletion was mapped to a minimally deleted region (MDR) of ~45 kb encompassing two genes; TRAF3, a negative regulator of the non-canonical NF-kB pathway, and AMN. Using cIg-FISH to screen a 158 patient cohort for abnormalities of TRAF3 we identified 7 (4.4%) patients with bi-allelic deletions and 19 (11.8%) patients with a single copy. Sequencing of the entire TRAF3 locus in 62 patients and the HMCL identified inactivating mutations in 9 patient samples and 4 HMCL. Overall, the incidence of TRAF3 inactivation, bi-allelic deletion or LOH with mutation, is 8/46 (17.4%) in HMCL and 12/62 (19.4%) in patients, making it one of the most common genetic abnormalities identified in MM to-date. Expression of wild-type TRAF3 in HMCL with TRAF3 abnormalities inhibited the processing of NFKB2 from p100 (inactive isoform) to p52 (active). Inhibiting this process was associated with a G1/G0 cell cycle arrest and the induction of apoptosis. In contrast, over-expression of wild-type TRAF3 in HMCL without TRAF3 abnormalities had no effect. Since TRAF3 inactivation leads to the activation of NF-κB signaling, a process that is targeted by bortezomib, we hypothesized that MM with TRAF3 inactivation would be particularly sensitive to this drug. As predicted 17/19 (89%) patients, predicted to have TRAF3 inactivation, responded to bortezomib. This was accompanied by a prolongation of progression-free-survival (PFS) from 83 to 193 days (p<0.0001). *Conclusions*. TRAF3 is the most commonly inactivated tumor suppressor identified in MM to-date and almost all patients with this abnormality respond to bortezomib.

PO-135

METHYLATION PROFILES OF P16, P14 AND P15 GENES IN KOREAN PATIENTS WITH MULTIPLE MYELOMA

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Introduction. Dysruption of cell cycle control genes (p16, p14 and p15) is known to be involved in the tumorigenesis of multiple myeloma (MM). We investigated the inactivation status of p16, p14 and p15 genes using promoter methylation study and fluorescent *in situ* hybridization (FISH) study in patients with MM and analyzed the association of inactivation of those genes and clinical prognosis. *Materials and Methods*. Newly diagnosed 44 patients with MM and 34 adult patients with acute lymphoblastic leukemia (ALL) were enrolled. Promoter methylation study of p16, p14 and p15 gene was done by bisulfite modification - methylation specific PCR, using 2 sets of primers. Deletion of p16, p14 and p15 gene was detected by dual color FISH (Vysis, IL, U.S.A). *Results*. Methylation of the p16, p14 and p15 promotor were detected in 30/43 (69.8%), 5/43 (11.6%) and 14/43 (32.6%) patients with MM, respectively. Methylation of any of p16, p14 or p15 promotor was observed in 96.6% of MM patients. Methylation of the p16, p14 and p15 promotor were detected in 16.0%, 5.4% and 46.0% of patients with ALL, respectively. Deletion of p16, p14 and p15 was detected in none of the

patients with MM, and in 32.4% of the patients with ALL. Of note, in the cases (15/44, 34.1%) showing methylation of more than one CpG sites of the p16 promotor, significant correlations of methylation with old age≥60yr and lower overall survival rate (p<0.05) were observed. Heavy methylation of p16 protomor was a independent predictive variable for overall survival [Hazard Ratio 7.5, 95% CI 2.1-26.3, p=0.002]. Other adverse prognostic factors by univariate analysis were old age (≥60yr , p=0.005), male gender (p=0.031), anemia (<8.5 g/dL, p=0.038), elevated β2-microglobuline (≥4.5 mg/dL, p=0.014), high serum creatinine levels (≥2.0 mg/dL, p=0.008). *Conclusion*. Almost all of MM patients showed the methylation of any of p16, p14 or p15 promotor, which suggest the potential applicability of hypomethlyating agents to MM. The promoter methylation of p16, p14 or p15 was a major contributor to the disruption of cell cycle regulation in MM, whereas both the deletion and/or the promoter methylation of p16, p14, and p15 contributed to the disruption of cell cycle regulation in ALL. In our study, methylation of more than one CpG sites of the p16 promotor was an independent adverse prognostic factor in MM. We infer that quantitative methylation study for p16 promotor is helpful for the evaluation of prognosis.

PO-136

PRL-3 IS OVEREXPRESSED IN MM AND INVOLVED IN CELL MIGRATION

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Introduction. Gene expression profiling studies of two IL-6-dependent myeloma (MM) cell lines (OH-2 and IH-1) showed that PRL-3, a metastasis-associated tyrosine phosphatase, was induced by several mitogenic cytokines. We hypothesized that PRL-3 could be a common downstream target for several cytokines in MM and decided to examine its role in MM cells. *Materials and methods*. Expression of PRL-3 was evaluated with Affymetrix microarrays, quanitative RT-PCR, Western blots and immunohistochemistry. FISH and confocal microscopy were used for genetic and functional studies, and PRL-3 expression was downregulated by siRNA. The phenotypic effects were evaluated by thymidine incorporation and in a migration assay. *Results*. Expression of PRL-3 mRNA and PRL-3 protein increased after stimulation with cytokines. Higher expression in IL-6 dependent cell lines may indicate the importance of the microenvironment for expression of this gene. Low or no expression was found in 11 non-myeloma haematological cell lines. The PRL-3 gene expression was up- regulated in myeloma cells from 256 newly diagnosed patients compared to in normal bone marrow-, MGUS- and SMM plasma cells. Among 7 subgroups identified by unsupervised hierarchical cluster analysis,PRL-3 gene expression was significant higher in the 3 groups denoted as Proliferation, Low Bone Disease, and MMSET/FGFR3.Most MM cell lines had two copies of the PRL-3 gene, thus indicating that cytokine induction and not gene amplification was responsible for the over expression of PRL-3.PRL-3 protein was expressed in 19 of the 21 bone marrow biopsies, and confocal microscopy demonstrates that the PRL-3 protein seemed to cycle between cytosol and nucleus in a cell cycle-dependent matter. SiRNA induced down regulating of the PRL-3 gene resulted in reduced cell migration in the INA-6 cell line, but had no effect on proliferation. *Conclusion*. We here report for the first time that PRL-3 is over expressed in multiple myeloma, and that the PRL-3 gene may be involved in the molecular pathogenesis of specific myeloma patient subgroups. PRL-3 may have a role in myeloma cell migration and this may represent a new potential therapeutic target.

PO-137

PATHOGENIC ATM MUTATIONS OCCUR AT A LOW FREQUENCY IN MULTIPLE MYELOMA

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Introduction. Ataxia Telangiectasia (A-T) patients have biallelic inactivation of the ATM gene and exhibit a 200 fold increased frequency of lymphoid tumours. ATM mutations have been found in a number of adult lymphoid malignancies but there is no data on the occurrence of ATM mutations in multiple myeloma tumours. We report here a 50 year old patient with a milder A-T phenotype who developed multiple myeloma and subsequently we have screened forty five cases of multiple myeloma for ATM mutations. Methods The 62 ATM coding exons and flanking intronic sequences, following PCR, were analysed by Denaturing high performance liquid chromatography analysis (HPLC) and DNA sequencing. Functional analysis was performed on any novel sequence change in the ATM protein by transfecting the altered ATM construct into an ATM null lymphoblastoid cell line and measuring the kinase activity in response to gamma irradiation. *Results*. A 48 year old man with a milder variant of ataxia telangiectasia developed myeloma. Mutational analysis confirmed the presence of two ATM mutations; the 9022C>T (R3008C) missense mutation and the IVS10-6T>G splicing mutation. Western blotting revealed expression of a reduced level of full length ATM protein and almost normal level of chromosomal radiosensitivity. The partial ATM function is consistent with *leaky* expression of a low level of normal ATM from the IVS10-6T>G allele resulting in a milder A-T phenotype and relative longevity in this patient. 2 out of 45 myeloma patients were found to have mutations in ATM and a further 4 of the 45 tumours were found to have new polymorphisms. In one tumour the missense mutation S2394L was identified, modelled in an expression system and the protein was shown to have no ATM kinase activity. This patient also had the polymorphic sequence change P1054R. One further myeloma patient had the ATM splice site mutations IVS40-1G>C leading to loss of exon 41. *Conclusion*. This study shows that ATM mutations can occur at a low frequency in sporadic multiple myeloma tumours, at a higher frequency than the normal population and may indicate a role of ATM mutations in the pathogenesis of some multiple myelomas.

PO-138

ASSESSING THE PURITY, SENSITIVITY AND SPECIFICITY OF TWO PLASMA CELL SELECTION METHODS

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Introduction. Isolating tumor cells from plasma cell proliferative disorders (PPD) has traditionally been accomplished using CD138 positive immunomagnetic bead selection. In our hands purity is 90-99% for new and relapsing myeloma patients as confirmed by a 3 color isotypic immunofluorescence slide-based method. Recently, reports of cell selection (138^+) bias and an effect on gene expression profiling (GEP) have emerged. We therefore compared the effect of CD138 positive selection versus a negative selection method on yield, purity and GEP. *Methods*. Positive selection utilized CD138 antibody and magnetic particles while negative selection used a cocktail of antibodies CDs 2, 14, 33, 41, 45RA and 66b. Selections were done using the RoboSep Cell Separation System (Stem Cell Technologies, Vancouver, BC). Eighteen bone marrows drawn after informed written consent were tested. The bone marrow was pooled and split into two equal aliquots. Plasma cell (PC) purity, cell number, and residual tumor cells left in the non plasma cell fractions were determined. The PC fractions were then placed into TRIZOL and stored at -80 until isolation for GEP. GEP was performed using Affymetrix U133 plus 2.0 chips where sufficient RNA (10/18) was available from both selection methodologies. The quantity and quality of the RNA was assessed using the absorption ratio and the Agilent Nanochip Assay. *Results*. The post sort PC fractions contained approximately the same number of cells (r2=0.99) although the purity of the positive selection was overall better (mean 89% vs. 82%). Both methods left residual PCs behind in the non-PC fraction, although fewer were left

after positive selection. GEP analysis using unsupervised clustering demonstrated the paired samples were more like each other than the methodology used. However, consistently discernible differences exist with subsets of genes over-expressed in positively and negatively selected samples reflecting normal PC/monocyte, and granulocyte contamination respectively. Interestingly, both selection methods produced the same profile with no contaminating signatures observed when a high number of clonal PCs were present in the starting material. *Conclusion*. These results demonstrate the CD138 selection method yields slightly better cell recoveries with less contaminating cells, confirmed by immunofluorescence and GEP, than that of the negative selection cocktail.

PO-139

DISEASE STAGE, SAMPLE PURITY AND IMPACT ON GENE EXPRESSION PROFILING (GEP) STUDIES IN PLASMA CELL PROLIFERATIVE DISEASE (PPD)

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Introduction. The impact of contaminating normal hematopoeitc cells on GEP studies of PPD can be significant even after sample purification. In this study, we examine the effect of disease stage and sample purity on GEP and their interpretation. *Methods.* GEP of CD138[+]-selected plasma cells from 15 normal donors (NPC), 22 MGUS, 24 smoldering MM (SMM) and 101 MM for the Mayo Clinic was analyzed. A contamination signature (CS) was derived from clustering and functional analysis of differentially expressed genes across these stages of PPD as determined by ANOVA with multiple testing corrections. The presence of the CS was correlated with disease stage and sample purity determined by slide-based cytoplasmic immunoglobulin (Ig) light-chain staining. We then assessed the impact of the CS on the interpretation of the recently published MGUS-like MM constituting about 29% of MM and associated with better prognosis (UAMS dataset). *Results*. We identified a CS comprising monocyte/leucocyte markers and Ig-related gene. Most MM samples with the CS had low purity (<90%). These genes were strongly correlated, had strongest expression in NPCs, and were present in almost all MGUS, most of SMM but few MM samples. Interestingly, the CS was present even in MGUS with high purity (>95%). Applying the CS to the UAMS dataset, 126 (36%) samples have the CS and were enriched for MGUS-like MM (36% versus 20%, 2-tail Fisher¢s exact p=0.0013). Our assignment of contaminated samples by CS differed from the original study assignment using different metrics. Patients with a CS had significantly better survival. When samples with CS were removed, the remaining MGUS-like MM no longer had better survival. Conversely, MGUS-like signature was not observed among MM patients with purity greater than 90% in the Mayo dataset. *Conclusions*. For GEP studies of minimal plasmacytosis states, CD138 selection alone is insufficient, and flow sorting with additional markers (*e.g.* CD56 and CD19) to identify clonal cells is required. Post-hoc methods to abrogate the effect of contamination are sub-optimal. Current pre-analysis metrics may not effectively identify samples with CS. MGUS-like MM as a distinct biological entity will require further clarification.

PO-140

COMPUTER VISION FOR FISH SCREENING IN MYELOMA

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Introduction. New fluorescent *in situ* hybridization (FISH) methods are being developed for use with handheld medical diagnostic platforms. This will allow rapid FISH screening of large cell populations for a fraction of the cost incurred by traditional methods, and enable researchers and clinicians to identify important MM-related translocations, such as t(4;14), without the need for expensive optical hardware. However, the application of these new methods is limited by the need for time-consuming human analysis of the resulting fluorescent images. *Methods.* One way to mitigate the need for human analysis is the use of computer vision software to rapidly identify cells in population images and extract relevant statistics regarding the number and type of translocations present. We present a computer vision system based on the human visual processing pathway that can extract cell and probe information from the FISH images generated by new techniques and gold-standard analysis procedures. FISH images are given to the system and automatically decomposed into a map of cell and probe locations. These locations are compared with clustering and distance metrics in an automated fashioned to rapidly categorize large populations of white blood cells. *Results*. We show that the system can extract the number and location of cells and probes (both dual-fusion and break-apart) directly from FISH images. The resulting population statistics were in concordance with those generated by a human expert, even for images with cells not directly in the optical focal plane and images containing low-level measurement noise and/or biological sample artifacts. *Conclusions*. It has been shown that certain translocations have a dramatic effect on the survival rate and response to treatment of multiple myeloma patients. New computer vision systems can rapidly detect the number and type of chromosomal abnormalities in FISH samples, and should increase the ability of clinicians to customize treatment for individual patients. This work was supported by the Natural Sciences and Engineering Research Council (NSERC), the Informatics Circle of Research Excellence (iCORE), a Western Economic Diversification grant, and the Canadian Institutes of Health Research (CIHR).

PO-141

DETECTION OF CHROMOSOME 13 DELETION EVENTS IN MM USING MLPA

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Chromosome 13 (Chr13) deletion events have been associated with various malignancies including Multiple Myeloma (MM) and Osteosarcoma (OS). There is disagreement in the published literature over the extent of deletions observed. Both interstitial deletions and complete monosomy of the chromosome have been implicated in each disease. In MM Chr13 deletions are indicative of a poor prognosis therefore characterisation of the deletions is important. We are interested in Chr13 deletions in both MM and OS as they exhibit different phenotypes, bone breakdown and formation respectively. On Chr13 there are various tumour suppressor genes, and genes involved in bone homoeostasis, which may be differentially expressed depending on the deletion event occurring. Fluorescence *in situ* hybridisation (FISH) is frequently used in cytogenetic analysis, but this method is expensive and utilises only a limited number of probes at once. Multiplex Ligation-dependent Probe Amplification (MLPA) is a relatively new technique utilising a PCR-based method, which uses up to 40 specific probes simultaneously and can be used to detect and quantify multiple deletions and/or amplifications for a given sample. We demonstrate Chr13 deletion events for both MM and OS cell lines using MLPA in comparison to healthy control lymphocytes. In total, 44 MLPA probes (including key genes and STS markers) have been hybridised to the DNA samples and data analysis has been aided by FISH using a Chr13 paint. Thirty probes span the length of Chr13 with control probes for chromosome 10. We have detected Chr13 deletions in the MM cell lines JIM-1, JIM-3, LP1 and U266 as well as the OS cell lines MG63 and SaOS2. Specific deletion patterns for each sample have been identified using the MLPA probes. These will be explained in depth during the presentation. No deletions have been detected in the cell lines AGLCL, IM9, HS Sultan, RPMI 8226, Karpas 620 and HOS, but detection of deletion is restricted to the probes utilised. Using the various techniques has both confirmed and refuted previous cytogenetic analysis. However, these data indicate that interstitial deletion events in MM may be more common than monosomy 13, and further confirmation is required in a patient cohort.

PO-142

THE FAITHFUL GENOTYPING OF SAMPLES FOLLOWING AMPLIFICATION USING THE QIAGEN REPLI-G MINI KIT

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Introduction. The most common genetic variation in the genome is the alteration of a single base, a single nucleotide polymorphism (SNP). SNP genotyping is an important technology to determine genetic variation and the role of SNPs in disease pathology and drug response. However, the available quantity of genomic DNA (gDNA) can be a major limitation in molecular genetic analysis. Whole genome amplification (WGA), based on multiple displacement amplification (MDA) provides a technique to increase precious and depleted gDNA resources. This method relies on isothermal amplification using the Phi29DNA polymerase and

random hexamers. Obtaining a balanced and faithful amplification is of great importance when applying whole genome amplified gDNA (WGA gDNA) to molecular genetic analysis. It has been shown that quantity and quality of input DNA is critical in determining the genotyping performance of WGA gDNA. Furthermore, a balanced amplification is essential to prevent allelic dropout, which would result in low genotyping success rates in SNP analysis. The aim of this study was to evaluate the SNP genotyping assay performance of pre- and post WGA gDNA. *Materials and methods*. DNA was extracted from 23 myeloma cell lines and 80 peripheral blood samples, obtained from clinical multiple myeloma samples. WGA was performed using the Qiagen Repli-g mini kit with 25 ng of gDNA input. Quality and quantity of pre- and post-WGA gDNA was determined. Twenty-eight SNPs were analyzed in pre- and post-WGA gDNA using a SNPlex genotyping platform. *Results*. There was no significant difference in genotyping performance between pre- and post WGA gDNA, indicating that the quality of gDNA was similar in WGA gDNA and starting material. The overall genotype concordance between pre- and post- WGA gDNA samples was excellent. *Conclusions*. WGA gDNA shows excellent SNP genotyping assay performance, similar to that of pre-amplified gDNA. These results suggest that WGA gDNA is suitable for SNP genotyping assays.

PO-143

MIR-15A AND MIR-16-1 EXPRESSION IN MULTIPLE MYELOMA

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Introduction. Multiple Myeloma (MM) is an incurable B-cell malignancy arising from isotype-switched plasma cells (PCs), located in the bone marrow (BM). Conventional and intensified treatment followed by stem cell transplantation can lead to complete remission (CR), however the majority of patients eventually succumb to their disease. MM cells are characterized by a profound genetic instability, leading to chromosomal abnormalities. The most common abnormality is chromosome 13q14 deletion, which is an early event in MM pathogenesis. Since deletions at chromosome 13q14 are associated with poor prognosis it is of great importance to determine chromosome 13q14 deletions by fluorescence *in situ* hybridization (FISH) in patients with newly diagnosed MM. Furthermore, it has been suggested that a tumor suppressor gene is located on the 13q14 locus. Micro RNAs (miRNAs) are a new class of small noncoding single stranded RNAs of ~22 nucleotides. miRNAs are able to bind to partially complementary sites in messenger RNAs (mRNAs). As a result, the mRNA is degraded or remains untranslated, leading to decreased levels of mRNA and protein respectively. miRNAs are thought to be involved human tumorogenesis and it has been suggested that miRNAs can function as tumor suppressors and oncogenes. Micro RNA (miRNA)-15a and miRNA-16-1, located on chromosome 13q14, could have tumor suppressor activity. It has recently been shown that miRNA-15a and miRNA-16-1 are able to bind BCL2, an anti-apoptotic protein, at a conserved target site in the 3' UTR. Downregulation or deletion of miRNA-15a or miRNA-16-1 has been observed in CLL patients, and may result in increased expression of BCL2, subsequently inhibiting apoptosis. The aim of this study is to gain insight in the role of miRNA-15a and miRNA-16-1 in the pathogenesis of multiple myeloma. *Materials and methods*. Abnormalities of chromosome 13q14 were determined using FISH analysis. Determination of mature miRNA-15a expression levels in CD138 magnetic cell selected (MACS) myeloma plasma cells will be performed using the TaqMan MicroRNA Assay. miRNA-15a and miRNA-16-1 expression levels will be analyzed in a cohort of 30 MM patient samples obtained from clinical multiple myeloma samples. *Results*. The results of miRNA-15a and miRNA-16-1 expression analysis, combined with FISH data of chromosome 13q14 in this cohort of 30 patients will be presented.

PO-144

FISH CONFIRMS CLONALITY OF PROGENY IN 3-D CULTURE OF MM

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Introduction. Multiple myeloma (MM) is identified by an immunoglobulin gene rearrangement (IgH VDJ), a unique signature defining all members of the MM clone. MM is also characterized by karyotypic instability, including chromosomal amplifications, deletions, and translocations. This abnormal cytogenetic signature provides a second marker to identify members of the malignant clone. We have designed an *in vitro* 3-dimensional (3-D) tissue culture model within which *ex-vivo* MM B and plasma cells proliferate and generate progeny. *Methods*. Cells from BM aspirates were grown in 3-D culture designed to recapitulate the cellular niches found in the BM. Subsequently, cells were harvested from ECM and cytospin slides were prepared. May-Grunwald Giemsa stained cells were classified based on lymphocyte or plasma cell (PC) morphology and cell position on the slide was recorded. To identify cytogenetic abnormalities, the slides were destained and subsequent fluorescent *in situ* hybridization (FISH) analysis was performed. *Results*. Although chromosomal abnormalities may not mark 100% of the PC population in a given patient, their presence provides a marker to define clonal identity with the dominant population of PC. The progeny of MM BM cells grown in 3-D cultures include both B and PC. After 2 weeks of culture, progeny cells were screened for cytogenetic abnormalities known to be present in the MM cells of the original BM aspirate, as detected by FISH. Although complex chromosomal patterns characterized the *ex-vivo* plasma cell populations in BM aspirates, these were reproduced identically in the progeny from 3-D cultures of those BM cells. Thus, chromosomal abnormalities which mark the malignant clone as identified in the diagnostic, pre-culture BM aspirate are maintained in the 3-D culture and characterize the progeny cells. *Conclusions*. This work supports the idea that the abnormal chromosomal signature seen *ex-vivo* also marks the progeny of malignant cells in 3-D culture. This 3-D culture system coupled with FISH analysis of the cellular MM components arising in culture is a powerful new technique to investigate the malignant compartments within the BM microenvironment, their response to therapy within a 3-D architecture, and the BM niches that sustain each compartment of the malignant clone.

PO-145

RNA-FISH DETECTS CLONAL IGH IN PCS OF MM PATIENTS

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Introduction. The fluorescent *in situ* hybridization (FISH) technique has widespread applications in both basic science and diagnostic clinical research. Most recently, RNA probes have been used to detect transcribed genes in individual cells. Multiple myeloma(MM) is characterized by the presence of a monoclonal protein and chromosomal translocations involving the Ig heavy (IgH) locus. These clonal events allow us to design a RNA-FISH strategy to observe the presence of specific mRNA in plasma cells(PC). This describes our method for using specialized cRNA probes specific for genetic loci. *Materials and Methods*. Bone marrow(BM) aspirates were obtained using standard methods. Cytospin slides were prepared from mononuclear cells using RNAse-free techniques and stored in ethanol (-20oC) until the preliminary clonal information is gathered and the cRNA probe prepared. The probe was synthesized using BM plasma cells cDNA. The IgH clone was determined using a combination of seven PCR targets. Each of the seven sense primers had a unique sequence for common variable regions (V1 - V7) present in the human genome. All reaction shared a common antisense primer, which was of IgG origin. The appropriate amplicon was sequenced. The cRNA probe was prepared by ligating the select PCR product into a dual-promoter plasmid. The plasmid in our experiment contained the T7 and SP6 RNA promoter sites. The plasmid was transformed using TOP-10 competent cells, cloned, amplified and DNA extracted. The antistense cRNA probe was transcribed using T7 RNA polymerase. DIG-labeled dUTP was incorporated in the nucleotide mix during the transcription. The probes were hybridized to the slides. A FITC tag was attached by a DIG/antibody combination. A mounting medium with DAPI was added to the cells and the slides were analyzed using a fluorescent microscope. The same cells were cIg-FISH analyzed to confirm the cells were of plasma origin. *Results*. The clonal plasma cells were clearly FITC positive around the nucleus and also within the cytoplasm of the clonal PCs. Non-PCs on the slide were negative for cRNA hybridization. *Conclusions*. The RNA-FISH provides a specific method for analyzing clonal PC.

PO-146

PHARMACOGENOMICS STUDY OF BORTEZOMIB ACTIVITY IN MYELOMA

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Introduction. Clinical development of bortezomib in multiple myeloma (MM) included pharmacogenomic gene expression profiling (GEP) of pre-treatment tumor biopsies in order to evaluate the feasibility of developing predictive classifiers in prospective clinical trials and to better define the biological pathways related to drug sensitivity. These studies allowed the identification and independent validation of statistically significant classifiers of response and survival.[1] Here we describe specific genes and pathways associated with bortezomib efficacy in relapsed MM. *Methods.* The myeloma GEP dataset consists of 264 samples from a subset of patients enrolled in Phase2 and Phase3 clinical trials of bortezomib. Patient subsets showed clinical characteristics similar to those of the overall study populations, although there was a bias toward patients with higher tumor burden. The dataset shares numerous biological features with those of single-center myeloma GEP studies, including a distribution of TC subtypes (Translocation CyclinD) nearly identical to that of newly diagnosed MM. We tested for genes associated with either response (R) or progressive disease (PD) and overall survival (OS) after bortezomib therapy. These clinical endpoints were also analyzed with various biological pathway algorithms including Ingenuity Pathway analysis, PARIS[2] and GSEA.[3] These results were also compared with that of cell lines showing differential sensitivity to bortezomib. *Results*. A comparison of R and PD highlighted numerous differentially expressed genes; approximately 200 were over-expressed in R and 500 were over-expressed in PD (t-test <0.01). R-associated genes include signaling molecules like TRADD, CFLAR and TANK, while PD-associated genes included oncogenes (NRAS), apoptotic regulators (DAP3) and cancer antigens (CTAG). Pathway tools helped to integrate and extend these observations, consistently identifying NFκB activation and adhesion as R-associated, while protein synthesis and mitochondrial pathways were PD-associated. Pathways such as NFκB, adhesion and cytokines appear linked specifically to bortezomib and not dexamethasone sensitivity. These approaches were also applied to patient survival. In contrast to analyses of single genes, the pathway tools highlighted links between response and survival, including adhesion pathway expression. *Conclusions*. This study highlights the diversity of biological pathways expressed in MM and the utility of clinical genomics to associate pathways with activity of single-agent bortezomib.

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PO-147

ASSOCIATION OF CLONAL AND SUBCLONAL CHROMOSOMAL ABERRATIONS WITH D-TYPE CYCLIN EXPRESSION AND EVENT FREE SURVIVAL IN MULTIPLE MYELOMA

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Aim. We assessed whether the height of CCND expression correlates with the presence of clonal or subclonal aberrations of 11q13, t(11;14) and t(4;14) and event-free survival in Multiple Myeloma (MM). *Patients and Methods*. 128 newly diagnosed MM-patients (65 training (TG) / 63 independent validation group (VG)) and 14 normal donors (ND) were included. Bone marrow aspirates were CD138-purified. RNA was *in vitro* transcribed and hybridised to Affymetrix HG U133 A+B GeneChip (TG) and HG U133 2.0 plus array (VG). CCND1 and CCND2 expression was verified by real-time RT-PCR and western blotting. iFISH was performed on purified MM-cells. Clonal aberrations were defined as being present in >60%, subclonal aberrations in 20-60% of MMC in a given patient. Expression data were gcrma-normalised and a Kruskal-Wallis rank sum test used (Bioconductor). *Results*. 11q13+. CCND1 (208711_s_at, 208712_at) is significantly higher (p<0.0001), CCND2 (200953_s_at, 200951_s_at) significantly lower (p<0.0001) expressed in MMC harbouring clonal, compared to subclonal, or no gain of 11q13. t(11;14). CCND1 is significantly higher (p<0.0001), CCND2 significantly lower (p<0.0001) expressed in MMC harbouring clonal, compared to subclonal, or no t(11;14). t(4;14). CCND1 is significantly lower (p<0.0001), CCND2 significantly higher (p<0.0001) expressed in MMC harbouring clonal compared to subclonal, or no t(4;14). The expression of CCND3 (201700_at) is not significantly different between the 3 groups for all aberrations investigated. The expression of CCND2 correlates with short event-free survival (EFS), but not if patients with t(4;14) are excluded. There is no significant difference in EFS for patients harbouring the respective aberrations in a clonal or subclonal pattern. *Conclusion*. (1) An additional copy of 11q13 or a t(11;14) correlates with increased CCND1- and decreases CCND2-expression, a t(4;14) is associated with an increase of CCND2- and decrease of CCND1-expression. (2) In each case, the height of the CCND-expression is significantly different whether the respective aberration is clonal or subclonal. (3) Thus, when interpreting expression data in the context of chromosomal aberrations, it is important to consider if plasma cells carry a respective aberration in subclonal / clonal pattern.

PO-148

SYNTHESIS OF GENETIC AND MOLECULAR PROGNOSTIC MODELS IN MYELOMA (MM)

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Introduction. Two powerful prognostic models of MM were published recently by the IFM group and UAMS. One uses genetic factors t(4;14) and/or 17p13 deletion by FISH combined with beta-2 microglobulin (B2M) (IFM model). The other is a model based on the gene expression of 17 genes (17-gene model). In this study, we attempt to compare these models. *Methods.* The UAMS gene expression dataset of 351 newly diagnosed MM treated with total therapy II (TTII) was analyzed. Cases with spiked expression of FGFR3 and/or WHSC1 were labeled as t(4;14). Samples with the lowest 5% of TP53 expression were labeled as TP53 deleted. A proliferation index (PI), based on the median expression of 12 proliferation-related genes, was used as a surrogate for B2M. The 17-gene index was calculated using log2 of the ratio between the mean expression of 12 genes and 5 genes associated with the worst and best prognosis respectively (log2Q4/Q1). 214 UAMS patients treated with total therapy III (TTIII) were used for validation. *Results*. Using a combination of t(4;14), low TP53 expression and PI greater than median, patients could be divided into 3 groups with significantly different survival (log-rank p<0.0001). The distribution of patients across the 3 risk groups was similar to the IFM study. However, the survival of each of these risk groups could be further separated by the high-risk criteria defined by the 17-gene model (logQ4/Q1 > 0.85). Only logQ4/Q1 > 0.85, and t(4;14), but not low TP53 expression, high PI or CKS1B expression, emerged as independent risk factors on Cox proportional hazard analysis. Indeed t(4;14) was able to further dissect high-risk patients defined by the 17-gene model. Using t(4;14) and the 17-gene model, a high-risk group with very short survival (16.6 months on TTII) can be identified. This model was validated in patients treated with TTIII. *Conclusions*. While the molecular prognostic model is extremely powerful, genetic factors are still important. A model combining molecular and genetic factors identifies subset of patients with very short survival.

GROUP 2: Pathophysiology - microenvironment

PO-201

CHARACTERISTICS OF CD138 EXPRESSION ON PLASMA CELLS IN MULTIPLE MYELOMA (MM) PATIENTS

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The CD138^{+} cells were analyzed in 70 MM patients. Flow cytometry method using fluorochrome -conjugated monoclonal antibodies was applied. Plasma cells were determined by means of CD45, CD38 and CD138 antigens. Monoclonal antibody against CD138 FITC (Serotec) was applied. There were performed determinations of: CD138 expression intensity using RFI index, CD138 expression range using Cv (coefficient variability) index,. size and granularity of investigated CD138^{+} cells. Following findings were revealed: 1.Heterogeneity of CD138 expression of analyzed patients respecting CD138 expression intensity, with: presence of plasma cell population showing high and homogeneous expression (n=12), presence of plasma cell population with homogeneous low/middle CD138 expression intensity (n=43), presence of plasma cell population with heterogeneous CD138 expression intensity forming two cell subpopulations: CD138$^{+/++}$ cells and CD138± cells (n=15), 2.Occurrence of negative correlation between RFI value and Cv magnitude. (r=-0.7422, p=0.0001). It means, that plasma cell population with a high RFI is characterized by a low variability of expression intensity while population of cells with low RFI shows a high variability of expression intensity, 3.Occurrence of positive correlation between size of CD138^{+} cells and degree of their granularity (r=0.571, p=0.0001). No correlation was found between CD138 expression intensity on plasma cells and their size (r=0.537, p=0.65), and correlation between CD138 expression intensity and granularity of plasma cells was close to statistical significance (r=0.53, p=0.058). *Conclusions*. The expression of CD138 on plasma cells of MM patients is heterogeneous with respect to its intensity. It does not depends on the size of CD138^{+} cells, however there is possible to distinguish three most frequently occurring expression patterns: high- homogenous expression in whole cell population, a low/middle expression with a minor homogeneity of CD138 expression intensity and third expression pattern consisting of cell population with high and low CD 138 expression intensity (i.e. composed of two subpopulations). The correlation CD138 expression with clinical picture of the disease will be determined in this study.

PO-202

LONG-TERM FOLLOW-UP ANALYSIS ON THE PROGNOSTIC INFLUENCE OF ANTIGENIC MARKERS IN MULTIPLE MYELOMA: A STUDY ON 712 PATIENTS UNIFORMLY TREATED WITH HIGH-DOSE THERAPY

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Introduction. In contrast to other haematological malignancies, little attention has been paid to the potential prognostic impact of antigenic expression in malignant plasma cells (PC) from patients with multiple myeloma (MM). *Material and Methods*. A large series of 712 patients enrolled in the Spanish GEM-2000 protocol were included in the analysis. All patients were uniformly treated with six alternating cycles of VBCMP/VBAD followed by high-dose therapy -melphalan 200 mg/m^2 supported by ASCT-. Flow-immunophenotypic analysis was carried out at diagnosis by using an appropriate panel of quadruple combinations of the monoclonal antibodies against CD19, CD20, CD28, CD33, CD38, CD45, CD56, CD117 and CD138 antigens. Acquisition of information of at least 1,000 stained PC was assessed on a dual-laser FACSCalibur flow cytometer using the CellQuest software program (BD Biosciences, San José, CA, USA). Negative or positive expression was defined according to the basal level of fluorescence of the PC in a control tube. *Results*. The median progression-free survival (PFS) and overall survival (OS) for the whole group were 37 months (95% confidence interval, CI: 34-41) and 67 months (95% CI: 58-75) respectively. Events of disease´relapse / progression were 436 (61%) and 276 patients (39%) have died. In order to ascertain the prognostic influence of PC phenotype, uni- and multivariate analyses for PFS and OS were performed. Positive expression of the CD117 antigen (present in the 35% of the patients, n=249) was associated with a favourable outcome defined by both longer PFS (44 vs 33 months, p=0,03) and OS (median not reached, NR vs 63 m, p=0,007). By contrast, the CD28 positive group (38% of cases, n=270) showed an adverse prognosis, associated with a shorter OS (55 vs NR, p=0,02), but not significant differences for PFS (32 vs 38, p=0,1). Patients with CD19^{+ve} (7%), CD33^{+ve} (19%) or lacking CD56 (44%) also showed a shorted survival, although the differences did not reach statistical significance. Three antigenic pair-combinations were associated with an adverse prognosis: CD56-veCD28^{+ve} profile (PFS: 27 vs 38 m, p=0,03 and OS: 37 vs 68 m, p=0,001); CD56-veCD117-ve profile (PFS: 31 vs 40 m, p=0,01 and OS: 63 m vs NR, p=0,05) and CD117-veCD28^{+ve} (PFS: 30 vs 38 m, p=0,028 and, OS: 51 vs NR, p=0,006). Moreover, the triple combination of CD56^{+ve}CD117^{+ve}CD28-ve defined a group of 131 patients (20%) with a significantly better course of the disease (PFS: 45 vs 35 m, p=0,006 and OS: NR vs 63 m, p=0,0001). Finally, in the Cox proportional-hazard model, this antigenic profile (CD56^{+ve}CD117^{+ve}CD28-ve) was selected as independent prognostic factor together with advanced ISS stage, age >60 years, thrombocytopenia (≤130-109/L), hypercalcemia (≥11 mg/dL), high bone marrow infiltration by flow-cytometry (≥10% BMPC) and high proportion of S-phase PC (≥2,5%). *Conclusion*. Although none of the individual antigens analyzed had independent prognostic value, the combination of CD56, CD117 and CD28 in malignant PC defines a phenotypic profile with significant influence on OS in the multivariate analysis. Supported by Cooperative Research Thematic Network (RTICs; RD06/0020/0006), Instituto de Salud Carlos III, and MM Jevitt, SL firm.

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REGULATORY T CELL SUBSETS IN MYELOMA

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Introduction. The immunologically hostile microenvironment of Multiple Myeloma (MM) contributes to the limited success of immunotherapy strategies. In addition to direct tumour-induced immunosuppression, tumour cells may generate suppressor cells. Regulatory T-cells (Tregs) profoundly suppress immune responses and induce tolerance. The aim of this study is to determine if Treg subsets are increased in the peripheral blood (PB) of patients with MGUS/MM & how this may correlate with increasing disease burden. *Materials and Methods*. PB samples from 176 patients with MGUS/MM (Newly diagnosed (ND), n=38; Plateau/low disease burden (LD), n=64; Relapsed/refractory (R/R), n=30 & MGUS, n=44) with a median age of 69 years (range 39-89 yrs) were analysed by flow cytometry and compared to PB from 34 age/sex matched controls. Using a sequential gating strategy, naturally occurring Tregs (nTregs) were identified as CD4^{+}/CD25^{+}/FoxP3+ T-cells and expressed as a percentage of the CD4^{+}T-cell population. Double negative T cells (DN Tregs) were identified as CD3^{+}/CD4^{-}/CD8^{-}/αβ-TCR^{+}/γδ-TCR- and expressed as a percentage of the CD3^{+} lymphocyte population. Sera from the same patients were analysed by ELISA for IL10 and TGF-beta. *Results*. nTReg cells were significantly increased in patients with MGUS/MM compared with controls (Controls 1.5%±0.2, MGUS 2.2%±0.3, ND 2.1%±0.2, LD 3.2% ±0.7 & R/R 3.7%±0.5; p=0.003). In a functional suppression assay, nTReg from patients with MGUS/MM demonstrated suppressive activity of both autologous and allogeneic T-cells similar to nTReg cells from control PB (p=NS). In contrast, DN TRegs were significantly reduced in patients with myeloma (ND1.0%±0.2, LD 1.1%±0.1, R/R 1.3%±0.2) compared with MGUS & controls (2.1% ±0.9 & 3.3%±0.7, respectively; p=0.02). There were no significant differences in serum IL10 and TGF-β levels but MGUS demonstrated higher levels of IL10 (80 ±32 pg/mL) compared with controls (28±14 pg/mL; p<0.05), ND patients (18±8 pg/mL), LD (23±11pg/mL) and R/R (29±16 pg/mL). *Conclusions*. These results provide further evidence of immune dysregulation in MM. The association with advanced disease stage suggests a causal association. The data presented provides a platform for a novel cell therapy approach to lessen the immune dysregulation seen in MM and potentially augment adoptive immunotherapy.

PO-204

IRF-4 SHOWS ABERRANT OVER-EXPRESSION IN NEOPLASTIC PLASMA CELLS

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Introduction. IRF-4 (also known as MUM-1) is a member of the interferon regulatory factor family of transcription factors whose expression is critical for the transition from pre B-cell to immature B-cell and for differentiation of mature B-cell to plasma cell. IRF-4 expression is usually assessed by immunohistochemistry, RT-PCR or western blot analysis but these approaches do not allow characterisation of cell populations. We have developed a multi-colour flow cytometry assay incorporating an indirect intracellular staining using the goat polyclonal antibody IRF-4 (Santa Cruz). This permits simultaneous analysis of IRF-4 in combination with five cell surface markers, allowing quantitation of the IRF-4 levels present in specific B-cell and plasma cell populations. The aim of this study was to assess the expression profile of IRF-4 in neoplastic and normal B-cells and plasma cells. *Methods*. Leucocytes prepared from whole bone marrow using ammonium chloride lysis were incubated with surface markers CD52 PE, CD38 PerCPCy5.5, CD19 Pe-Cy7, CD138 APC and CD20 APC-Cy7. Cells were then fixed and permeabilised before incubation with unconjugated IRF-4, washing and incubation with an Alexa488 secondary antibody. *Results*. IRF-4 expression showed the expected profile with low levels in normal B-cells, higher levels in progenitor B-cells (median 3.6-fold higher than mature B-cells) and strong uniform expression in normal BM plasma cells (median >20-fold higher than mature B-cells). Plasma cells from untreated myeloma patients showed a median 1.9-fold higher IRF-4 expression level than normal plasma cells (p=0.003, normal n=11, neoplastic n = 14). Neoplastic plasma cells in MGUS patients also expressed higher levels of IRF-4 than normal counterparts present in the same bone marrow indicating that up-regulated IRF-4 is at least partially inherent to the neoplastic cells. *Conclusions*. The results demonstrate that there are quantitative differences in IRF-4 expression levels between normal and neoplastic plasma cells. The ability to simultaneously quantitate IRF-4 expression and assess cell surface markers that allow classification of B-cell and plasma cell sub-populations will facilitate the analysis of proteins involved in plasma cell differentiation. It will also potentially improve phenotypic characterization of lymphoproliferative disorders with plasma cell differentiation.

PO-205

FLOW CYTOMETRY DETECTION OF FREE LIGHT CHAINS

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Introduction. Previous work has elegantly described the presence of FLC only producing cells in bone marrow smears (Ayliffe *et al.*'07). This led to the hypothesis that we may be able to, using sensitive flow cytometry, identify FLC producing cells within the bone marrow aspirates. Further we may be able to identify discrete sub populations of cells which exhibit FLC only production. *Materials and Methods*. Bone marrow aspirates were washed fixed and permeabilized (Caltag Fix and Perm). Cells were stained with CD38 / CD138 (APC/ PerCP-Cy5.5, BD Biosciences) to identify neoplastic populations and subsequently stained for intact immunoglobulin expression and FLC expression (The Binding Site). Stained cells were analysed using a Becton Dickinson FACScan. *Results*. 2/20 samples analysed identified cells with differential immunoglobulin expression: Sample Patient 1: IgA lambda (26% bone marrow infiltration) by comparison of IgA (FITC) v FLC Kappa (RPE) and IGA (FITC) v FLC Lambda (RPE) intact immunoglobulin only and FLC and intact immunoglobulin populations could be identified. Sample Patient 2: IgG lambda (45% bone marrow infiltration) by comparison of IgG (FITC) v FLC Kappa (RPE) and IgG (FITC) v FLC Lambda (RPE) the majority of the clone did not seem to be expressing FLC, however, a discrete population of cells which stained positively for FLC lambda and negatively for intact immunoglobulin could be identified. A unique property of these highly stained cells was the apparent lack of CD38 staining together with physical similarities to small B cells. *Discussion*. High levels of plasma cell infiltration (>20%) were required to be able to visualise any sub populations using flow cytometry. However, clearly in these two samples homogeneous immunoglobulin production was not identified. The exact nature of the small B cell like FLC positive cells in patient 2 is not known. Work is ongoing to further elucidate their identity.

PO-206

DETECTION OF NORMAL AND ABNORMAL PLASMA CELL BY FLOW CYTOMETRY

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Introduction. Multiple myeloma (MM) and monoclonal gammopathy of uknown significancy (MGUS) are rutinely distinguished on the basis of paraprotein concentration, the level of plasma cell infiltrate and the presence or absence of other clinical features. Flow cytometric detection of plasma cells according to the expression of CD38 and CD138 is not enough to discriminate between normal and abnormal plasma cells. The aim of this study was to find the numbers of normal and abnormal plasma cells and their relations with type of disease. *Materials and methods*. 30 newly diagnosed untreated multiple myeloma patients (60,3±10,99 years old) and 16 monoclonal gammopathy patients (61,88±15,12 years old) were enrolled in the study. Bone marrow aspirates were analysed by flow cytometric immunophenotyping. Plasma cells were indentified by expression of CD38, CD138, CD45 and also CD56 and CD19. *Results*. We found distinct populations of plasma cells. Discrimination between normal $CD19^+$ plasma cells and abnormal $CD56^+$ plasma cells was done. This normal/abnormal ratio (N/A) was used to describe a distribution of $CD38^{++}$ plasma cells. Patients with monoclonal gammopathy had 0,9±1,26% (average±SD) of $CD38^+CD138^+$ cells with N/A ratio 3,36±5,42. Patients with myeloma had 7,19±10,28% of $CD38^+CD138^+$ cells (0,02-37,9%) with N/A ratio 0,72±2,28. In most cases plasma cells of MM patients did not express CD45. On the other hand, MGUS plasma cells expressed CD45; these plasma cells were usually $CD19^+$. In some MM patients (1,2%) $CD38^+$ $CD138^+$ plasma cells did express neither CD19 nor CD56. *Conclusions*. Results confirmed that a majority of normal $CD19^+$ plasma cells in MGUS patients can be found. Numbers of all plasma cells in MGUS in comparison with MM patients are lower. In MM patients plasma cells are usually $CD56^+$. Further follow-up of patients can confirm the N/A ratio as a predictive factor for transformation MGUS into MM.

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PO-207

REPORT OF THE EUROPEAN MYELOMA NETWORK (EMN) WORKSHOP ON MULTIPARAMETRIC FLOW CYTOMETRY IN MULTIPLE MYELOMA AND RELATED DISORDERS

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A one-day workshop where a group of 16 members of the European Myeloma Network (EMN), from 9 different European countries met, took place the last 16th of November, 2005 in Copenhagen. The aims

of the workshop were to identify areas of consensus and areas where disagreement still exists as regards the clinical utility of multiparametric flow cytometry for the study of multiple myeloma (MM) and to provide technical recommendations. At the meeting, both flow cytometry experts and clinicians with extensive experience in the area of MM, were present and specific indications for the use of flow cytometry were discussed. At diagnosis, immunophenotyping is suitable for discriminating between neoplastic and reactive plasmacytosis but morphological investigation remains the most appropriate method for enumerating plasma cells to discriminate between MGUS and myeloma. Flow cytometry can be used to assess expression of potential therapeutic targets, and potentially predict the risk of transformation from MGUS to myeloma. Minimal residual disease (MRD) detection by flow cytometry is less sensitive than patient-specific PCR analysis but equally predictive of outcome and applicable to a greater proportion of patients. Therefore multiparametric flow cytometry is a feasible and sufficient method for the evaluation of response to therapy. In this report consensus approaches for gating reagents and strategies for screening and analysis are outlined for the detection, enumeration and characterization of plasma cells at diagnosis and follow-up. This paper is intended to provide a foundation for further development of diagnostic multiparametric flow cytometry and the precise medical indications in multiple myeloma.

PO-208

THE MATURATION OF MYELOMA CELLS DOES NOT CORRELATED WITH SURVIVAL DURATION

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Introduction. We evaluated the prognostic value of MPC-1-negative and MPC-1-negative CD45-positive immature myeloma (MM) phenotype. *Patients and methods*. In a retrospective study of newly diagnosed MM patients treated without autologous stem cell transplantation (ASCT) or with ASCT the phenotypic analysis was performed by 3-color flow cytometry using the CD38 plasma gating methods. We used the classification of monoclonal marrow plasmacytosis (MOMP) (Kawano *et al.* Int J Hematol 1995;61:179) and the revised classification (MOMP-2005) (Otsuyama *et al.* Int J Hematol 2006;83:39). The Kaplan-Meier method and the log-rank test was used for analysis of survival duration. *Results*. We studied 123 cases of MM (45 in the international staging system (ISS) stage 1, 32 in stage 2, 46 in stage 3). 83 patients underwent chemotherapy such as melphalan/prednisolone etc, and 40 underwent ASCT. 1. MOMP classification. In patients treated without ASCT, the median survival time of MOMP-3 (62 months) was significantly longer than MOMP-2 (31 months) ($p<0.05$). In patients treated with ASCT, the median survival time of MOMP-1 (48 months) was significantly shorter than MOMP-2 (77 months) ($p<0.05$). There was no difference between other pair of combinations of MOMP-1 to -4 groups. 2. MOMP-2005 classification. In patients treated without ASCT, there was no difference among the groups of MOMP-I to -IV. In patients treated with ASCT, the median survival time of MOMP-I (48 months) was significantly shorter than MOMP-2 (77 months) ($p<0.05$). 3. ISS classification. In patients treated without ASCT, the median survival time of ISS stage 1 group (median survival not yet reached) was significantly longer than stage 2 group (31 months) and stage 3 group (14 months) ($p=0.0001$ and $p<0.0001$, respectively). In patients treated with ASCT, the median survival time of ISS stage 1 group (101 months) was significantly longer than stage 2 group (56 months) and stage 3 group (48 months) ($p=0.04$ and $p=0.002$, respectively). *Conclusions*. Overall survival does not correlate to increase in immature MM cells. Short survival of MM patients at ISS stage 3 is independent of the MM maturation classified by expressions of MPC-1, CD45, and CD49e.

PO-209

CANCER GERMLINE ANTIGEN-SPECIFIC T CELLS ARE PRESENT AND FUNCTIONAL IN MANY PATIENTS WITH MULTIPLE MYELOMA AND PARAPROTEINAEMIAS

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The expression of Cancer Testis Antigens (CTAgs) is normally restricted to the pre-meiotic spermatogonia cells of the testis. The testis is an immunologically privileged site and so immunological tolerance to CTAg is not established. However, CTAg expression is also detected in many types of malignant disease including plasma cells from patients with multiple myeloma. CTAg expression has been shown to prime a T cell immune response in many patients with solid tumours and this may offer a novel target for immunotherapy in patients with myeloma. We have used immunodominant peptide epitopes from a range of CTAgs to screen for CTAg-specific T cells in the blood of patients with multiple myeloma at various stages of their disease. Initial studies demonstrated that T cells from 15 out of 37 patients responded to one or more CTAg peptides and the magnitude of the CTAg-specific $CD8^+$ T cell response ranged between 0.0004% and 0.1% of the total $CD8^+$ T cell pool. Serial analysis showed that these immune responses were detectable in individual patients at multiple time-points during the course of their disease. For the last two years we have extended our initial study to further characterise the CD8 T cell response and have also characterized CD4 T cell responses. In some patients we determined the membrane phenotype of the CD8 CTAg-reactive T cells as $CD45RA^+$ and CCR7-, an effector memory differentiation state. CD8 CTAg-specific responses have also been detected in patients with clinically benign forms of paraproteinaemia indicating that T cell immunity may play a role in the control of disease progression. Analysis of bone marrow in one patient indicated a higher proportion of CTAg-specific CD8 T cells within bone marrow and the phenotypic profile of these cells is being determined. In addition we have characterised CD4 T cell responses in 19 normal age-matched matched controls, 20 MGUS patients and 24 Multiple Myeloma patients. Functional T cells specific for CTAg are therefore present in a large proportion of patients with multiple myeloma and offer the possibility of a novel approach for immunotherapy in this disease.

PO-210

C-REACTIVE PROTEIN PROTECTS MYELOMA CELLS FROM APOPTOSIS VIA ACTIVATING ITAM-CONTAINING FCGRII

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Introduction. Elevated levels of C-reactive protein (CRP) are present in patients with infections, inflammatory diseases, necrosis, or malignancies including multiple myeloma (MM), and accumulating evidence strongly suggests that CRP contributes to the pathogenesis of cardiovascular disease. These findings led to our hypothesis that CRP may have a functional effect on tumor cells. *Methods.* The present study was undertaken in a myeloma setting to determine whether CRP might affect tumor cell growth and survival. *Results*. CRP promoted cell proliferation and reduced primary cell apoptosis in a dose-dependent manner. Next, we examined the effects of CRP on myeloma cell lines under stressed conditions and showed that CRP protected myeloma cells from apoptosis induced by serum starvation or IL-6 deprivation. More importantly, CRP also protected myeloma cells from apoptosis induced by dexamethasone or melphalan. The protection was significant since CRP reduced cell death by 50 to 70%, and may be clinically relevant because the results were reproduced in myeloma-SCID mouse models. Injection of CRP prior to treatment of myeloma-bearing mice with dexamethasone or melphalan significantly undermined the therapeutic effects of these chemotherapy drugs. CRP protected tumor cells from apoptosis by downregulating Bax expression, inhibiting phosphorylation of Bcl-2, and upregulating phosphorylation of Bad, which led to inhibited caspase-9, caspase-3 and PARP activation induced by dexamethasone. We next examined cell surface receptors for CRP and found that CRP bound FcgRIIA and/or FcgRIIC, but not FcgRIIB. Specific siRNAs that inhibited FcgRIIA or FcgRIIC but not FcgRIIB expression, and antibodies against FcgRIIA/C that blocked receptor-ligand interaction abrogated CRP-mediated protection of cell apoptosis. These results indicate that CRP mediated its effects via activating immunoreceptor tyrosine-based activation motif (ITAM)-containing FcgRII. By binding to these receptors, CRP increased the level of phosphorylated Akt, ERK1/2, and IkBa; relocalized NFkB p65 to the nucleus; and inhibited p38 kinase activity. CRP also enhanced myeloma cell secretion of IL-6 and synergized with IL-6 to protect myeloma cells from chemotherapy drug-induced apoptosis. *Conclusions*. Our results provide strong evidence for a novel effect of CRP on myeloma cells. This study also implicates CRP as a potential target for MM therapy.

PO-211

LFA-1/FAK/PI3-K/AKT MEDIATES SURVIVAL OF MM CELLS

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Introduction. Multiple myeloma (MM) is still an incurable disease and adhesion of MM cells to bone marrow stromal cells is one of the hallmarks of the disease. Lymphocyte function associated antigen 1 (LFA-1) is an adhesion molecule that mediates lymphocyte adhesion, but its role in MM is only poorly understood. The aim of the presented study was to improve knowledge about LFA-1 and associated pathways in MM for the development of molecular targeted therapies. Methods and *Results*. We show that LFA-1 is expressed on U266, RPMI-8226, OPM-2, and NCI-H929 MM cell lines and on primary cells of eight tested patients. The LFA-1 inhibitor LFA878 induces apoptosis in all four cell lines as revealed by annexin V staining and caspase 3 cleavage. Apoptosis is not hampered by adhesion to stromal cells. Additionally, the soluble ligand, intracellular adhesion molecule 1 (ICAM-1), which is increased in the serum of MM patients, does not protect from melphalan-induced apoptosis. Western blots demonstrate down-regulation of FAK, PI3-K, and Akt upon LFA878 treatment. Additionally, sequential inhibition of the pathway by simultaneous application of Src family kinase or PI3-K inhibitors significantly increases LFA878 induced apoptosis. *Conclusion*. LFA-1/FAK/PI3-K/Akt is a survival pathway in MM and targeted inhibition provides new therapeutic options.

PO-212

VEGF AND IL6 SECRETION BY STROMAL CELLS AND MYELOMA CELLS

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Introduction. Multiple Myeloma (MM) is characterized by accumulation of monoclonal plasma cells (PCs) in the marrow. The marrow enhances proliferation and suppresses apoptosis in these PCs and forms a supportive microenvironment, actions mediated in part through cytokines such as VEGF and IL6. MM cell lines, when grown in co-culture with marrow derived stromal cells (BMSCs) results in increased levels of VEGF and IL6. This effect is mediated primarily by the BMSCs although PCs also secrete these cytokines. We examined if differences exist between PCs from different disease stages of MM in terms of cytokine stimulation. *Materials*. PCs were obtained from fresh marrow aspirates from patients with MGUS, SMM or active MM. Normal PCs were obtained at the time of orthopedic surgeries. PCs were grown in co-culture with BMSCs and VEGF and IL6 levels were determined using ELISA. *Results*. A total of 71 samples were studied including normal, MGUS, SMM and myeloma. The median VEGF levels with plasma cells alone was 30.2 pg/mL, with stromal cells alone was 737 pg/mL and with the co-culture was 1057 pg/mL, representing a median increase of 41% with co-culture. The median IL-6 levels with plasma cells alone was 22.3 pg/mL, with stromal cells alone was 2457 pg/mL and with the co-culture was 5982 pg/mL, representing a median increase of 99% with co-culture. There was no significant difference between the disease groups in terms of the baseline VEGF and IL6 secretion by plasma cells or the increase in VEGF and IL6 with co-culture. *Conclusions*. We demonstrate up regulation of VEGF and IL-6 when primary MM cells are grown in contact with BMSCs highlighting the importance of these cytokines in the marrow microenvironment. Using BMSCs derived from the same source we analyzed differences between PCs from different disease stages. PCs from different disease stages and normal PCs induced the same degree of stimulation of VEGF and IL-6. It is possible that difference in numbers of plasma cells across these disease stages contributes to the increase in cytokine levels previously shown with disease progression. Differences between disease stages in terms of the marrow stromal cells are being studied.

PO-213

EPIGENETIC DYSREGULATION OF WNT SIGNALING PATHWAY IN MULTIPLE MYELOMA

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Wnt signaling has recently been implicated in the pathogenesis of cancer. We studied the activity of Wnt signaling and the methylation status of a panel of seven soluble Wnt antagonists including WIF1, DKK3, APC, SFRP1, SFRP2, SFRP4 and SFRP5 by methylation-specific PCR in myeloma cell lines and primary myeloma samples. Of the 4 myeloma cell lines, Wnt signaling was shown to be constitutively activated in LP1 and WL2, correlating with hypermethylation and hence silencing in three and four of the seven Wnt antagonists respectively. 5-Aza-2'-deoxycytidine treatment of these two cell lines showed progressive demethylation of methylated Wnt inhibitors, re-expression of transcripts and down-regulation of Wnt signaling. Methylation of the seven genes was found in 4% to 22% of primary myeloma marrow samples. Twenty-one (42%) patients had methylation of at least one of these 7 genes, of which 13 (61.9%) had ≥2 genes methylated. No association between methylation and clinical characteristics and outcome was demonstrated. In conclusion, Wnt signaling is constitutively activated in myeloma cell lines due to methylation silencing of multiple soluble Wnt antagonists. Methylation of these soluble Wnt antagonists, occasionally multiple genes, in primary myeloma samples implicated gene hypermethylation in myeloma pathogenesis. Our study also underscored the importance of studying methylation of a panel of genes regulating a cellular pathway instead of isolated genes.

PO-214

EXPRESSION OF FUNCTIONAL FOLATE RECEPTOR ALPHA IN A SUBSET OF MULTIPLE MYELOMA PATIENTS

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Cellular uptake of folate in humans is mediated by either a cell surface folate receptor (FR) or reduced folate carrier (RFC). RFC is expressed in nearly all cells but FR expression is restricted to certain cell types. Unlike RFC, which utilizes a bidirectional anion exchange mechanism to transport folate, FR after binding folic acid is taken up into cells by receptor-mediated endocytosis. FR expression, especially the alpha isoform, is highly upregulated in malignant epithelial tumors. Based on these observations folic acid-conjugated therapeutic agents have been developed as target therapies in solid tumors. Data on the expression of folate receptors in hematopoietic malignancies is limited, although in certain myeloid leukemias FR beta isoform is upregulated. We examined the expression of FR in bone marrow aspirates obtained from 36 multiple myeloma patients utilizing a polyclonal antibody that detects the alpha isoform and a fluorochrome-conjugated folate that specifically binds FR. Mononuclear cells obtained from fresh bone marrow samples were analyzed for FR alpha expression in CD138 positive and CD138 negative fractions by flow cytometry. Fifteen samples (42%) showed FR alpha expression in greater than 10% of the cells within the CD138-positive population. Only 2 samples (5%) showed no FR expression. Interestingly, many of the samples that showed a high level (>20%) of FR alpha expression within the CD138-positive population were also FR alpha positive in the CD138 negative population. CD34 positive early hematopoietic cells and bone marrow mononuclear cells isolated from normal donors did not express FR alpha In order to further confirm our findings and to verify that the receptors detected on myeloma cells were functional, we incubated 15 of the myeloma samples with fluorochrome- conjugated folate followed by extensive washing prior to flow cytometry analysis. The results of these experiments showed specific binding, indicating that the receptors were functional. Although further studies need to be performed to precisely define which subsets of myeloma patients express high levels of folate receptors, our observations suggest the possibility for development of FR-targeted therapeutics for treatment of multiple myeloma.

PO-215

EPIGENTIC SILENCING OF CD9 DURING MM PROGRESSION

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Introduction or aims. The surface expression of CD9, a glycoprotein of the tetraspanin family influencing several biological processes, inversely correlates with progression in several solid tumors. We investigated

CD9 expression in MM cells during disease progression. Further, we addressed the involvement of DNA methylation and histone deacetylation in the CD9 silencing and the possible consequences of this silencing in terms of sensitivity to NK cell mediated lysis. *Materials and methods*. Immunophenotyping for CD9 and CD38 was performed on primary bone marrow plasma cells of MM (n=62) and MGUS (n=6) patients. CD9 membrane expression on the murine 5TMM cells during disease progression was detected by a double staining for CD9 and anti-idiotype. CD9 mRNA levels were determined by realtime quantitative PCR. To determine the methylation status, the CD9 promoter region containing 20 CpG sites from bisulfite modified gDNA was amplified. NK cell mediated cytolysis of the 5T33MM cell lines was measured by a 51Cr release assay. *Results*. We showed CD9 expression in the majority of MGUS and MM patients with non-active disease, while most cases with aggressive disease were CD9 negative. The CD9 expression of murine 5T33MM and 5T2MM cells was also significantly downregulated during development of disease. The CD9 promoter region of CD9 non-expressing 5T33MMvt cells was found to contain twice as much methylated CpG sites compared to the CD9 expressing 5T33MMvv cells. Co-treatment of the 5T33MMvt cells for 48h with the demethylation agent 5-Aza-2'deoxycytidine and the histone deacetylase inhibitor LBH589 resulted in a strong synergistic reactivation of CD9 at mRNA and protein level. The 5T33MMvv cells were found to be more susceptible to NK mediated cytolysis than the 5T33MMvt cells. 5T33MMvt cells were stably transfected with the lentiviral transferplasmid pHR'tripCMV-mCD9-SIN, resulting in an induction of CD9 expression as evidenced by FACS. The latter cells were more sensitive to cell mediated lysis than control transduced cells. *Conclusions*. We demonstrate an inverse correlation between CD9 expression and disease progression in MM. Epigenetic silencing through DNA methylation and histone deacetylation seems to be involved in this CD9 downregulation. Furthermore, we provide evidence that as CD9 expression becomes down-regulated during disease progression, MM cells become less susceptible to NK cell-mediated cytolysis.

PO-216

BIM EXPRESSION IN MM IS REGULATED BY IGF-1 AND EPIGENTICS

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Introduction or aims. Insulin-like growth factor-1 (IGF-1) is an important factor in proliferation, cell survival and migration of MM cells. We ascertained the role of IGF-1 in the microenvironment induced modulation of the gene expression of MM cells. *Materials and methods*. Gene expression of murine 5T33MMvv cells treated or not for 6 and 12h with IGF-1 was compared using Affymetrix cDNA microarrays. Real Time Quantitative PCR was used to confirm results on mRNA level, while FACS stainings or Western blots were performed to confirm on protein level. *Results*. Statistical analysis of the microarray data resulted in 138 consistent down-regulated and 80 upregulated genes after stimulation with IGF-1. One of the genes significantly and consistently downregulated in the MM cells was Bim. Bim is a pro-apoptotic protein belonging to the BH3-only group of Bcl-2 family members. Three major forms of Bim are known: BimEL, BimL and BimS. The downregulation was confirmed on mRNA level at 12h (63% overall inhibition). At protein level an inhibition of 100, 56 and 100% was found for respectively BimEL, BimL and BimS after 24h. Co-treatment of 5T33MMvv and 5T33MMvt cells for 24h with the demethylation agent 5-Aza-2'deoxycytidine and the histone deacetylase inhibitor LBH589 resulted in a strong induction of Bim expression at protein level. In the future, we will investigated the contribution of IGF-1 to the epigenetic silencing of Bim by i) comparing the Bim promoter methylation pattern of 5T33MMvv cells treated or not with IGF-1 using bisulfite PCR sequencing and ii) using epigenetic modulating agents. To address the functional involvement of Bim, 5T33MMvt cells were stably transfected with Bim siRNA resulting in a knockdown of 83% as evidenced by FACS. Drug resistancy and apoptosis assays will be performed on the parental and Bim siRNA transfected cells. *Conclusions*. We demonstrated downregulation of the pro-apoptotic protein Bim on transcriptional and translational level by IGF-1 treatment. Furthermore, epigenetic silencing of Bim through DNA methylation and histone deacetylation was shown. We hypothesize that using epigenetic modulating agents this IGF-1 effect on Bim and thus the increase in MM cell survival will be reversed.

PO-217

SERUM YKL-40 CORRELATES WITH SERUM INTERLEUKIN-6 IN MULTIPLE MYELOMA

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Introduction. The secreted glycoprotein YKL-40 (CHI3L1, HC gp-39) is a potential player in the tumor-host interactions affecting several aspects of multiple myeloma (MM) including angiogenesis. YKL-40 expression is seen in vascular smooth muscle cells, and the protein is suggested to function in angiogenesis by stimulating the migration and reorganization of vascular endothelial cells and by promoting vascular smooth muscle cell attachment, spreading and migration. The aim of this study was to investigate the association between serum YKL-40 (S-YKL-40) and the degree of bone marrow (BM) angiogenesis in MM including serum concentrations of angiogenic cytokines. *Materials and Methods*. S-YKL-40 was measured using an ELISA in 54 MM patients at diagnosis. BM angiogenesis was estimated by three different methods including vascular grading, microvessel density (MVD), and Chalkley count. Microvessels were identified using immunohistochemical staining for CD34-positive endothelial cells. The serum concentrations of the angiogenic cytokines interleukin-6 (S-IL-6), hepatocyte growth factor (S-HGF) and basic fibroblast growth factor (S-bFGF) were measured in both peripheral blood (PB) and BM. *Results*. 57% of the patients had a S-YKL-40 elevated above the upper limit in an age specific 90 per cent reference range for healthy adults. Neither vascular grading, MVD or Chalkley count was associated with S-YKL-40 level. As described in Table I, patients with elevated S-YKL-40 had higher BM levels of IL-6 and HGF compared to patients with normal S-YKL-40 although the difference was only significant for IL-6. At the same time, a negative association between S-YKL-40 level and BM levels of bFGF was seen (Table 1). The association between S-YKL-40 and S-IL-6 was further analysed using S-YKL-40 as a continuous variable showing a strong correlation with S-IL-6 in both BM (rho = 0.51; p=0.03) and PB (rho = 0.60; p< 0.0001) consistent with S-IL-6 not differing between BM and PB in the individual patient. *Conclusions*. In patients with newly diagnosed MM the S-YKL-40 level lack association with the degree of BM angiogenesis, but S-YKL-40 show a strong positive correlation with S-IL-6 consistent with a possible IL-6 mediated regulation of YKL-40 secretion demonstrated in previous studies.

Table 1.

	Normal S-YKL-40		*Elevated S-YKL-40*		
	N	*Median (range)*	*N*	*Median (range)*	*p-value*
S-IL-6, ng/L					
PB	23	3 (1-13)	31	11 (0-43)	< 0.0001
BM	9	3 (1-11)	10	9 (1-37)	0.04
S-HGF, ng/L					
PB	23	1 (0-4)	30	3 (0-43)	0.03
BM	4	8 (3-17)	8	16 (3-43)	0.5
S-bFGF, ng/L					
PB	23	5 (1-25)	31	7 (1-31)	0.6
BM	7	163 (131-589)	8	55 (36-198)	0.008

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EVALUATION OF SELECTED BIOLOGICAL PARAMETERS IN STAGES OF MULTIPLE MYELOMA EXAMINED ACCORDING TO DURIE-SALMON (D-S) AND INTERNATIONAL PROGNOSTIC INDEX (IPI)

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Introduction. Multiple myeloma is an unusually heterogeneous disease with individually different course, response to therapy and prognosis. Up-

to-date stratification systems have, however, an important limitation in their insufficient incorporation of those parameters, that express intrinsic biological properties of myeloma cells and the microenvironment of the bone marrow. This study is focused on the evaluation of the differences between serum levels of selected biological parameters and stages of multiple myeloma (MM) evaluated according to the system of D-S and IPI. *Materials*. The analysed group consisted of 108 patients evaluated at the time of MM diagnosis. For the assessment of serum levels of examined molecules were used: REA, RIA, ELISA and the technique of quantitative sandwich enzymatic immunoassay. Statistical analysis was carried out using Kruskal-Wallis, Mann-Whitney and Pearson Chi-Square test (p<0.05). *Results*. Statistically significant differences were found out between all D-S stages in case of β2-microglobulin (p<0,0001), thymidinekinase (p<0,0001), ICTP (p<0,0001), OPG (p<0,024), HGF (p<0,010) and syndecan-1 (p<0,0003); stage I vs II in the case of b2-microglobulin (p<0,016), thymidinekinase (p<0,005) and syndecan-1 (p<0,0002); stage I vs III: β2-microglobulin (p<0,00003), thymidinekinase (p<0,00018), ICTP (p<0,00191), OPG (p<0,012), HGF (p<0,006) and syndecan-1 (p<0,0001); stage II vs III: b2-microglobulin (p<0,00001), thymidinekinase (p<0,003), IL6-R (p<0,027), ICTP (p<0,00001). There was no relation between the D-S stages and VCAM-1, ICAM-1, PINP, VEGF and Fas serum levels. Statistical analysis revealed significant relationship also in comparison between all IPI stages and serum levels of thymidinekinase (p<0,001), IL-6R (p<0,016), ICTP (p<0,0001), HGF (p<0,001), VEGF (p<0,012), Syndecan-1 (p<0,011) and Fas (p<0,005); stage I vs II in the case of ICTP (p<0,005) and Fas (p<0,029); stage I vs III: thymidinekinase (p<0,003), ICTP (p<0,00001), HGF (p<0,014), syndecan-1 (p<0,12), and Fas (p<0,003); stage II vs III: thymidinekinase (p<0,003), IL-6R (p<0,012), ICTP (p<0,001), HGF (p<0,001), VEGF (p<0,003) and syndecan-1 (p<0,029). There was no relation between the IPI stages and VCAM-1, ICAM-1 and PINP. *Conclusion*. This study established, that both of the most prevalent stratification systems have a very good relationship to evaluated biological parameters of multiple myeloma, although in the case of IPI the relationship found was of a more intimate character.

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PO-219

MANGANESE INDUCES MYELOMA CELL ADHESION TO FIBRONECTIN

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Introduction. Adhesion of MM cells in the BM is important for the growth and survival of the myeloma cells. It is previously shown that adhesion is strongly enhanced by the cytokines HGF, IGF-1 and SDF-1alfa, which are present in the BM microenvironment. Very late antigen 4 (VLA-4) is one of the main adhesion receptors that mediate cytokine-stimulated cell binding to fibronectin (Fn). In this study we have examined the effect of manganese on adhesion of MM cells to Fn. *Material and methods*. Cell adhesion experiments were performed with the human myeloma cell lines INA-6, CAG, OH-2, IH-1, ANBL-6 and JJN-3. The INA-6 cell line was chosen for a closer examination of manganese-induced adhesion. Flow cytometric analysis with the mAb HUTS-21 was used for recognizing high affinity beta1 integrins. *Results*. Manganese induced adhesion in all the six cell lines tested and the amount of adhesion correlated with the expression of VLA-4 in the cell lines. We also studied the different properties between cytokine- and manganese-induced adhesion and found that while the cytokine-stimulated INA-6 cells only had the ability to bind fibronectin, manganese-stimulated cells adhered to both fibronectin and VCAM-1. The cell adhesion to Fn induced by manganese was stronger than that induced by cytokines. We found that manganese, and not cytokines, had the ability to convert VLA-4 to a high-affinity state, which could explain the difference in adhesion properties. The binding kinetics between the two forms of adhesion was also different. Manganese had the ability to induce adhesion more rapidly than the cytokines, while the latter adhesion persisted longer. *Conclusion*. We have identified manganese as an important adhesion factor for MM cells. The manganese-induced VLA-4 mediated adhesion to fibronectin differs in properties from the cytokine-induced adhesion. Since the location to the BM is important for the survival, proliferation and functional program of MM cells and since the BM is the major storage for manganese in the body, a high level of manganese here could be beneficial for the survival of MM cells.

PO-220

CYTOKINE-ACTIVATED VLA-4 ADHESION OF MYELOMA CELLS TO FIBRONECTIN INVOLVES HEPARAN SULFATES

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Background. Cell surface adhesion molecules (integrins, syndecans et c.) are believed to play pivotal role in localization of multiple myeloma (MM) cells to the bone marrow (BM). Adhesion to BM matrix proteins induces pro-survival signaling cascades, increases proliferation and promotes drug resistance. Adhesion of MM cells is substantially increased following stimulation by cytokines. Most important cytokines are HGF, IGF-1 and SDF-1α. In human MM cells the integrin VLA-4 (α4beta1) is one of the main adhesion receptors that mediate cytokine-stimulated tumor cell binding to fibronectin (Fn). Syndecans, a family of cell surface glycoproteins, are involved in adhesion owing to their negative charge and ability to attach to *heparin*-binding domains of matrix proteins. Syndecan-1 (CD138) is the only syndecan highly characteristic for myeloma cells. Possible interaction/cross-talk of integrins and syndecans is widely discussed, intriguing data have been demonstrated for carcinoma cells, but little evidence exist in MM so far. Methods and *Results*. To determine if heparan sulfates are essential in the VLA-4-mediated adhesion, we performed the adhesion assay with MM cell line INA-6 in the presence of heparin (20 ug/mL), and with MM cells pre-treated with chlorate (30 mM) (an inhibitor of heparan sulfate formation) or heparitinase (10 mU/mL). Cells labeled with fluorescent dye were put into the plates, precoated with human Fn and the results were read in 1 hour after removal of non-adherent cells. Heparin dramatically reduced the HGF- and IGF-1- mediated adhesion to Fn. So did sodium chlorate and heparitinase. Adding 15 mM sodium sulfate abolished the inhibitory effect of chlorate. Chondroitinase did not affect the cell adhesion to Fn. Localization of integrins and syndecan-1 in the cell membrane was studied with fluorescent laser scanning confocal microscopy in INA-6 cells stimulated with HGF (150 ng/mL), IGF-1 (100 ng/mL), SDF-1 (75 ng/mL). Cells were put onto confocal dishes precoated with Fn. In 40 min non-adherent cells were removed, cells were incubated with PE-labeled anti-CD138 (syndecan-1) and FITC-labeled anti-CD49d (α4 subunit of VLA-4). Scans were taken at the interface. In unstimulated cells α4 signal appeared as a central spot at the adhesion surface, surrounded by a ring-like CD138 signal, with clear separation of both signals in the majority of cells. With cytokine stimulation the signals from α4 and CD138 got co-localized in the majority of the cells in a ring-formed pattern, α4 moving laterally and merging with CD138-positive area. This effect was most clearly seen with IGF-1 stimulation. The same tendency was found in a primary MM cell sample. *Conclusion*. Heparan sulfates are important for VLA-4-dependent cytokine-stimulated adhesion of MM cells to fibronectin. This may involve lateral movement of VLA-4 molecules leading to colocalization of syndecan-1 and VLA-4.

PO-221

BINDING OF TACI AND APRIL TO SYNDECAN-1 CONFER ON THEM A MAJOR ROLE IN MULTIPLE MYELOMA

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Introduction. BAFF and APRIL stimulate the growth of multiple myeloma cells (MMC). BAFF and APRIL share two receptors - TACI and BCMA - and BAFF binds to a third receptor, BAFF-R. We previously reported that TACI expression is a good indicator of a BAFF-binding receptor in human myeloma cell lines (HMCL), unlike BCMA that is expressed on all HMCL. BAFF-R is lacking. More recently, Ingold *et al.* (J Exp Med; 2005) and Hendriks at al (Cell Death Differ; 2005) identified proteoglycans as the APRIL-specific binding partners. Bischof *et al.* (Blood; 2006) demonstrated that TACI binds also heparane sulfate (HS) proteoglycans. TACI binds HS chains of syndecan-1, syndecan-2 and

syndecan-4. Syndecan-1 is expressed by plasma cells and epithelial cells and is involved in several cellular processes relying on interactions with extra-cellular matrix proteins, growth factors, chemokines and adhesion molecules. *Materials and Methods*. We investigated the binding of BAFF, APRIL and their receptors on a large panel of 16 HMCL and primary MMC using flow cytometry. The link between BAFF/APRIL family members and HS proteoglycans was investigated using heparin or heparatinase pretreatments. Growth factor activity of BAFF and APRIL on HMCL was studied by tritiated thymidine incorporation. *Results*. We identified a large binding of APRIL, unlike BAFF, at the surface of all syndecan-1+ HMCL. Regarding growth factor activity, APRIL was more efficient than BAFF to stimulate the proliferation of MMC. An explanation could be a link between APRIL and TACI through syndecan-1 as it was reported for FGF and FGFR. All syndecan-1+ HMCL bind TACI-Fc, whereas no binding of BCMA-Fc or BAFF-R-Fc molecules at the surface of HMCL could be detected. The binding of APRIL and TACI-Fc was abrogated by heparin pretreatment or by pretreatment of MMC with heparatinase. These results were confirmed using primary tumor plasma cells of myeloma patients. Our data establish a functional link between syndecan-1 and the APRIL/TACI pathway, a signaling route that induces survival and proliferation in MMC. Syndecan-1 appears to strongly promote APRIL/TACI signaling. *Conclusion*. These results identify syndecan-1 as coreceptor for APRIL and TACI promoting APRIL/TACI signaling in MMC.

PO-222

SOLUBLE MICA/B IN MM: A POTENTIAL IMMUNE ESCAPE MECHANISM

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Introduction. NKG2D is an activating NK receptor expressed by NK cells, $CD8^+$ αβ T cells and γδ T cells. NKG2D ligands include MICA and MICB, commonly upregulated on tumor cells including Multiple Myeloma (MM). Shedding of MICA/B has been reported, leading to decreased expression of NKG2D on effector cells. Here, we evaluated the presence of soluble MICA/B (sMIC) in patients with MM. *Methods.* Sera from 51 control persons, 13 patients with monoclonal gammopathy of undetermined significance (MGUS), 66 patients with stable MM and 29 patients with progressive MM were analysed for sMIC using a commercial ELISA. FACS analysis was performed for expression of NKG2D on fresh effector cells (5 controls, 6 MM). To evaluate the effect of sMIC, effector cells were cultured for 2 days with or without sMICA±IL-15 and reanalysed. *Results*. There was a significantly higher level of sMICA in MM as compared to MGUS or controls (in pg/mL: controls 112±99, MGUS 126±62, stable MM 210±144, progressive MM 315±498, $p< 0.01$). In contrast, sMICB was elevated only in progressive MM (in pg/mL: controls 75±68, MGUS 79±58, stable MM 80±82, progressive MM 177±273). FACS analysis of effector cells revealed an NKG2D expression on 84±6% NK cells, 84±8% αβ T cells and 79±7% γδ T cells in healthy controls. In contrast, NK cells of patients expressed NKG2D in 74±8%, αβ T cells in 83±5% and γδ T cells in 66±14% ($p< 0.05$ for NK cells and γδ T cells). *In vitro* addition of sMICA decreased NKG2D expression in all effector cells, additional IL-15, however, counteracted this effect. *Conclusion*. Soluble NKG2D ligands are increased in MM compared to healthy controls and MGUS. Also, NK cells and γδ T cells reveal a lower surface expression of NKG2D in MM which might be sMICA mediated. *In vitro* results suggest that IL-15 restores NKG2D expression in the presence of sMICA, thus representing a potential agent for immunomodulation in MM.

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KIT PROTEIN (CD117) EXPRESSION IS NOT CORRELATED WITH KIT GENE AMPLIFICATION IN MULTIPLE MYELOMA PATIENTS

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Intoduction. KIT (CD117) is a type III receptor tyrosine kinase operating in cell signal transduction in several cell types. Aberrant KIT expression has been reported in several solid tumours including glioblastoma multiforme, testicular teratocarcinomas, GIST tumours , small-cell lung cancers. Exon 11 KIT gene mutation is the mechanism leading to expression of CD117 in GIST tumours while in glioblastoma multiforme is. highly frequent KIT gene amplification. Previous studies demonstrated that approximately 30% of multiple myelomas express CD117 when detected by flow-cytometry or immunohistochemistry analysis and that CD117 positivity don't have clinical implication. We first evaluate by FISH analysis amplification of the KIT gene in a series of 35 multiple myelomas. Moreover we evaluated if imatinib can induce apoptosis in purified primary CD117 positive plasmacells. *Methods*. Immunohistochemistry with an anti-KIT polyclonal antibody (DAKO) were performed on a series of 35 bone marrow biopsies. FISH analysis for the detection of KIT gene amplification were performed on CD138-purified plasmacells with Chromosome 4 Alpha Satellite/FITC probe (Resnova) and bacterial artificial (BAC) bA74L18 and bA586A2 probes labeled with Cy3 (Amersham, Piscataway, NJ) by nick translation for KIT gene. Purified plasmacells of two CD117 positive myelomas were coltured with imatinib and annexin (FITC) were evaluated with a FACScan flow cytometer. *Results*. Definite membrane-associated CD117 positivity was found in 10 cases (28.5%). The cut-off value was defined at 20%. FISH analysis was performed on the 10 cases with KIT protein expression and demonstrated lack of amplification of the KIT gene. Imatinib was unable to induce apoptosis in both purified primary CD117 positive plasmacells *Conclusion*. KIT gene amplification is not responsible of expression of KIT protein in multiple myelomas. Apoptosis was not observed in primary CD117 positive plasmacells when cultured with imatinib. . Previous studies demonstrated that imatinib is not an active agent in refractory/relapsed myelomas but that it may be a potential antiresorptive therapy for bone disease. Further studies are necessary to establish if KIT protein is activated by mutation of KIT gene and to evaluated if imatinib, alone or in association with other drugs, may be a therapeutic agent in multiple myeloma.

PO-224

EPIGENETIC SILENCING OF THE WNT ANTAGONIST DICKKOPF-1(DKK1) IN MULTIPLE MYELOMA

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Introduction. Studies by Shaughnessey and coworkers have demonstrated that the WNT signaling inhibitor Dickkopf-1(DKK1) is secreted by multiple myeloma (MM) cells and may contribute to osteolytic bone disease by inhibiting osteoblasts differentiation. Moreover, previous studies from our own laboratory have shown that the WNT signaling pathway is aberrantly activated in MM. This activation was shown to enhance MM proliferation and, most likely, involves para- and/or autocrine stimulation by WNTs. As a consequence, DKK1 could act as a negative regulator of WNT signaling in MM cells suppresing tumor progression. Consistent with this hypothesis, we observed that DKK1 expression is often lost in advanced myeloma and is absent in most MM cell lines. *Materials*: In our current study, we examined the level of expression of DKK1 and B-catenin in bone marrow biopsy specimens from a panel of MM patients at different stages of disease. To address the mechanism responsible for the loss of DKK1 expression observed in advanced myeloma, we have studied the pattern of CpG island methylation of the DKK1 promoter in MM cell lines and primary MMs in relation to DKK1 expression.*Results*. We observed loss of DKK1 expression in subset of patients with advanced MM, particularly in cases with blastic transformation and a high proliferation rate. Loss of DKK1 was correlated with an increased nuclear expression of B-catenin. Furthermore, we found methylation of the DKK1 promotor in several MM cell lines as well as in MM cells from patients with advanced MM. Treatment with the demethylating agent; 5-aza-2-deoxycytidine restored DKK1 expression. *Conclusions*. Our results establish methylation of the DKK1 promotor as a cause of DKK1 silencing in MM cell lines and in patients with advanced MM.

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CD32B IN MULTIPLE MYELOMA AND SYSTEMIC AL-AMYLOIDOSIS

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Introduction. Seeking targets for monoclonal antibody therapy, we tested CD138$^+$ cells from patients with systemic AL-amyloidosis and multiple myeloma for CD32B, a B-cell receptor for immunoglobulin Fc regions and inhibitory regulator of humoral immunity whose gene (FCGR2B) is on chromosome 1q23. *Materials and Methods.* We used CD138$^+$ plasma cells from 34 patients with AL-amyloidosis for flow cytometry, for Fc-receptor gene expression by gene-chip analysis, and for FCGR2B1 and B2 isoforms by reverse transcriptase (RT-)PCR. We evaluated CD138$^+$ plasma cells from 65 patients with multiple myeloma with flow cytometry and karyotype and fluorescence *in situ* hybridization (FISH). We also FACS-sorted CD138$^+$/CD32B$^+$ and CD138$^+$/CD32B$^-$ fractions from patients with plasma cell leukemia (PCL) to compare the fractions for gain of 1q25 (+1q25) by FISH and for quantitative FCGR2B1 expression by real-time PCR. *Results*. On the surface of 99% of CD138$^+$ cells from patients with AL-amyloidosis bright CD32B expression was found at both diagnosis and relapse, and CD32B gene-expression was significantly higher than CD32A, CD16 and CD64 by Affymetrix U133 Plus 2.0 microarray (p<0.01). The FCGR2B1 isoform was highly expressed by RT-PCR. On CD138$^+$ myeloma cells, CD32B was highly expressed at diagnosis (>95%) but was significantly lower in those with ISS stage II/III myeloma, poor-prognosis cytogenetics, plasma cell leukemia, or myeloma in relapse (all p<0.01). Human myeloma cell lines were CD32B- by flow cytometry and RT-PCR. There was no difference in the percent of CD138$^+$/CD32B$^+$ cells in those with or without +1q25 by FISH. There was no difference in +1q25 by FISH between CD138$^+$/CD32B$^+$ and /CD32B$^-$ sorted paired fractions from 5 cases with PCL; however, real-time PCR demonstrated that relative expression of FCGR2B1 was 1 to 2 logs lower in the CD138$^+$/CD32B$^-$ fractions. *Conclusions*. CD32B is present on CD138$^+$ cells in AL-amyloidosis and myeloma, and is a correlate of prognosis in myeloma and a target for monoclonal antibody therapy in both diseases. Loss of CD32B protein and message in myeloma may be due to mutations at 1q23, promoter or gene methylation, or microRNA activity, and may contribute to the evolution of the terminal phase phenotype.

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TYPE I MAGE, PROGRESSION, AND CYCLING IN MULTIPLE MYELOMA

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Introduction. The type I Melanoma Antigen GEne (MAGE) proteins CT7 (MAGE-C1) and MAGE-A3 were detected in >75% of primary myeloma specimens by both RT-PCR and immunohistochemistry (IHC). Higher levels of MAGE protein expression had a positive correlation with higher percentages of proliferating cells. These findings lead us to investigate the hypothesis that CT7 and MAGE-A3 may play direct roles in cell cycle dysregulation and disease progression. *Methods*. Bone marrow biopsies from 46 newly-diagnosed, symptomatic patients prior to treatment and 35 relapsed myeloma patients were evaluated by IHC for MAGE expression and for proliferation by dual staining for Ki-67 and CD138. The relation between type I MAGE expression and cell cycling was examined by inducing G1 arrest in myeloma cell lines (ARP-1 and KMS-11) with PD0332991, a potent, reversible inhibitor of cyclin-dependent kinases (CDK) 4/6. Proliferation was analyzed by bromodeoxyuridine (BrdU) incorporation and total DNA staining. Expression of Ki-67, CT7 and MAGE-A3 was evaluated by quantitative RT-PCR. *Results*. The proliferating fraction of myeloma cells was significantly higher in relapsed patients (19.0±3.5%) compared to newly-diagnosed (6.9±1.3%, p<0.0002). Protein expression of CT7 was similar in newly-diagnosed and relapsed patients (76.0% new, 77.1% relapsed). In contrast, MAGE-A3 was detected in a significantly greater percentage of relapsed patients (35.6% new, 77.1% relapsed, p=0.0003) demonstrating a positive correlation with disease progression *in vivo*. *in vitro*, G1 arrest of cell lines with PD0332991 resulted in 50-80% decreased BrdU incorporation, 70-80% decreased Ki-67 mRNA expression, and lower relative expression of CT7 and MAGE-A3 mRNA. Release from G1 arrest resulted in increased proliferation and increased relative expression of CT7 and MAGE-A3, suggesting specific interaction with the early G1 checkpoint regulated by CDK 4/6 activity. *Conclusions*. Expression of CT7 appeared to be an early event in the natural history of myeloma, whereas activation of MAGE-A3 expression correlated with disease progression. CT7 and MAGE-A3 expression was associated with abnormal proliferation *in vitro* and *in vivo*, suggesting a role in the cell cycle dysregulation characteristic of multiple myeloma. These results support the further investigation of type I mage as novel therapeutic targets in this disease.

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CYSTATIN-C: AN EARLY MARKER OF RENAL IMPAIRMENT AND AN INDEPENDENT PREDICTIVE FACTOR FOR SURVIVAL IN MULTIPLE MYELOMA. REDUCTION POST BORTEZOMIB THERAPY

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Introduction. Renal impairment is a common complication of multiple myeloma (MM). Standard assessment of kidney function in MM includes serum creatinine and creatinine clearance (Ccr) that probably underestimate the prevalence of renal impairment. Cystatin-C (Cys-C) is a cysteine-proteinase inhibitor, which participates in the intracellular protein catabolism. It is freely filtered in the glomeruli and totally reabsorbed in the proximal tubular cells; therefore, it is a perfect endogenous marker of GFR. The aim of this study was to evaluate the serum levels of Cys-C in MM and explore possible correlations with clinical data, including survival. *Patients and Methods.* We studied 127 newly-diagnosed symptomatic MM patients, 28 relapsed patients pre- and post-bortezomib therapy, and 15 healthy controls. Serum Cys-C was determined by particle enhanced immunonephelometry (Dade Behring, Liederbach, Germany). *Results*. In newly-diagnosed MM patients, serum Cys-C was increased compared with controls [median (range) 1.01 mg/L (0.24-5.61 mg/L) vs. 0.7 mg/L (0.59-0.95 mg/L); p<0.0001]. Seventy-one patients (55.9%) had higher Cys-C levels than the upper normal limit of 0.95 mg/L, while only 31 (24.4%) had elevated serum creatinine. Cys-C showed strong correlations with b2-microglobulin (r=0.675, p<0.0001), creatinine (r=0.695, p<0.0001), Ccr (r=-0.531, p<0.0001) and urea (r=0.509, p<0.0001), and weaker correlations with albumin (r=-0.273, p=0.002), hemoglobin (r=-0.312, p=0.001), LDH (r=0.206, p=0.022) and ferritin (r=0.277, p=0.002). Patients with ISS stage 3 had increased median Cys-C (2.1 mg/L) compared with stage 1 (0.95 mg/L; p<0.0001) and stage 2 patients (1.01 mg/L; p<0.0001). The median survival of all patients was 48 months and the median follow-up period was 17 months. The univariate analysis showed that Cys-C, β2-microglobulin, LDH, Hb, Ccr, and ISS stage predicted for survival. The median survival for patients with normal Cys-C levels (≤0.95 mg/L) has not been reached yet, while in patients with high Cys-C (>0.95 mg/L) the median survival was 34 months (95% CI 20.54-47.46; p=0.002). The multivariate analysis revealed that only Cys-C (p=0.03) and LDH (p<0.001) had independent prognostic significance. Patients with both high levels of Cys-C and LDH (>240 IU/L) had a median survival of 33 months (95% CI 22.32-43.68), while the median survival of patients with normal Cys-C and LDH has not been reached yet (p<0.001). Patients with relapsed myeloma had increased median Cys-C (1.36 mg/L) compared with controls (p<0.0001) and newly-diagnosed patients (p<0.01). Bortezomib therapy produced a significant reduction of Cys-C levels (median: 1.0 mg/L, p=0.008). Responders had a greater reduction than non-responders (p=0.01). *Conclusion*. Cys-C is an early marker of renal impairment which predicts independently for survival in MM. Bortezomib monotherapy seems to reduce Cys-C levels in responders.

PO-228

REGULATORY NETWORK IN THE BONE MARROW OF MULTIPLE MYELOMA

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The aim of study was to analyze prognostic significance of different tumor-host interactions in the bone marrow (BM) of multiple myeloma (MM) patients (pts) by immunohistochemical markers of sensitivity to

the IL-6; adhesion molecules; osteoclastogenesis; and angiogenesis. *Materials and Methods.* Sixty newly diagnosed MM pts (33 male/27 female pts, mean age 60 years, range 35-75) were distributed according to the clinical stage (CS, Salmon&Durie) as: I 8pts, II 22pts, III 30pts. IgG myeloma was diagnosed in 35pts; IgA in 12pts; light chains in 12pts. Regarding ISS score, the group included: ISS1 18pts, ISS2 13pts, ISS3 29pts. All patients were treated with conventional chemotherapy. All samples of BM biopsies were analyzed for the immunohistochemical expression of gp 130, VCAM, OPG and RANKL. The intensity of these staining was graded as weak (0-30% cells), moderate (31-60% cells), and strong (>60% cells). Analyzing the microvessel density (MVD), BM vessels were visualized by immunohistochemical staining for CD34. The number of vessels per 400x high power field (HPF) was counted in the area of the most dense vascularization. *Results*. The expression of gp130 was higher in III vs. I CS (32 vs.15%, p<0,05). High level of the expression of the VCAM indicated a significantly shorter overall survival (36 vs. 18m, log rank, p<0,001). MVD was significantly higher in III vs. I CS (15 vs.7,5/ x400 field, p<0,001); and in ISS3 vs. ISS1 (17,5 vs. 9,7/ x400 field, p<0,05). The expression of FGFR-3 was found significantly higher in III vs. I CS (47,5 vs. 25%, p<0,05); and in ISS3 vs. ISS1 (60 vs. 22,5%, p< 0,001). Significantly stronger expression of RANKL was detected in III vs. I CS (67,5 vs. 38,5%, p<0,05), and in pts with ISS3 vs. ISS1 (55 vs. 38,5%, p<0,05). This correlated with low expression of OPG in III CS (Me 27,5%, range 10-40%), and ISS3 (Me 20%, range 5-30%). Strong activity of angiogenesis and osteoclastogenesis in III CS indicated significantly shorter overall survival (26 vs. 43,5 m, log rank, p<0,05). *Conclusion*. Sensitivity to the growth cytokines, interaction with adhesion molecules, angiogenesis and osteoclastogenesis are representing important predictive factors in MM.

PO-229

CIRCULATING PROTEASOME LEVELS IN MULTIPLE MYELOMA

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Introduction. The proteasome is a proteolytic complex for intracellular degradation of ubiquitinated proteins which are involved in cell cycle regulation and apoptosis. A constitutively increased proteasome activity has been found in certain types of malignant cells including multiple myeloma (MM). Circulating proteasome levels (cProteasomes) can be measured in serum or plasma samples by ELISA techniques. Preliminary data also suggest that proteasome concentrations in peripheral blood are elevated in patients with malignant diseases and correlates with tumor burden. *Methods.* We developed an ELISA technique to detect circulating 20S proteasome in serum or plasma samples. Since there are only limited data on the circulating proteasome levels in MM and no data on its prognostic value, we examined circulating proteasome levels in a large cohort of 50 controls, 20 individuals with monoclonal gammopathies of undetermined significance (MGUS) and 141 patients with newly diagnosed smouldering (SMM) or active (AMM) MM. The prognostic relevance of cProteasomes was evaluated in previously untreated patients with AMM in a multivariate analysis including established prognostic parameters chromosome 13 deletion, beta2-microglobulin, C-reactive protein and high-dose chemotherapy. In 50 patients the effect of treatment on cProteasomes was analyzed. *Results*. Serum proteasome concentrations were significantly elevated in MM compared to controls (p<0.001), in MM versus MGUS (p=0.03) and in AMM (n=101) versus SMM (n=40) (p<0.001). In patients with AMM, there was a significant (p<0.001) decrease from pre- to post-treatment proteasome concentrations in responders to chemotherapy, but not in nonresponders. Circulating proteasome levels were identified as a prognostic factor for overall survival in patients with AMM in the univariate (log-rank: p<0.001) Kaplan-Meier analysis. Median overall survival was not reached versus 30.2 months (p=0.03) in patients with low versus elevated post-treatment cProteasomes, respectively. In the multivariate Cox proportional hazard regression analysis cProteasomes were the most powerful prognostic factor for overall survival (hazard ratio 4.38) among the parameters deletion 13 and high-dose therapy. *Conclusions*. We demonstrate that increased serum proteasome concentrations correlate with advanced disease, decrease after effective chemotherapy and are an independent prognostic factor in MM.

PO-230

ABERRANT PTEN IN MM MONOCYTES: ROLE IN MM ANGIOGENESIS

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Introduction. Multiple myeloma (MM) is accompanied by enhanced bone marrow angiogenesis. We have recently demonstrated that pleiotrophin, which is highly produced by MM cells, transdifferentiates monocytes into endothelial cells and its expression is inhibited by the tumor suppressor PTEN which prevents angiogenesis. As a result, we hypothesized that PTEN is an important regulator of early angiogenesis by controlling transdifferentiation of monocytes into endothelial cells. *Materials and Methods.* First, we studied the level of PTEN expression in peripheral blood (PB) monocytes from MM patients and healthy controls using immunomagnetic bead CD14-selected PB cells that were analyzed by flow cytometric analysis with anti-PTEN antibodies. Next, we determined whether monocytes can transdifferentiate into endothelial cells using the monocytic cell lines THP-1 and U937 in the presence of pleiotrophin and M-CSF. We also performed similar experiments using transwell plates with MM cells. The cells were analyzed for tube-like structure formation and presence of endothelial cell specific markers using western blot and RT-PCR analyses. *Results*. PTEN was expressed in CD14-purified PB monocytes from healthy controls whereas there was a marked reduction in its expression in MM patients' PB monocytes as analyzed by flow cytometry. RT-PCR and immunoblot analyses revealed that U937 cells expressed PTEN whereas THP-1 monocytes did not. In the presence of M-CSF and pleiotrophin, THP-1 monocytes but not U937 cells transdifferentiated into endothelial cells. Moreover, in the presence of MM cells, only THP-1 but not U937 cells stimulated transdifferentiation of monocytes into endothelial cells that was blocked by anti-pleiotrophin antibody. *Conclusion*. We postulate that early production of tumoral blood vessels in MM results from pleiotrophin that is highly produced by MM cells. This protein in combination with M-CSF induces transdifferentation of monocytes into endothelial cells and this event appears to be controlled by PTEN expression. Therefore, we prepared U937 cells stably expressing wt PTEN, dysfunctional PTEN and PTEN-siRNA, and are producing THP-1 expressing the same constructs in order to confirm that PTEN plays a critical role in this early vasculogenic event that is likely to be a critical event in the development and progression of MM.

PO-231

MONOCLONAL GAMMOPATHY ASSOCIATED WITH GAUCHER DISEASE.REPORT OF 16 CASES

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Introduction. In addition to its common bone and visceral manifestations Gaucher Disease (GD) have been frequently associated with polyclonal and monoclonal gammopathy (MG). Moreover, previous reports have suggested an increased risk of B cell malignancy, specially multiple myeloma (MM) in these patients. The objective of our study was to determine the frequency and presentation of MG in GD patients and the possible effect of enzyme replacement therapy (ERT) on MG. *Material and Methods*. French Observatoire on Gaucher Disease (FROG Study) is a cross-sectionnal epidemiological study on adult GD involving 64 French centers. Standard clinical, biological (including serum protein electrophoresis) and imaging data performed as part of usual management within the 3 previous years were collected at the time of a routine visit. In patients with suspected MG specific additional data were studied : immunofixation, dosage of immunoglobulins, bone marrow aspiration. *Results*. From 05/2005 to 06/2006 one hundred and seven GD (105 GD type 1, 2 GD type 2) were included. Sixteen cases of MG (15%) were observed (10 male, 6 female). Mean age of patients with MG (60.5±9.1 years) was significantly higher (p<10-4) than those without MG (42.5±12.8 years). Thirteen cases (12,2%) were classified as MGUS and 3 (3.8%) as B cell malignancies : 1 Multiple Myeloma, 1 Chronic Lymphocytic Leukaemia, 1 Non Hodgkin Lymphoma. Immunochemical typing revealed 11 IgG, 2 IgM, 1 biclonal (IgG + IgA) and 1 triclonal (IgG + IgA + IgM), 12 kappa and 4 lambda. Among thirteen patients

receiving ERT we observed 7 cases of decrease and 6 cases of stabilisation of MG. Patient with MM was treated with ABMT and achieved a very good partial response. *Conclusion*. Our study confirm the high frequency of MG in GD patients. Moreover, as in general population, increase of frequency of MG seems to be related with age. Risk of B cell malignancy need specific follow up. ERT can eventually reduce the level of MG. However further studies are needed to know if this effect may reduce the risk of incidence of B cell malignancy.

PO-232

MULTIPLE MYELOMA, IGA KAPPA TYPE, WITH THE DEMONSTRATION OF ALPHA HEAVY CHAIN PROTEIN

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Introduction. We report here the unusual association of a monoclonal IgA kappa and alpha heavy chain protein, identified using immunoelectrophoresis while missed by conventional techniques for detecting and classifying monoclonal components. *Material and Method*. A 67 year old woman was referred to a University Hospital for medical advice about a fortuitously discovered monoclonal IgA kappa and Bence Jones proteinuria. Her serum and urines were reassessed for monoclonal gammopathy using capillary zone electrophoresis (Paragon CZE 2000, Beckman Coulter), immunoelectrophoresis (IEP), immunofixation electrophoresis (IFE), capillary immunofixation/subtraction (IF-ES) and when necessary immunoselection. Serum free light chains and immunoglobulins were measured by nephelometric immunoassay. *Results*. With CZE the electropherogram showed two spikes located in the beta 2 fraction and one spike in the slow gamma 1. All these three spikes disappeared using IF-ES with immune sera directed against alpha and kappa chains. IEP with an immune serum directed against light and heavy chains, revealed three arches crossing the gamma arch, with the beta 1 moving one evocating an IgA with a lower molecular weight than normally. IFE showed an IgA kappa band and two other bands reacting exclusively with anti-kappa but not with anti-alpha or anti-free kappa. IEP with monospecific immune sera showed that two arches reacting with anti-alpha reacted also with anti-kappa while the beta 1 moving one did not react with immune serum directed against kappa chains. IEP with monospecific immune serum directed against free light chains showed the presence of free monoclonl kappa light chains. Immunoselection confirmed the presence of an alpha protein devoid of light chains.The quantitation of free light chains confirmed the presence in the serum of free monoclonal kappa chains, with a concentration of 743 mg/L for free kappa and a ratio of 47.3. Bence Jones protein of the kappa type was demonstrated in the urines using IEP. *Conclusions*. Using conventional techniques, the alpha heavy chain protein was not even suspected. Only immunoelectrophoretic techniques were able to demonstrate the coexistence of a complete monoclonal IgA and an alpha heavy chain protein in the serum of a patient with multiple myeloma.

PO-233

VALUE OF APPROPRIATE HISTOLOGY IN FOLLOW-UP OF MYELOMA

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Introduction. The multiple myeloma(MM) guidelines in the United Kingdom do not advocate performing bone marrow trephine biopsy(BMTB) during follow-up. In a recent study, we found that the plasma cell(PC)% in BMTB performed at the time of autologous stem cell transplant(ASCT) strongly correlated with survival. Hence, we undertook this study. *Materials and Methods.* We studied 106 samples to address if BMTB was superior to bone marrow aspiration (BMA) in documenting presence of disease and its volume at follow-up in cases of MM. The PC% on BMTB had been estimated on CD138 immunostain. Furthermore, BMTBs had been immunostained for CD56, cyclin D1, light chains and epithelial membrane antigen. The mean PC% in BMA and BMTB was 13.1+2.6% and 31.8+5.8% respectively. While BMTB opinion was based on PC%, cytology and immunohistology, BMA opinion was based on PC% and cytology alone. *Results*. Overall in 92(89%) samples, there was detectable disease at the corresponding time. The positive predictive value of both BMA and BMTB was 100%. However, the negative predictive value for BMTB and BMA was 57% and 22% respectively. Among MM excluding non-secretory cases, the BMTB-PC% showed a significant correlation with paraprotein levels, whereas BMA-PC% did not. *Conclusion*. We strongly recommend performing BMTB and adequately investigating these samples with immunohistochemistry during follow-up of MM.

PO-234

OH-2: A MYELOMA CELL LINE WITHOUT IGH TRANSLOCATION

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Introduction. The human multiple myeloma cell lines (HMCL) used in most labs are only representing the group of patients that have an IGH translocation. Since more than 40% of patients do not harbour an IGH translocation, it is an aim to characterize and even make new HMCLs without this aberration. *Materials and Methods.* Metaphase spreads of OH-2 were hybridized with split rearrangement probes for the three immunoglobulin loci IGH, IGK and IGL. Array-comparative genomic hybridization (CGH) with 1Mb resolution was used to detect global genomic aberrations and verify diploid status. *Results*. OH-2 is an HMCL that was established in our lab several years ago of the pleural fluid from a myeloma patient in end stage of the disease. The phenotype of OH-2 cells has been extensively studied and presented in several papers. The cells are completely dependent on IL-6 or other growth-promoting cytokines and grow only in medium supplemented with human serum. Metaphase FISH with split probes showed that OH-2 is a cell line without any IGH, IGK or IGL rearrangements. The cell line is hyperdiploid, as shown by array-CGH with triploid chromosome 3, 7, 14, 15 and 21. It has monosomi 13 and expresses cyclin D1, D2 and D3. *Conclusion*. The cell line OH-2 might be a good model HMCL to represent the patient groups without an IgH translocation, but with a hyperdiploid genetic profile. It thereby belongs to the translocation-cyclin D classification group *D1+D2*.

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MYELOMA MICROENVIRONMENT CREATES ABNORMAL APC FUNCTION AND MAY FAVOR GENERATION OF TH17 CELLS

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Multiple myeloma (MM) is associated with significant immune dysfunction. The biological basis of this dysfunction remains ill defined. We have previously observed significantly decreased number of T regulatory (Treg) cells, as measured by Foxp3 expression, in both MGUS and MM compared to normal donors. We have further analyzed elements of bone marrow (BM) microenvironment that may be responsible for dysfunctional Treg cells in MM. Since Treg cells require interaction with antigen presenting cells (APC), we evaluated effects of IL-6 and TGF-β on the ability of APC to help suppressive activity of Treg cells. Prior exposure of mature dendritic cells to IL-6 and TGF-β abrogated Treg cell suppressive activity from 47% inhibition to 23% increase in T cell proliferation (p=0.01). To evaluate the ability of monocyte-derived DCs from multiple myeloma patients to provide adequate help to generate robust T cell responses, we further investigated the ability of purified monocytes from myeloma patients to generate appropriate T cell responses by producing Th1 type of cytokines upon activation. We incubated purified CD14 expressing monocytes with or without LPS and measured production of TNF-α by ELISA. Purified monocytes from normal donors (N=8) produced very little TNF-α and upon activation with LPS, a 13-fold increase was observed. On the other hand, monocytes from myeloma patients (N=4) produced significantly high levels of TNF-α at baseline without any activation, and produced less than a 2-fold increase in the production of TNF-α upon activation with LPS. These abnormal monocytic responses may lead to generation of TH17 cells that are pathogenic in number of immune-related disease states. Therefore, we evaluated the role of myeloma microenvironment-related cytokines, IL6 and TGF-beta on the generation TH17 cells via IL23. We

analyzed the production of IL-23 upon activation of PBMC from myeloma patient in the presence of IL-6 and TGF-β.The PBMCs from myeloma patients produced 2 to 3-folds higher levels of IL-23 in presence of these cytokines compared to their absence suggesting their modulating T cell responses towards TH17 development. The cytokines from MM BM microenvironment may thus be responsible for the observed T cell dysfunction via abnormal APCs and by favoring TH17 cells via IL-23 production. These cytokines thus may be targets to modulate immune responses in myeloma to enhance immune function and devise effective vaccination strategies in the future.

PO-236

SIL-6R EFFECTED ON THE GROWTH OF MM CELLS VIA INDUCTION OF ACTIVATION OF JAK/STATS AND INCREMENT OF SIL-6R REDUCED THE APOPTOSIS OF U266 CELLS BY ANTICANCER DRUGS

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Introduction. Interleukin-6 (IL-6) plays a pivotal role in the pathogenesis of multiple myeloma (MM). Elevated sIL-6R levels have been documented in MM patients indicating that its production is coordinated as part of a disease progression. We also found that high levels of both IL-6 and sIL-6R were detected in patients who have no complete remission (CR). Thus, the role of sIL-6R reported as prognostic marker as well as gave us clinical implications in MM patients. However, mechanisms by which complex of sIL-6R/IL-6R on MM cells affected the growth of MM cells was still remained unknown. *Materials and Methods.* To investigate the effect of sIL-6R on MM patients, western blot analysis, EMSA, ELISA was performed using U266 Human MM cell line. To further examine the role of sIL-6R on MM patients treated with velcade, U266 cells reduced the production of sIL-6R using siRNA of sIL-6R were used. *Results*. sIL-6R did increased the phosphorylated STAT-1, -3 and Erk as well as the activity of NFkB. Both the expression of cyclin D1 and c-Myc was also increased. Subsequently, sIL-6R is capable of proliferating MM cells via activation of JAK/STAT pathway. To further examine whether sIL-6R affected the survivals against anti-cancer drugs of MM, the biologic behavior of U266 cells was examined after treating them with various concentration of sIL-6R. Increasing levels of sIL-6R on U266 cells, IL-6 dependent cell, reduced the activity of caspase-3 as well as the formation of cleaved PARP by velcade. Combined treatment of sIL-6R and anti-cancer drugs increased phosphorylation levels of STATs and Erk when compared with anti-cancer drug only. The activity of NFkB was also gradually increased in U266 cells pretreated velcade by adding increment of sIL-6R to conditioned cultured media. Velcade was dramatically effective on U266 cells transfected with siRNA of sIL-6R. In *Conclusion*. sIL-6R effected on the growth of MM cells via activation of JAK/STAT cell signaling as well as protective effects against velcade. We demonstrated that it is essential to consider not only the action of IL-6 itself, but also the effect sIL-6R may have on processes of MM patients.

PO-237

RGS1 REGULATES MYELOMA CELLS TRAFFICKING AND CHEMOTACTIC RESPONSES TO CXCL12

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Background. The exact mechanisms that regulate plasma cells (PC) responsiveness to CXCL12, their homing and egress from the bone marrow (BM) niches are poorly elucidated. PC homing to the BM from germinal centers are known to exhibit high chemotactic responses to CXCL12 despite no changes in their CXCR4 expression, suggesting a possible downstream regulation of CXCL12/CXCR4 signaling pathway. RGS1 is a GTPase that is known to be involved in B cell trafficking and their responsiveness to CXCL12. We speculated that retention or egress of PC from the BM is possibly regulated by RGS1 and that its up-regulation would result in extramedullary disease and progression to plasma cell leukemia. Methods and *Results*. We first examined CXCR4 and RGS1 expression in three human cell lines (8226, OPM2 and U266) and in primary myeloma cells isolated from a patient with plasma cell leukemia (PCL1). While similar levels of CXCR4 were detected in 8226, OPM2, U266 and PCL1 cells; RGS1 mRNA levels were significantly higher in PCL1 cells compared to 8226, U266 and OPM2. In order to investigate whether RGS1 was involved in CXCL12 derived PC migration, we cloned RGS1 cDNA into pDEST47-GFP vector and stably transfected it into 8226, U266 and OPM2 cells. While RGS1 overexpression did not affect myeloma cells survival, proliferation or CXCR4 expression, it clearly reduced their responsiveness to CXCL12 inhibiting their migration (transwell migration assay) and adhesion (fibronectin adhesion assay). We are currently investigating, *in vivo*, in NOD/SCID mice the BM homing of 8226/RGS1+ compared to cells transfected with control vectors. Finally we measured mRNA RGS1 expression in primary myeloma cells ($CD138^+$) isolated from the BM of medullary phase myeloma patients (n=4) and from plasma cell leukemia patients (n=4). With the exclusion of one outlier, the median expression of RGS1 mRNA was nearly 2 folds higher in plasma cell leukemia compared to medullary phase myeloma. *Conclusion*. Our work provides evidence that RGS1 negatively regulates myeloma cells responsiveness to CXCL12 gradients and supports a key role for RGS1 in MM cells egress from the BM mircoenvironment. Further studies are underway to fully understand the regulation of RGS1 expression in MM.

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C/EBPB REGULATES TRANSCRIPTION FACTORS BY CONTROLLING IRF-4

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Introduction. The development and maturation of plasma cells is dictated by multiple interacting transcription factors (TFs). C/EBPb is a TF regulated by IL-6 and has profound effects on regulation of growth, survival and differentiation. Mice deficient in C/EBPb show impaired generation of B lymphocytes suggesting that C/EBPb plays an important role in B lymphopoiesis. In this study we delineated the effect of C/EBPb on transcription factors critical for myeloma cell proliferation by over-expressing and silencing C/EBPb in myeloma cells. *Methods.* MM.1S cells were transiently transfected with C/EBPb or GFP using the Nucleofactor kit V solution (Amaxa Biosystems, Cologne, Germany). Following transfection, cells were subjected to western analysis. *Results*. Silencing of C/EBPb by transfection of a truncated form as well as drug induced down-regulation of C/EBPb in MM cell lines (MM1.S) resulted in growth arrest and apoptosis of MM cell lines. This was accompanied by a complete down-regulation of the anti-apoptotic BCL-2 molecule. Further, silencing of C/EBPb completely inhibited IRF-4 expression. In contrast, over-expression of C/EBPb increased protein levels of IRF-4 suggesting that IRF-4 is under direct control of C/EBPb. IRF-4, which is over-expressed in MM, is an essential transcription factor for the generation of plasma cells by regulating transcription factors like Blimp-1 and PAX-5, which are critical for plasma cell differentiation. Our studies showed that down-regulation of IRF-4 resulted in a complete abrogation of Blimp-1 and PAX-5 suggesting that the expression of these factors is completely C/EBPb/IRF-4 dependent. *Conclusions*. Our data indicate that C/EBPb is an important key regulator for survival and growth of MM cells. We show for the first time that C/EBPb is a critical regulator upstream of IRF-4. Down-regulation of the C/EBPb/IRF-4 complex results in complete disruption of the network of transcription factors necessary for MM growth and survival. Targeting C/EBPb may provide a novel therapeutic approach in the treatment of multiple myeloma.

PO-239

GR ALTERNATIVE SPLICING AND GC RESISTANCE IN MM

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Glucocorticoid (GC) resistance remains a major problem in treatment of Multiple Myeloma (MM). Response to glucocorticoid therapy is directly related to expression of glucocorticoid receptor (GR). In GC

resistant myeloma cell lines, we have previously identified a truncated GR. Termed GR-P, it occurs due to a failure to splice at the junction of exon 7 and intron G. To assess the clinical significance and prognostic value of GR-P, we have developed a real-time PCR assay to quantify the amount of wild type (GRα) and GR-P mRNA in myeloma patient samples from the ECOG study E1A00 protocol. There is wide range of expression of GRα and GR-P in the samples analyzed to date. The current sample size has not produced a statistically significant correlation between GR isoform phenotype and GC responsiveness and more samples will be accrued for these studies. In laboratory correlates to the clinical studies, we have examined the effect of GC treatments on GR isoform expression in the MM.1S myeloma cell line. Utilizing real time PCR, in a time course of GC treatment, we observed the expression of GRα decrease with time while GR-P expression increases. These changes do not appear to be a result of apoptosis, but are rather a more immediate effect of the GC exposure, indicating that the GR isoform levels can be modulated acutely by GCs. To analyze the mechanisms of splicing which lead to GR-P expression, we have constructed a mini-gene of the region surrounding the splicing site of exon7/intronG. Transfection of the mini-gene into myeloma cell lines followed by and reverse transcriptase PCR will enable us to determine the splicing patterns that occur in cells with different GC response phenotypes. In conclusion, we believe that understanding the mechanism of GR-P regulation may be of prognostic and therapeutic value in elucidating GC resistant MM.

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FUNCTIONAL ANALYSIS OF THE STAT3, MAPK AND AKT PATHWAYS IN PRIMARY MYELOMA CELLS: HETEROGENEITY IN TUMOUR CELL SIGNALLING

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Signalling through the IL-6R/STAT3-, MAPK- and PI3K/Akt-pathways has been reported to critically contribute to growth and survival of multiple myeloma (MM) cell lines. Nevertheless, knowledge about their importance in primary tumour cells, and, thus, about their significance for targeted therapeutic intervention, is still limited. We therefore analysed these pathways in more than 20 primary MM samples by intracellular phosphoepitope staining and FACS. The cells were exposed to different activating conditions or inhibitors (Sant7, PD98059, Akti-1/2) whose effects on pathway activity and survival were studied. Specificities of the inhibitor-mediated biological effects were verified through siRNA-knockdown of the presumed targets in cell lines. Phosphorylation of STAT3, Erk1/2 and Akt was enhanced in primary MM cells by either PMA, IL-6/IGF1 or coculture with BMSCs, and efficiently down-regulated by the respective inhibitors. Inhibition of the IL-6R/STAT3 or MAPK pathway had relatively minor effects on the survival of primary MM cells. In contrast about 50% of the samples showed marked cell death after Akti-1/2 treatment, which was not increased through additional blockade of the other pathways. Whereas Akti-1/2-sensitive samples displayed Akt phosphorylation after stimulation with cytokines or BMSCs, resistant ones dit not. This matches observations in cell lines, were only two (MM.1s, MOLP8) of the four lines tested showed inducible Akt activity and displayed apoptosis on Akt1/2 inhibition. Similar results were obtained with Akt isoform-directed siRNA expression constructs. Our observations indicate a prominent role of Akt in the pathogenesis of a subgroup of MM whereas MAPK and STAT3 appear to be less relevant. The subgroup devoid of Akt signalling appears to be largely independent from all of the three pathways investigated here. These findings indicate a marked heterogeneity in tumor cell signalling and are therefore of relevance for the development of future targeted therapies.

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INDOLEAMINE 2,3-DIOXYGENASE (IDO) MAY NOT BE A MAJOR FACTOR FOR TUMOR IMMUNE EVASION IN MULTIPLE MYELOMA

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Introduction. Indoleamine 2,3-dioxygenase (IDO) is a tryptophan catabolising enzyme expressed by several cancers that induces immune tolerance. High IDO expression has been linked with short survival in some cancers, but data on the possible role of IDO are not available yet in multiple myeloma (MM). *Methods.* We used quantitative (Q) PCR to evaluate IDO in CD138[+] BM cells from MM patients (n=17), MM cell lines (n=6) and MM BM stromal cells (SCs) (n=5). *Results*. Low level expression of IDO was found in the CD138+ BM fraction of 17 myeloma patients using Q-PCR (median 0.52 fold compared to normal PBMNC; range, 0.08-15.03). Even in the patient with highest IDO expression, IDO mRNA levels were >100x lower than in DCs or HeLa cells stimulated with IFN-γ. Similarly, 6 myeloma cell lines had low IDO expression by qPCR (median 0.04; range 0.001- 0.68). Stimulation with IFN-γ led to an upregulation of IDO in 2 of these cell lines, as shown by qPCR and Western blot, but again with expression levels being >100x lower than in activated DCs. Analysis of the tryptophan/kynurenin ratio in cell line culture supernatants furthermore revealed little sign of enzyme activity, even after stimulation. IDO expression could not be induced in the IDO- cell lines. Interestingly, when comparing CD138+ and CD138- cell fractions from BM of 3 myeloma patients, a detectable, although weak, PCR band was demonstrated in the CD138- fraction only. By conventional PCR using purified cell subsets from the CD138- fraction, a weak band was amplified from monocytes and T-cells. In cultured BM SCs from myeloma patients, IDO expression was low at baseline, but could be upregulated by interferon-γ. IDO proved functional in this setting with reversal of the tryptophan/kynurenin ratio after 48 hours of interferon-γ. BM-derived SCs from myeloma patients thus seem to have similar characteristics with regard to IDO expression as SCs from normal donors and do not appear to be a major source of IDO in myeloma when examined in isolation. *Conclusion*. IDO is weakly expressed in myeloma plasma and stromal cells and may not contribute to immune paralysis in this disease.

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CYTOPLASMIC LOCALIZATION OF NUCLEAR FACTOR-KAPPA B IN MULTIPLE MYELOMA PLASMACELLS

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Nuclear factor-kappa B (NF-κB) is multifunctional transcription factor that regulates different signal transduction pathways such as cell survival and proliferation. A number of tumors display activated NF-κB, which contributes to promote cancer cell growth and resistance to chemotherapeutic drugs. NF-κB has been shown to be constitutively active in multiple myeloma (MM) cells, resulting in increased expression of Bcl-xL and IL-6. In addition NF-κB has an important role in regulation of IL-6 transcription in bone marrow stromal cells (BMSCs). Here, we analyzed the localization of NF-κB in bone marrow MM cells from 30 patients with MM at presentation or in relapse, in BMSCs from two MM patients as well as in two myeloma cell lines (XG1, RPMI 8226). NF-κB localization was evaluated by either immunohistochemistry, immunofluorescence or immunoblot using a monoclonal mouse anti-human p65 (Rel A) antibody that recognizes the p65 subunit. Surprisingly, nuclear localization of NF-κB was (weakly) detected in BMSC samples and in only one MM sample from a refractory MM patient, while the other samples, including the MM cell lines, exclusively express the cytoplasmic (inactive) form of NF-κB. We next analyzed the sensitivity of MM primary cells to different doses of the proteasome inhibitor Bortezomib (from 1 nanomolar to 10 micromolar), which is known to antagonizes NF-κB activity. We found a consistent dose- and time-dependent antitumor activity against both chemoresistant and chemosensitive myeloma cells in all the samples analyzed, independently of NF-κB localization. These results indicate that Bortezomib is active in MM cells regardless the NF-κB localization and suggest the existence of other molecular targets of proteasome inhibitors in MM.

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AN *IN VIVO* MODEL TO PROBE THE CELLULAR ORIGIN OF MULTIPLE MYELOMA

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The nature of the cell of origin of multiple myeloma (MM) remains enigmatic, however it is of crucial importance in the quest for a cure. To investigate the clonal origin of MM as well as the genetic requirements for pathogenesis, we have created a novel flexible mouse model system

that enables the delivery of stochastic, sequential, somatic mutations to precisely defined plasma cell precursors *in vivo*. To this end, we have used BAC transgenic technology to express two distinct types of avian leukosis virus (ALV) receptors, TVA and TVB, in the expanding centroblast of the dark zone and the committed plasmablast of the light zone of germinal centers, respectively. Mammalian tissues are refractory to transduction by retroviruses of the ALV family unless they ectopically express the cognate avian-derived receptors. Thus, we have expressed TVA driven by regulatory elements of A-myb, a transcription factor expressed in dividing blasts of the dark zone. TVB was expressed in the context of Blimp-1, a master regulator of plasma cell differentiation. Transgenic mice express each receptor in lymphoid tissues following immunization with T-dependent antigens. Whereas TVA expression is confined to dividing follicular B cells, TVB-expressing cells appear in the light zone of germinal centers but also in extrafollicular collections of plasmacytic cells as well as mature plasma cells of the bone marrow. At appropriate timepoints in the course of an immune response, a variety of genetic lesions are introduced via each receptor. Viral vectors can be engineered to carry dominant oncogenes or various inactivators of tumor suppressor genes. Receptors remain expressed following gene transduction, thus enabling the introduction of secondary lesions. Using this approach, we are attempting to recapitulate the early natural history of MM generation, using key lesions (eg. cyclin D1 overexpression) in the relevant cellular and physiological milieu.

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GENETIC INSTABILITY IN MULTIPLE MYELOMA: AN INVESTIGATION OF RHAMM-MEDIATED MITOTIC ABNORMALITIES IN MYELOMAGENESIS

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RHAMM is a multifunctional protein involved in cell motility, cytoskeletal dynamics, and most recently implicated in centrosome and mitotic spindle stability (Mol Biol Cell vol. 14, 2262-2276). The latter studies have revealed that RHAMM dysregulation correlates with mitotic abnormalities that may give rise to aneuploidy, and ultimately to transformation. In multiple myeloma (MM), RHAMM abnormalities in B and plasma cells correlate with reduced survival and thus may have a role in the genetic instability that is a hallmark of this disease. In this study we have developed tetracycline-inducible cell lines expressing full-length RHAMM (RFL). With this system, RHAMM expression can be finely controlled, offering advantages over non-inducible systems which have failed to generate clones expressing significant levels of RHAMM. We observed induced RFL by immunofluorescence as early as 5 hours post-induction, peaking at 24 hours. RFL was observed by confocal microscopy in association with centrosomes, mitotic spindles, the cytoskeleton, the nuclear and cell membrane. At higher levels of induction, RFL accumulates at the mitotic spindle: the majority of these spindles have a normal bipolar arrangement, however many are grossly enlarged and many appear asymmetric. In addition, a trend towards multipolar spindles in induced transfectants compared to non-induced controls was observed. Highly induced RFL-GFP cells visualized by live cell immunofluorescence microscopy arrest in mitosis and subsequently undergo apoptosis. By 72 hours less than one percent of these cells remain alive. The nature of the mitotic delay in the induced cells is being investigated, as is the extent to which cells with multipolar spindles give rise to the surviving population. At lower RFL induction levels cells survive up to 4 days. A detailed characterization of mitotic abnormalities in these cells in ongoing. RFL levels in these cells may be more reflective of MM. The surviving fraction is being assessed for genetic instability by cytogenetics and FISH. We speculate that oncogenesis in MM involves overexpression of RHAMM, and may additionally include upregulation of genes that abrogate its toxic effects, thereby promoting rescue of RFL overexpressing cells and enhancing survival of mitotically abnormal clonal variants having increasingly aggressive characteristics.

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A NOVEL REAL-TIME *IN VIVO* HOMING MODEL OF MULTIPLE MYELOMA

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Background. Multiple myeloma (MM) is characterized by widespread involvement of the bone marrow (BM) at diagnosis, implying a continuous (re)circulation of the MM cells in the peripheral blood and (re)entrance into the BM. The normal process of B cell homing is regulated by cytokines and receptors such as SDF-1, CXCR4, VLA4, LFA-1, VCAM-1 and ICAM-1. In order to better understand the role of homing in MM, we developed an *in vivo* model which allows the continuous real-time imaging of MM cells as they home and adhere to the BM, as well as quantifying the numbers of cells in the circulation. *Methods*. MM.1S were fluorescently labeled by incubation with the dialkylcarbocyanine membrane dye DiD (Molecular Probes). Cells were i.v injected in Balb/c mice. Appropriate arterioles in the ear pinnae of the mice were chosen for obtaining measurements, and the fluorescence signal on the MM cells was excited as the labeled cells passed through a slit of light focused across the vessel. Cell counts were obtained every 5 min from the time of injection. MM cell homing to bone marrow vasculature of the skull was analyzed using fluorescence confocal microscopy. Imaging duration was 1-3 hours per session. DiD was excited with a 635 nm diode laser. High-resolution images with cellular details were obtained at depths of up to 250 um from the surface of the skull. Images from several depths were obtained and z-stacking was performed to merge the images. Quantitative evaluation was made by dividing the bone marrow into pre-determined quadrants (areas 1 to 4) and counting numbers of fluorescent cells per field. To demonstrate that that this new model identifies changes in homing of MM cells, we used the CXCR4 inhibitor AMD3100 and anti-VLA-4 antibody to inhibit homing to the BM. MM cells were pre-incubated with AMD3100 (50 uM overnight, Sigma, MO) or anti-VLA-4 antibody (1hr incubation with 10 ug/mL, BD Pharmingen, CA) or control PBS under the same conditions. *In vivo* flow cytometry and confocal imaging were then performed on control and AMD3100 treated mice. *Results*. The number of cells in the control group decreased dramatically (86% decrease) after 1 hour indicating homing, whereas there was only a 47% reduction in the cells at 1 hour in the AMD3100 treated cohort, (p=0.002). Similarly, we demonstrated that the number of cells present in the perivascular bone marrow niches of the skull was significantly higher in the control mice as compared to the AMD3100-treated group at 1 hour after injection. The mean cell count in the AMD3100 treated mice decreased to 38% as compared to controls, p=0.01. Likewise, the use of anti-VLA-4 antibody demonstrated significant retention of MM cells in the circulation 1 hr after injection (4% reduction in circulating cell count vs 82% for control). *Conclusion*. We describe a new model that detects *in vivo* real-time homing of MM cells from the peripheral circulation into BM niches, which can be used to study the trafficking of MM cells into and out of the BM, as well as the effect of novel agents on this dynamic process.

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ABILITY OF CAPILLARY ZONE ELECTROPHORESIS IN DETECTING MONOCLONAL LIGHT CHAINS

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Introduction. To precise the ability of capillary zone electrophoresis (CZE) in detecting monoclonal light chains in serum, we reviewed the files of patients presenting with monoclonal light chains as M-components. We report here the results of a study combining mainly the characteristics of the capillary zone electrophoretic profile, the shape and location of the light chains precipitates in immunoelectrophoresis and the quantitation of free light chains (FLC), at the time of diagnosis. *Methods*.S 145 patients presented with monoclonal light chains using CZE (Paragon CZE 2000°, Beckman Coulter), immunoelectrophoresis (IEP) with antibodies against FLC (Helena) and a semi-automated immunofixation-electrophoresis technique (SPIFE 2000, Helena). Immunoglobulins and FLC were quantified by immuno-nephelometry (BN2, Dade Behring) with reagents from Dade Behring and The Binding Site respectively. Patients were then classified according to the aspect of the beta and / or gamma zone of the electrophoretic profile and the location and shape of the precipitates with IEP. *Results*. For 69 samples, the electrophoretic profiles presented with characteristics liable to be related to a monoclonal component and thus leading to further investigations. On 76 electrophoretic profiles (52.4%) no abnormalities could bee seen in the beta and / or gamma zone. In 25 cases (33%), IEP with immune antibodies against free kappa or lambda light chains demonstrated the presence of a gamma located arc with a Gaussian shape typical of a monoclonal component. The corresponding concentrations of FLC were below 500 mg/L, thus below the detection limit of monoclonal IgG with CZE.

In 28 cases (37%), IEP demonstrated beta located arcs (10 in beta 1, 14 in beta 2 and 4 double-humped arcs) a not easy region for monoclonal components to be detected. In 6 samples, IEP demonstrated alpha located arcs and eventually in 17 cases multiple-humped arcs stretching from alpha 1 to beta 2 or gamma zone. *Conclusion*. The results of this study demonstrate that the low sensitivity of CZE in detecting monoclonal free light chains is either due to light chains concentration well below the limit of detection of M-components or to location in alpha or beta zones where M-components are not easily detected.

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PLASMA CELL TUMORS IN TRANSGENIC MICE

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Aims. Accurate mouse models of human plasma cell tumors such as multiple myeloma (MM) are needed to study events involved in the initiation and progression of these neoplasms, identify genes that confer tumor susceptibility, and test intervention strategies that might lead to a better clinical outcome. The cellular oncoprotein MYC, the plasma cell growth, differentiation and survival cytokine IL-6, and death suppressors of the BCL2 family are key pathogenetic factors in human MM, suggesting that the transgenic expression of these factors in plasma cells in mice generates pre-clinical model systems of plasma cell neoplasia that may be of great relevance for human beings. *Materials and Methods.* We have studied plasma cell tumor formation in the following transgenic mouse strains: (i) BALB/c.H2-Ld-IL-6 mice bearing a widely expressed human IL-6 transgene (originally developed by T. Kishimoto, Osaka University; backcrossed onto BALB/c by M. Potter, NCI, Bethesda, MD); (ii) BALB/c mice carrying the EiSV-Bcl-2-22 transgene (developed by A. Harris and J. Adams, WEHI, Melbourne; backcrossed onto BALB/c by S. Silva, Karolinska Institute, Stockholm); (iii) C57BL/6 mice bearing a human MYC gene driven by human Igλ or Igλ enhancers (λ-MYC and κ-MYC mice); (iv) gene-insertion mice that harbor a His6-tagged mouse Myc gene in three different locations of the mouse Ig heavy-chain locus (referred to as *iMyc* mice); (v) and iMyc mice carrying a 3'KE-Bcl-XL transgene (developed by B. Van Ness, University of Minnesota). *Results*. All transgenic mouse strains exhibit *spontaneous* development of plasma cell tumors in extraosseous lymphoid tissues. Intercrossing two different transgenes; *e.g.*, Myc + IL-6 or Myc + Bcl-XL, results in a predictable acceleration of plasma cell neoplasia. Bone marrow infiltration with malignant plasma cells, osteolytic lesions and pathological fractures are frequently observed in double transgenic mice; *e.g.*, in iMyc/Bcl-XL mice, but are less common in their single transgenic counterparts. Plasma cell leukemia is a consistent feature of mice with advanced tumors. *Conclusions*. Our findings demonstrate that genes involved in human myelomagenesis are also involved in plasma cell neoplasia in mice. Future studies need to address the principal shortcoming of the presently available mouse models: the failure to recapitulate the primary bone marrow manifestation of tumor growth that is the hallmark of human MM.

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EXPANSION AND CHARACTERIZATION OF BONE MARROW MESENCHYMAL STEM CELLS IN MULTIPLE MYELOMA PATIENTS

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Introduction. Multiple Myeloma (MM) is a B-cell malignancy characterized by the proliferation of clonal plasma cells within the bone marrow (BM) and presenting osteolytic lesions in the skeleton of 80% of patients. This malignancy is characterized by complex karyotypic aberrancies and MM cells strongly interact with the microenvironment regulating their homing, proliferation and survival. *Objective.* Since BM mesenchymal stem cells (MSC) are key components of the microenvironment, we have evaluated the biological characteristics of MM-MSC. To improve the bone formation, mesenchymal stem cells (MSC) transplant should be a good cell therapy in complete remission patients. *Methods.* BM were obtained from 9 healthy donors and 24 MM Patients, 14 females and 10 males with a medium age of 61, 5 years and 60,5 years respectively. MSC derived from these two groups were tested for their phenotype, their expansion, their capacity to support the hematopoiesis and finally their ability to differentiate into adipocytes and osteocytes. *Results*. Using the CFU-F assay to determine the presence of MSC in the BM sample, we observed a high number of MSC in BM-MM in comparison with healthy BM (34,7±12,8 versus 6,3±2,3). A high number of CFU-F seems associated with an increase of CRP and leucopenia and correlate with the tumor mass. MM-MSC have a higher proliferative capacity during the primoculture of 13,4±0,9 days versus 20,1±0,7 days for normal MSC. After three passages, the MM-MSC were able to support the hematopoiesis and expressed CD105 (SH2), CD73 (SH3), CD44, CD166 and were negative for CD34, CD45, CD38, CD138 and HLA-DR. These cells were also able to differentiate into adipocytes and osteocytes demonstrated by Oil Red O or the Alizarin Red staining and by the production of calcium deposit, alkaline phosphatase (ALP) and osteopontin (OPN). We also demonstrate that a sufficient number of MSC of 1.106 MSC/kg could be easily obtained after three passages and be grafted. At this time karyotypic abnormalities were not observed on the expanded MM-MSC. *Conclusions*. BM derived from MM patients contains a higher proportion of MSC with a great proliferative capacity probably due to the presence of growth factors and cytokines produced by the plasma cells. Their cytokine profile and their immuno-modulatory potential are now in progress. No influence of previous chemotherapy on the presence and characteristics of MSC were observed. The MM-MSC have similar characteristic than healthy MSC including phenotype, hematopoiesis support and differentiation potential. Thus, autologous transplant of MM-MSC seems to be considered.

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HGF PROMOTES MIGRATION OF MYELOMA CELLS

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Introduction. Multiple myeloma (MM) cells home to the bone marrow where they grow, proliferate and produce osteoclast-activating factors. MM cells disseminate throughout the skeleton. Migration is not a passive event, but is regulated by stimuli from the environment. We wanted to investigate the impact of hepatocyte growth factor (HGF) on MM cell migration. We tested the effect of HGF alone, and in combination with stromal cell-derived factor (SDF)-1alpha. We also tested HGF in combination with specific blocking agents to find signalling mediators downstream of HGF. *Matherials and Methods.* We studied cell migration by use of INA-6 cells in a Transwell two-chamber assay, confocal- and transmission microscopy and actin measurement by flow cytometry. HGF produced by mesenchymal stem cells (MSC) was detected by PCR and Western blot. *Results*. HGF in the lower chamber of the Transwell increased migration of INA-6 cells 5-fold compared to controls, whereas an equal concentration of HGF in both chambers or HGF in the upper well only, did not enhance migration significantly. Cell migration was thus directed towards an HGF concentration gradient. INA-6 cells also migrated towards an increasing concentration of SDF-1alpha. A combination of HGF and SDF-1alpha in the lower chamber of the Transwell gave a synergistic effect on cell migration. Interestingly, when SDF-1alpha was added to the lower well, HGF gave a strong increase in migration irrespective of whether it was added to the lower, the upper or to both wells.We demonstrated that HGF-stimulated migration involved VLA-4 integrin, and was PI3K, MEK and NF- kappa B dependent, but did not involve G-proteins. We also showed that MSC produced HGF that is bioactive for MM cell migration. *Conclusion*. Our investigation points to HGF as a potent attractant for MM cells, equally important as SDF-1alpha. Our study suggests that novel therapies targeting the HGF/c-Met signalling pathway may inhibit MM cell migration that is associated with progressive MM.

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ANGIOGENESIS IN MULTIPLE MYELOMA: ANGIOGENIC SWITCH AND RELEXION OF PLASMA CELL NUMBER?

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Introduction and Aims. Angiogenesis is a hallmark of active multiple myeloma. However, two etiologic hypotheses have been proposed: (1) an angiogenic switch (i.e. differential or *de novo* expression of pro/antiangiogenic genes in MM), and, alternatively (2) an effect of increased plasma cell number. Here, we investigate the angiogenic signature of multiple myeloma cells (MMC), normal bone marrow plasma cells (BMPC), and polyclonal plasmoblasts (PPC). PATIENTS AND *Methods*. Samples from 128 newly diagnosed MM-patients (65 training (TG)/63 independent validation group (VG)), 14 normal donors (ND), 12 plasmablast-samples were included. Bone marrow aspirates were CD138-purified by activated magnetic cell sorting and FACS-sorting. RNA was *in vitro* transcribed and hybridised to Affymetrix U133 GeneChips. We focused our expression analysis on a comprehensive set of 89 pro- and 44 antiangiogenic genes (141 and 64 probests) known to be involved in regulation of tumor angiogenesis. After gcrma-normalization of data, Empirical Bayes (EB), PANP and PAM algorithms were used. *p*-Values were adjusted using the Benjamini-Hochberg method (Bioconductor). *Results*. (i) A distinct set of proangiogenic genes was expressed by BMPC as well as MMC (*e.g.* VEGFA, HIG1). (ii) Using EB, only one pro-angiogenic gene (hepatocyte growth factor, HGF) was found to be significantly overexpressed in MMC compared to BMPC. HGF is not expressed in BMPC. (iii) A distinct set of antiangiogenic gene (*e.g.* TIMP2) is expressed in BMPC. (iv) Several expressed antiangiogenic genes (*e.g.* TIMP2, PF4) are downregulated in MMC compared with BMPC. *Conclusion*. Normal plasma cells express pro- and antiangiogenic genes. MMC display a specific additional angiogenic signature based on aberrant expression of angiogenic (*e.g.* HGF) and downmodulation of antiangiogenic genes. Similarities in the angiogenic profile of MMCs of different stages of disease indicate the acquisition of an angiogenic phenotype early in the development of the disease. Our data support a dual mechanism of angiogenesis based on the *angiogenic switch* as well as a higher production of angiogenic factors by an increased number of plasma cells.

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HEPATOCYTE GROWTH FACTOR (HGF) PLASMA LEVEL AND THROMBOSPONDIN BONE MARROW PLASMA LEVEL ARE OPTIMAL CANDIDATES FOR ANGIOGENESIS MONITORING IN MM PATIENTS UNDERGOING AUTOLOGOUS TRANSPLANTATION

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Introduction. Angiogenesis is involved in the development and progression of multiple myeloma (MM). It has already been shown that angiogenesis as assessed by micro-vascular density in the bone marrow of MM patients plays a significant role in the prognosis of MM. Vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and basic fibroblast growth factor (bFGF) plays a key roles as angiogenesis activators in MM. Role of angiogenesis inhibitors in MM remains very unclear. The most important inhibitors seem to be thrombospondin, endostatin and angiostatin. The aim of this study was to evaluate the role of VEGF, HGF, bFGF, thrombospondin, endostatin, and angiostatin peripheral plasma (PP) and bone marrow plasma (BM) levels in a group of patients with MM who underwent ASCT. *Methods.* We studied PP and BM levels of factors in 82 MM patients (43M/39F, median age: 51 years) who underwent an ASCT after high dose melphalan conditioning. PP and BM levels of VEGF, HGF, bFGF, thrombospondin, endostatin, and angiostatin were measured in time of diagnosis MM and after transplantation using ELISA method. Patients were divided into three groups according to treatment response: group A) 33 patients who achieved at least very good partial response (VGPR); group B) 28 patients who achieved partial response PR; group C) 11 patient who did not achieved even PR. The levels of factors in the time of diagnosis and after transplant were compared using nonparametric pair Wilcoxon test. *Results*. HGF concentration at the time of diagnosis in PP (median=620 pg/L) and in BM (median=1519 pg/L) decreased significantly after ASCT in patients with VGPR in PP (median=415 pg/L; p=0,014) as well as in BM (median=1174 pg/L; p=0,041). Significant differences in HGF levels at at the time of diagnosis and after ASCT in patiens with PR and with no response were not shown. Similarly, the VEGF levels at diagnosis time in PP (median=124 pg/L) and in BM (median=129 pg/L) decreased significantly after transplant in patients with VGPR in PP (median= 56 pg/L, p=0,016) and in BM (median=68 pg/L, p=0,006). Significant differences in VEGF levels at diagnosis time and after transplant in patients with PR and with no response were not shown. Thrombospondin concentrations at diagnosis time in BM (median=678 pg/L) increased significantly only in patiens with VGPR compared with post-ASCT value (median=801 pg/L, p=0,048).Thrombospondin levels in PP did not differ significantly at diagnosis time compared with the values after transplant in VGPR, PR and no response patients. Similarly bFGF, endostatin and angiostatin concentrations at diagnosis time did not differ significantly compared with values after transplant in PP and BM in patiensts with VGPR, PR and no response. Conlusions: Our results confirmed that key angiogenesis activators in MM are HGF and VEGF. Decreasing of their levels occurs only if the treatment is successful. From activators, HGF seems to be the best candidate for angiogenesis monitoring, because it is possible to monitor it in PP. The fact or finding that thrombospondin in patients with successful treatment increased after ASCT in BM is very important. It means, that angiogenesis after treatment is more inhibited in patients with VGPR than in other. Hovewer thrombospondin concentrations in PP are influenced with activated endothelium and platelets, so only BM level of thrombospondin is good candidate for angiogenesis monitoring. Supported with research program MSMT of Czech republic Nr. LC 06027

PO-252

ANGIOGENESIS AND PROLIFERATION INDEX IN MULTIPLE MYELOMA

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The aim of study was to analyze relation and prognostic significance of angiogenesis and proliferation index in the bone marrow (BM) of MM patients (pts) by immunohistochemical markers of angiogenesis and proliferation. *Material and methods*. Sixty newly diagnosed MM pts (33 male/27 female pts, mean age 60 years, range 35-75) were distributed according to the clinical stage (CS, Salmon&Durie) as: I 8pts, II 22pts, III 30pts. IgG myeloma was diagnosed in 35pts; IgA in 12pts; light chains in 12pts. Regarding ISS score, the group included: ISS1 18pts, ISS2 13pts, ISS3 29pts. All patients were treated with conventional chemotherapy. All samples of BM biopsies were analyzed for the immunohistochemical expression of FGFR-3 and Ki-67. In order to analyze the microvessel density (MVD), BM vessels were visualized by immunohistochemical staining for CD34. The number of vessels per 400x high power field (HPF) was counted in the area of the most dense vascularization. *Results*. MVD was significantly higher in MM pts in III CS vs. I CS (15 vs.7,5/ x400 field, p<0,001); and in pts with ISS3 vs. ISS1 (17,5 vs. 9,7/ x400 field, p<0,05). The expression of FGFR-3 was found significantly higher in III CS vs. I CS (47,5 vs. 25%, p<0,05); and in pts with ISS3 vs. ISS1 (60 vs. 22,5%, p< 0,001). The proportion of Ki-67 positive plasma cells was significantly higher in III CS vs. I and II CS (10 vs. 5%, p<0,01), and in pts with ISS3 vs. ISS1 (13 vs. 5%, p<0,01). Strong activity of angiogenesis and proliferation in III CS indicated significantly shorter overall survival of those pts vs. I CS (26 vs. 43,5m, log rank, p<0,05). Similarly, the overall survival of pts with ISS3 was significantly shorter vs. ISS1 (19,5 vs. 36m, log rank, p<0,001). In conclusion, the assessment of the activity of angiogenesis and proliferation index represents important predictive factor as possible targets of novel therapeutic strategies in MM.

PO-253

TUMOUR ANGIOGENESIS CORRELATES WITH PLASMA LEVELS OF CXCL12 IN PATIENTS WITH MULTIPLE MYELOMA

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Introduction. Angiogenesis, or the process of blood vessel formation from pre-existing vasculature, plays an important role in the pathogenesis of multiple myeloma (MM). MM patients with active disease exhibit increased bone marrow (BM) angiogenesis compared with those in remission and patients with monoclonal gammopathy of uncertain significance (MGUS). Although the precise molecular mechanisms underlying the progressive increase in angiogenesis in MM remain unclear, a number of MM plasma cell (PC)-derived growth factors, cytokines and chemokines have been implicated. Previous studies from our laboratory show that MM PC are an abundant source of the chemokine CXCL12 which is elevated in patients with active bone disease. Recent studies

suggest a role for CXCL12 and its receptor, CXCR4, in normal and pathological angiogenesis. In light of this, we wished to examine whether CXCL12 levels also correlated with degree of angiogenesis in patients with active MM and MGUS. *Materials and Methods*. Trephine and peripheral blood (PB) samples were collected from a total of 106 newly-diagnosed, untreated MM and MGUS patients and age-matched healthy subjects. Microvessel density (MVD) was used as a measure of angiogenesis and was determined by enumerating the number of small (<10μM) vessels present within trephine biopsies following CD34 immunostaining. CXCL12 levels in PB plasma samples were determined using a commercial CXCL12-specific ELISA. Tube formation assays were performed using Human Umbilical Vein Endothelial Cells (HUVECs). *Results*. Using conditioned media from the MM PC line RPMI-8226 and the highly specific small molecule CXCR4 inhibitor, 4F-Benzoyl-TE14011 (T140), we have shown that MM PC-derived CXCL12 directly stimulates *in vitro* tube formation. Furthermore, using trephine and PB plasma samples from MM and MGUS patients, we have demonstrated that PB plasma levels of CXCL12 positively correlate with the degree of bone marrow angiogenesis (MVD) in MM and MGUS patients, suggesting that MM PC-derived CXCL12 plays an important role in the increased BM angiogenesis associated with MM progression. *Conclusion*. This study demonstrates the novel finding that CXCL12 plays a role in the elevation of BM angiogenesis in MM patients. Therefore, the CXCL12/CXCR4 axis represents an exciting therapeutic target for the development of anti-angiogenic therapies in MM.

PO-254

REPORT: ANALYSIS OF BETA-2 MICROGLOBULIN (b2M), FREE HLA CLASS I HEAVY CHAIN (FHC) AND THE ASSOCIATED PROTEIN PATTERN IN MULTIPLE MYELOMA (MM) PATIENTS FROM THE NORDIC MYELOMA GROUP STUDY #5/94

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Background. Autologous stem cell transplantation is now considered the standard of care in young patients with multiple myeloma (MM) and the most consistent prognostic factor described at diagnosis has been blood levels of beta-2 microglobulin (β2m). Recently, the levels of β2m-free HLA class I heavy chain (FHC) has been shown to correlate with β2m but as expected not influenced by renal failure seen in MM. These data indicate that serum FHC may be a more useful disease marker than β2m in MM. The aim of this study was to evaluate the prognostic impact of FHC in a cohorde of 102 patients included in the NMSG study #5/94 and to identify β2m and/or FHC associated protein expression patterns identified by global array analysis by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS). *Methods*. Serum samples from 102 patients with MM undergoing high dose therapy and autologous stem cell transplantation were retrospectively analyzed for concentration of β2m and FHC. The serum specimens were further evaluated by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF-MS) to profile protein expression up to 20 kDa. *Results*. Serum β2m and FHC was correlated and β2m but not FHC was found to be a most significant predictor of overall survival. Using the SELDI technique with prefractionation of samples before profiling, we identified mass spectrometry peaks significantly correlated to β2m and FHC and such circulating biomarkers with a likely pathophysiological role may be used as predictors of outcome. *Conclusion*. Data from this study did not confirm FHC as a prognostic variable indicating that the prognostic impact of β2m levels is not only a tumour marker but also an indirect consequence of other disease related events including impaired kidney or other organ function as well as host-tumour interactions. The feasibility of a chip-based proteomic profiling technique to identify plasma proteins of prognostic significance will be demonstrated.

PO-255

IMMUNOHISTOCHEMICAL ANALYSIS OF HGF IN PLASMA CELL DISEASE

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Introduction. Hepatocyte growth factor (HGF) stimulates survival and proliferation of malignant plasma cells, and is a potential contributor to the bone disease of multiple myeloma. HGF is produced by the myeloma cells, and by surrounding cells in the bone marrow microenvironment, and may thus act in an autocrine or paracrine manner. Furthermore, we have previously demonstrated that elevated levels in serum are associated with poor prognosis. We aimed to investigate the presence of HGF in biopsies from patients with monoclonal plasma cell disease. *Material and Methods*. 68 bone marrow and 28 plasmacytoma biopsies from a total of 82 patients with monoclonal plasma cell disease were evaluated by immunohistochemical staining for HGF. Percentage of positive cells and intensity of staining were evaluated using light microscopy. *Results*. Cytoplasmic staining of HGF in the plasma cells was demonstrated in 88 out of 96 biopsies (92%), with low expression, defined as 10-49% positive cells, in 20 biopsies, and high expression, defined as 50-100% positive cells, in 68 biopsies. Staining was abrogated by preabsorption of the antibody with corresponding peptide. There was no significant difference in percentage of positive cells, or staining intensity, between patients with multiple myeloma (n=70), monoclonal gammopathy of undetermined significance (MGUS) (n=5) or solitary plasmacytomas (n=7). HGF-positive plasma cells were also encountered in normal bone marrow (n=3), although the small percentage of plasma cells in these biopsies does not allow for proper quantification. Among myeloma patients with low and high HGF expression, median overall survival was respectively 51 months and 23 months (p=0.016). iv) *Conclusion*. HGF immunostaining in a majority of malignant plasma cells, as well as plasma cells of MGUS patients, was demonstrated. High bone marrow expression was significantly associated with shorter overall survival in myeloma patients. In light of earlier findings of plasma cell production of HGF in less than 50% of myelomas and MGUS, we suggest that the high frequency of intracellular presence of HGF in our biopsies reflects not only synthesis, but also internalization of HGF produced by other cells in the bone marrow microenvironment.

PO-256

THE Y-BOX BINDING PROTEIN YB-1 IS ASSOCIATED WITH PROGRESSIVE DISEASE AND MEDIATES SURVIVAL AND DRUG RESISTANCE IN MULTIPLE MYELOMA

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Current knowledge about the molecular mechanisms underlying disease progression and drug resistance in multiple myeloma (MM) is still limited. Here, we analyzed the potential pathogenetic role of the Y box binding protein YB-1 in MM. YB-1 is a member of the cold shock domain protein superfamily, which is known to be involved in various cellular functions, such as translational control of protein synthesis, DNA repair and proliferation. Immunohistochemical analyses revealed that neither normal bone marrow (BM) plasma cells, premalignant plasma cells of patients with a monoclonal gammopathy of unknown significance (MGUS) nor MM cells with a mature morphology showed expression of YB-1 *in situ*. In contrast, YB-1 was strongly expressed *in situ* in a subset of MM samples and *in vitro* in all of the evaluated MM cell lines. The YB-1 expressing cells were characterized by their immature plasma cell morphology and higher proliferation rates. We observed that siRNA-mediated knockdown of YB-1 led to induction of apoptosis in MM cells even in the presence of BM stromal cells. Furthermore, we found that transient overexpression of YB-1 mediated resistance towards doxorubicin-induced apoptosis in MM cells. Thus, YB-1 contributes to disease progression, survival and drug resistance in MM and might therefore provide an attractive therapeutic target for the treatment of this cancer.

GROUP 3: Pathophysiology-bone disease

PO-301

A MURINE MODEL TO STUDY BONE DISEASE IN MULTIPLE MYELOMA

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Introduction. Multiple Myeloma (MM) is associated with significant skeletal changes including ospeoporosis and bone lesions in more than 80% of patients. Bone resorption is caused by an enhanced osteoclast number and activation. However, the molecular and cellular basis for these effects are not completely understood. Here we describe an *in vivo* model that allows us to evaluate the relationship and interactions between MM cells and cellular elements of bone formation and resorption. *Material and Method*. In this model, BM stroma and IL-6-dependent human MM cell line (INA-6) was injected into a human bone chip implanted into SCID mice. Bone chips in the control mice were injected with media alone. At different time points following injection, myelomatous and control bone chips were retrieved, fixed in paraformaldehyde, and evaluated by micro-computed tomography (mCT) and histological examinations for effects of MM cells on bone compartment. Additionally, we have measured the levels of human osteocalcin in murine serum to reflect the remodelling of human bone in mice. *Results*. We have observed lesions in myelomatous bone in mice at day 30 after the MM cell injections, without significant changes in the matched control bones. By 3-D histomorphometric parameters calculated from mCT (bone volume fraction = bone volume/total volume), a decreased bone volume fraction was observed at day 30 in bones with MM compared with normal bones, primarily due to reduction of bone density in the trabecular bone. Moreover, histological examination revealed a significant (p=0.011) increase in number of multinucleated TRAP-expressing osteoclasts in mice engrafted with MM versus control. Animals with bone containing myeloma cells also had significantly elevated serum markers of bone remodelling. To confirm the validity of this model to study effect of MM cells on bone as well as to show its utility for evaluation of novel agents active in MM bone disease, we treated mice with Zoledronic acid at 0,6 mg/kg, weekly for 2 months. Zoledronic acid was able to significantly inhibit the development of bone lesions in treated mice compared to untreated mice, evidenced by mCT, X-rays and TRAP staining. Additionally, we also observed that Zoledronic acid inhibited MM tumor growth, as measured by human IL-6sR released by INA-6 cells in mice sera, corroborating its reported anti-MM activity. *Conclusion*. This model therefore provides a reproducible and predictable *in vivo* system to study the biology of bone disease in MM, as well as to identify and evaluate therapeutic targets and agents targeting MM bone disease.

PO-302

IDENTIFICATION OF A HIGHLY SPECIALIZED MICRO-ANATOMICAL STRUCTURE THAT IS A KEY PLAYER IN MYELOMA BONE DISEASE

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Introduction. Multiple myeloma leads to bone destruction by osteoclasts (OC) and absence of bone repair by osteoblasts (OB). This has stimulated intensive research on myeloma cell (MM) - OC/OB interactions *in vitro*. Here we report a previously unrecognized histological structure involved in the interactions between the cells of the bone marrow cavity (such as MM) and the OC/OB of the bone surface, and we show that this structure is disrupted in myeloma patients with overt bone disease. *Methods*. We obtained serial sections from 36 and 9 bone marrow biopsies of myeloma patients and controls, respectively, performed immunohistochemistry with multiple stains to identify simultaneously different cells on bone surfaces undergoing remodeling and in the adjacent bone marrow, performed 3D reconstructions, and quantified erosion surfaces (ES), osteoid surfaces (OS), and total bone surfaces (BS). *Results*. 3D recontructions revealed a histological structure consisting of (i) an NCAM$^+$ cell-layer which separates the OC/OB on the bone surface from the bone marrow, and which appears as a continuous roof covering the OC/OB and joining the bone lining cells in the periphery; (ii) capillaries connected to the bone marrow side of this roof, thereby including the underlying bone remodeling compartment in the vascular space; (iii) pre-OC along these capillaries, suggesting a possible route of pre-OC to the bone remodeling site. Interestingly, nearly all the biopsies from myeloma patients with more than 5 osteolytic lesions (X-ray analysis) showed disrupted *roofs* on top of the remodeling surfaces. Furthermore, these biopsies showed ES/BS ratios between 40 and 60% and OS/ES ratios between 0.2 and 1.5, whereas biopsies with intact *roofs* showed ES/BS ratios below 20% and OS/ES ratios of at least 2. These respective values indicate massive resorption uncompensated by bone repair in biopsies with disrupted roofs. *Conclusions*. (i) Interactions between cells of the bone marrow cavity and OC/OB on the bone surface are not direct, but involve previously unidentified vascular structures. (ii) These structures collapse in multiple myeloma, allowing direct MM-OC/OB contacts. (iii) This collapse coincides with unbalanced bone resorption/formation (and with the formation of MM-OC hybrid cells: see our abstract on this topic). (iv) These vascular structures (and their collapse) should receive attention in models of pathophysiological bone remodeling, such as myeloma, and could be a new therapeutic target.

PO-303

CELLS OF THE MYELOMA CLONE DIRECTLY CONTRIBUTE TO THE FORMATION OF OSTEOCLASTS IN MYELOMA PATIENTS

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Introduction. Bone destruction is a major clinical manifestation of multiple myeloma. It is widely accepted that this destruction is not caused by the malignant cells themselves, but by osteoclasts, multinucleated cells of monocytic origin, considered to be the only cells able to degrade bone. Recent reports made us hypothesize that in multiple myeloma, cells of the myeloma clone may directly contribute to osteoclast formation. *Methods*. We examined osteoclasts for the presence of chromosomal translocations specific of the myeloma cell clone, by using combined immunohistochemistry and FISH on bone sections. We performed osteoclast-myeloma co-cultures, and traced myeloma nuclei in osteoclasts by using chromosomal translocations, Brdu, and the Y chromosome of male myeloma cells in osteoclasts of female origin. *Results*. We demonstrate that resorbing osteoclasts of myeloma patients contain nuclei with translocated chromosomes of myeloma B-cell clone origin in addition to nuclei without these translocations. These nuclei of malignant origin are transcriptionally active, appear fully intermixed with the other nuclei, and are not associated with CD138-stained plasma membrane remnants of myeloma cells, as would happen if they came from dying myeloma cells phagocytosed by the osteoclasts, as occasionally seen. The contribution of malignant nuclei to the osteoclast nuclei population analyzed in our study, ranged from 33% to 48%. Osteoclast-myeloma clone hybrids contained more nuclei than normal osteoclasts, and their occurrence correlated with the proximity of myeloma cells, as well as with disruption of a cell layer that separates normally osteoclasts from the bone marrow (see our abstract on *vascular compartments*). Similar hybrid cells were generated in myeloma cell-osteoclast co-cultures, indicating that they can originate through fusion between myeloma cells and mature osteoclasts. *Conclusion*. A substantial number of osteoclasts of myeloma patients are actually osteoclast-myeloma clone hybrids. Osteoclast-myeloma clone hybrids reflect a previously unrecognized mechanism of bone destruction in which myeloma cells participate directly.

PO-304

GENETIC POLYMORPHISMS OF EPOXIDE HYDROLASE, MAP KINASE, RNA HELICASE DDX18 AND TNF-α LINKED TO LIKELIHOOD OF BONE DISEASE IN MYELOMA

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Background. Bone disease in myeloma occurs as a result of the complex interactions between myeloma cells and the bone marrow micro-environment. To date, no studies have evaluated the potential impact of genetic polymorphisms upon this microenvironment. *Patients and Methods.* Peripheral blood DNA from 282 patients enrolled in the Total Therapy 2 (TT2) protocol was studied using the previously reported Affymetrix 3k BOAC custom chip to evaluate relevant genetic polymorphisms. Patients were classified based upon both full skeletal x-rays and MRI findings. The lower cut-off used was absence of focal abnormalities on x-ray and/or <7 focal lesions on MRI. *Results*. The top 200 SNPs were first evaluated based upon univariate P values linked to limited or extensive bone disease. The top 50 SNPs with the smallest P values were then selected (eliminating closely linked genes) for recursive partitioning analysis. The recursive partitioning was conducted both with and without the insertion of known biologically relevant SNPs. The pruned tree developed with recursive partitioning proved to be quite stable and incorporated 4 dominant SNPs: rs 3766934 Epoxide hydrolase; sr3783408 MAP kinase; sr 1062637 RNA helicase DDX18; and sr3181366 TNFSF8-TNF-α. For these analyses patients were divided into training (2/3 patients) and validation sets. In the training set correct classification was 82% with 99% sensitivity. In the validation set correct classification and sensitivity were 62% and 89% respectively. The unpruned tree contains 10 SNPs, including rs 259843 STAT 6 signal transducer, the correct classification was 85% with sensitivity 89% and specificity 77% in the training set. In the validation set correct classification, sensitivity and specificity were 60%, 72% and 35%, respectively. It is noteworthy that epoxide hydrolase protects against reactive oxygen species (ROS), which can regulate MAP kinase activation, which is upstream in a pathway leading to MIP-1α expression, a gene significantly linked to bone disease. *Conclusions*. This first assessment of genetic polymorphisms linked to myeloma bone disease in myeloma has led to the identification of polymorphisms with both potential biologic relevance and utility in prognostic models of myeloma bone disease. Further studies may allow identification of potential therapeutic targets. Research funded by the International Myeloma Foundation.

PO-305

DYSREGULATION OF PARATHYROID HORMONE (PTH), 25-HYDROXYVITAMIN D (VIT-D) AND CALCIUM/PHOSPHORUS HOMEOSTASIS IN MULTIPLE MYELOMA (MM) PATIENTS (PTS): WHAT ARE THE RISKS?

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ONJ is a serious complication in MM pts; incidence is time-dependent with highest risk seen after long-term use of bisphosphonates (BP) in older pts, often after dental surgical procedure (Badros, *et al.* JCO 2006). Stopping BP in ONJ pts and around dental procedures had minimal impact on both healing and prevention of ONJ and resulted in an increased risk of skeletal complications. In this study, we evaluated prospectively Vit-D, intact PTH, calcium, phosphorus, creatinine, albumin, alkaline phosphotase (Alk phos), and bone-specific Alk phos, and

Table 1.

	Frequency	*Vit- D 15 ng/mL*	*Vit- D 15-29 ng/mL*	*Vit- D > 30 ng/mL*	*P value*
No of Pts	100	40	35	25	
Age; median (range)	59 (29-80)	59 (29-80)	59 (30-74)	60 (43-78)	
Sex; Male/female	58/42	20/20	14/21	8/17	
Race; C/B/H	54/44/4	16/22/2	19/16/0	16/6/0	0.005*
Vit- D and calcium supplementation	30	0	10	20	
Disease status CR+nCR/PR/PD/New dx	19/36/36/9	4/14/15/2	6/12/15/2	2/10/6/5	NS
Lytic Bone disease/Pain/Fracture/ Hx of ONJ	93/58/33/14	39/26/13/7	31/20/11/3	23/12/9/4	
PTH; 10-69 pg/mL; median (range)	93	40 88 (13-1108)	35 95(21-219)	25 47 (2-226)	0.03‡
Creatinine; mg/dL 18 pts had Cr > 2 mg/dL & 5 on dialysis	100	1.3 (0.5-9)	1.4 (0.5-9)	1.3(0.9-4.5)	
Albumin; 3.2-4.6 g/dL median (range)	100	3.5 (1.9-4.7)	4 (2.6-5.3)	3.9 (2.6-5.3)	0.006
Calcium; 8.8-10.2 mg/dL median (range)	100	9.3 (8.1-14.9)	9.3 (7.2-13.1)	9.4 (7.5-11.2)	
Phosphorus; 2.3-3.3 mg/dL median (range)	100	3.4 (2.5-5.9)	3.9 (2.6-6.3)	3.5 (2.5-5)	0.02
ALK Phos 38-126 U/L; median (range)	100	80 (31-274)	91 (42-182)	74 (50-192)	0.05
Bone ALK Phos 14-42 U/L median (range)	40	25 (0-121)	23; (12-49)	19 (11-60)	0.04
NTX; 13-78 nM BCE/mM Cr median (range)	37	2873 (500-17186)	3225 (1076-24181)	2880(651-11604)	
CTX; 250-5100 pmol/L median (range)	41	23 (0-173)	21 (11-166)	21 (11-73)	

Caucasians have a lower vit--D levels controlling for age and sex.

biochemical markers of bone turnover N- and C-terminal cross-linking telopeptide of type I collagen (NTX and CTX) in 100 consecutive MM pts between September 1-Oct-15, 2006. Measurements were obtained before bisphosphonate infusions. The variables were correlated with retrospectively retrieved details of MM-related bone complications including lytic lesions, pain, fractures and history of ONJ. Pts' characteristics are summarized in Table 1; there were 18 pts with light chain disease and 5 non-secretory. Median time from diagnosis was 3.7 years (range: 0.7-17) and 0.3 yrs for newly diagnosed pts. Ninety-six pts received bisphosphoante therapy including sixty-four who received zoledronic acid; 57 pts received therapy on monthly bases and 39 pts were on 3 monthly schedule after a median of 5 yrs of monthly infusions (range: 3.5-17). Only 25 pts had adequate vit-D levels and 40% and 35% had deficient and insufficient levels, respectively. These levels will only decrease during the winter season as peak vit-D levels are reached by the end of the summer. Less than 40% of the pts recalled receiving clear instructions about calcium and vit-D supplementation and only 30% were taking both. A negative correlation exists between PTH and vit-D (r=-0.23, p=0.03), corrected calcium for serum albumin (r=-0.24, p=0.02) and phosphorus (r=-0.2, p=0.05). PTH was significantly higher in pts with renal insufficiency, p=0.02. There was a statistically significant correlation between PTH and bone turnover markers [Alk phos, bone specific-Alk Phos, NTX,; r=0.5, p<0.0001; r=0.8, p<0.0001 and r=0.3, p=0.04, respectively] but no correlation with CTX, (r=0.18, p=0.2). In a multivariate analysis of variance; PTH was higher in pts who experienced bone pain (p=0.02) and had renal insufficiency (p=0.0006); corrected calcium with positive correlation with phosphours (r=0.2, p=0.02) was higher in pts with history of ONJ (p=0.03) and renal insufficiency (p=0.02). Multiple logistic regression model showed that pts without fractures were 5 times less likely to develop ONJ (p=0.03) and NTX/CTX had marginally significant correlation with history of ONJ (p=0.09, 0.08; respectively). In 5 pts with recurrent ONJ, median Vit-D level was 10 (range:7-28), PTH was 130 (104-338), Alk phos of 117 (54-134), calcium was 9.6 (8.2-10) and phosphorus was 3.6 (range: 2.7-5.9). There was no difference in bone turnover markers between pts receiving monthly versus 3 monthly BP infusions (p=0.6). In conclusion, we observed a high prevalence of low vit-D and high PTH levels in MM pts; renal insufficiency further affected levels. PTH was associated with bone pain and increased markers of bone resorption. High calcium/phosphorus, a known promoter of vascular calcification, may play a role in BP induced ONJ. These correlations only suggest but do not prove causality. Physicians should be obliged to test and ascertain optimal value for vit-D, PTH, calcium and phosphorus levels in MM pts; further research should determine if such corrections will improve outcome.

PO-306

SERUM DKK1 IS ASSOCIATED WITH BONE LESIONS IN MM PATIENTS

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Dickkopf-1(DKK-1) gene and its protein act as Wnt-signalling inhibitor in Multiple Myeloma (MM). It has been related in this disease with inhibition of osteoblastic function and differentiation. Serum concentrations of this protein can be related with bone disease in MM patients, but this fact has not been profoundly investigated. *Aims*. To study the serum DKK1 concentrations in a series of MM and MGUS patients, to evaluate a possible role in the discrimination between both group of patients, the relationship with bone disease data, including serum biochemical resorption markers and its influence on survival. *Material and Methods*. 81 MM, 51 MGUS and 21 Benign Osteoporosis (BO), whose serum were collected at diagnosis and stored at -80°C. The median follow-up of the series was 100.4 months. The serum DKK1 was measured by double-sandwich enzimoimmunoassay, standardized in our laboratory. We have also analyzed bone resorption markers (CTX(β-crosslaps), Crosslinks and bone formation markers (Osteocalcine(OC), bone alkaline phosphatase(bAP)). *Statistical methods*. Non-parametric test (Mann-Whitney U, Kruskal-Wallis and Spearman correlation); survival curves of Kaplan-Meier were compared by long-rank test. *Results*. Serum DKK1 values were significantly higher in MM patients than in MGUS patients (p<0,01) (median: 47.8 ng/mL vs. 35.2 ng/mL), but didn't show differences with BO group (median:45.6 ng/mL). MGUS patients has values lower than BO group, but not significantly. In MM patients with very aggressive bone lesions (multiple lytic lesions and/or pathological fractures), the DKK1 concentrations were significantly higher than the others MM (median:100.5 vs.36.6m.; p<0.01). Interestingly, serum DKK1 was different between the MGUS patients and MM patients without bone lesions (median: 43 vs.35.2m.; p<0.05). DKK1 concentrations shown significant relationship with clinic parameters as serum calcium and bone pain. Serum DKK1 concentration only was correlated with sRANKL, but not with others serum biochemical parameters of bone resorption Serum DKK1 has no impact on survival in our MM patients. *Conclusions*. The measure of serum DKK1 is feasible and shown relationship with several clinic parameters of bone disease, but we do not find relation with the most known biochemical bone markers. This data can facilitate the further design of a score of several bone resorption markers that measure accurately the bone lesion in MM.

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PO-307

EXPRESSION AND LOCALISATION OF RANKL IN MULTIPLE MYELOMA CELL LINES

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Factor that induces osteoclast formation and contributes to the osteolytic phenotype of multiple myeloma (MM). RANKL protein can exist as both a membrane-bound and a soluble factor. There is controversy as to whether myeloma cells produce RANKL. We have compared the expression and localisation of the RANKL protein in MM and osteosarcoma (OS) cell lines to identify any differences that may result in the opposing phenotypes (MM - bone loss; OS - bone formation) and thus suggest a mechanism of action in MM. We have detected the expression of RANKL in RPMI 8226, U266, Karpas 620, JIM-1, JIM-3 and LP1 MM cell lines, using a monoclonal antibody, by western blotting and immunofluorescence. RANKL expression was also observed in the normal B cell line AGLCL and the T cell line Jurkat J16. Analysis of protein expression by western blotting has revealed glycosylated and unglycosylated forms of both the transmembrane and soluble proteins in insoluble and soluble fractions and in cytoplasmic and nuclear extracts from the MM and OS cell lines. Protein fingerprints differed between MM and OS cell lines. Immunofluorescent staining suggested that RANKL was

localised to the membrane surface and within the cytoplasm of the MM cell lines. Interestingly the OS cell lines demonstrated nuclear localisation of RANKL, which was verified using confocal microscopy. Surface expression of RANKL was also analysed using flow cytometry. Comparisons between the control cell lines and the MM cell lines have suggested an increased RANKL surface expression in the MM cell lines however this was not significant (AGLCL-35%, U266-48%, Karpas 620-36% JIM-1 - 38% cells expressing surface RANKL). Analysis of the OS cell lines showed a decrease in the percentage of cells expressing surface RANKL (MG63-6%, HOS - 3% and Sa_0S_2 - 4%). These data strongly suggest a role for the different isoforms and localisation of RANKL in the generation of the osteolytic phenotype in MM. Future work will explore these differences in clinical patient samples with reference to their bone loss status.

PO-308

CXCL12 STIMULATES OSTEOCLASTIC BONE RESORPTION IN A NOVEL MOUSE MODEL OF HUMAN MULTIPLE MYELOMA

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Introduction. More than 80 percent of patients with multiple myeloma (MM) develop focal osteolytic lesions throughout the skeleton. MM plasma cells (PC) stimulate focal bone loss by inhibiting osteoblastic bone formation and promoting the aberrant recruitment of osteoclast (OC) precursors from the peripheral blood (PB) to the bone surfaces. Recent studies from our laboratory and those of others, point to a significant role for the well characterized chemokine, CXCL12 in OC migration and activation. In this study, we have extended our initial findings by examining whether CXCL12 is capable of mediating an increase in osteoclast recruitment and osteolysis *in vivo*. *Methods and Materials*. The MM PC line, RPMI-8226, or RPMI-8226 cells over-expressing CXCL12 or RANKL, were directly injected into the intra-tibial space of athymic nude mice, mimicking MM PC infiltration. To determine the activating potential of MM PC-derived CXCL12, osteolysis was quantitated six weeks post injection by micro-CT and histological analyses. *Results*. Transplantation of RPMI-8226 into tibiae resulted in a 5% decrease in bone volume (BV) when compared with contralateral, vehicle control tibia. Importantly, transplantation of RPMI-8226 over-expressing CXCL12 resulted in a 14% decrease in BV, and an increase in osteolytic lesions and serum collagen I breakdown products (RatLaps™). Furthermore, the degree of bone loss in mice transplanted with RPMI-8226 over-expressing CXCL12 was similar to that seen following transplantation of RPMI-8226 cells over-expressing the potent pro-osteoclastogenic molecule RANKL. *Conclusions*. These studies confirm our previous *in vitro* findings that MM PC derived-CXCL12 stimulates the recruitment and bone-resorbing activity of OC, thereby contributing to the development of osteolytic lesions. Therefore, inhibition of CXCL12-CXCR4 may be an effective modality to inhibit osteolysis in MM patients.

PO-309

SERUM YKL-40 IS ASSOCIATED WITH BONE DISEASE IN MULTIPLE MYELOMA

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Introduction. The secreted glycoprotein YKL-40 (CHI3L1, HC gp-39) is a potential player in the tumor-host interactions affecting several aspects of multiple myeloma (MM) including bone destruction. Previous studies support a role for YKL-40 in remodelling of the extracellular matrix, in angiogenesis, and in cancer cell survival and invasion. The aim of this study was to investigate the association between serum YKL-40 (S-YKL-40) and the degree of bone disease in MM. *Materials and Methods*. S-YKL-40 was measured using an ELISA in 54 MM patients at diagnosis. Bone morbidity was assessed by radiography and scored semiquantitative as a total X-ray score. Ongoing bone metabolism was assessed using biochemical markers of bone formation (S-PICP, S-PINP, S-Bone ALP, S-OC) and bone resorption (S-CTX-MMP, U-NTX-1, U-PYD, U-DPD). The first 34 patients included were treated with conventional chemotherapy and followed for up to 30 months. Skeletal related events (SRE) were registered and subdivided in vertebral fractures and osteolytic events including non-vertebral fractures. *Results*. 57% of the patients had a S-YKL-40 elevated above the upper limit in an age specific 90 per cent reference range for healthy adults. Patients with elevated S-YKL-40 had a higher total X-ray score (p=0.005) and higher levels of S-CTX-MMP (p=0.003), U-PYD (p=0.04) and U-DPD (p=0.002), while U-NTX-1 and the markers of bone formation did not differ from the levels seen in patients with normal S-YKL-40. During follow-up 21 patients experienced SRE and 15 patients had osteolytic events. Using S-YKL-40 level, i.e. normal versus elevated, patients with elevated S-YKL-40 had shorter time to first osteolytic event (12 months versus not reached; Log rank test =0.03; HR = 3.70; p=0.046). This was confirmed when using S-YKL-40 (log2) as a continuous variable (HR = 1.45; p=0.048). The differences between groups for time to first SRE was only borderline significant. *Conclusions*. Newly diagnosed MM patients with elevated levels of S-YKL-40 have more severe bone destruction including increased bone resorptive activity and shorter time to progression of myeloma-related bone disease. A potential role for YKL-40 in the bone disease of MM must be considered.

PO-310

DCR3 INVOLVEMENT IN MULTIPLE MYELOMA BONE DISEASE

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Introduction. Multiple myeloma (MM)-bone disease is related to an unbalanced bone turnover with enhanced resorption, related to increased osteoclast (OC) recruitment and activity, and low bone formation. In an *in vitro* osteoclastogenesis model we previously demonstrated that OCs from peripheral blood mononuclear cells (PBMCs) of MM bone disease patients displayed a long life span in culture exclusively when generated in the presence of T cells. In this system the OCs do not undergo apoptosis although high concentration of apoptotic molecules, such as FasL, in the medium. However, why the OCs were protected from FasL-induced apoptosis remained unclear. Since Decoy Receptor 3 (DcR3), member of TNF receptor superfamily, is known to bind and neutralize FasL, in the present study we hypothesized a possible involvement of DcR3 in the inactivation of FasL in our system. *Materials and Methods*. Freshly isolated T cells and plasma cells from MM patients were subjected to mRNA and protein extraction to detect the expression of DcR3 by RT-PCR and western blot, respectively. The detection of FasL/DcR3 immunocomplex was performed by immunoprecipitation experiments. Cell viability was measured by MTT assay on mature OCs treated with different concentrations of anti-DcR3 antibody. Caspase-8 and casapse-3 cleavage were detected by western blot. *Results*. We found that T lymphocytes and malignant plasma cells from MM bone disease patients overexpressed DcR3. We also demonstrated the formation of the DcR3/FasL immunocomplex in T- and MM-cell lysates as well as in culture media, suggesting that DcR3/FasL interaction inactivate FasL in the media thereby OC were protected from the FasL-induced apoptosis. The viability of MM OCs was significantly reduced in presence of anti-DcR3 neutralizing antibody, indicating that the formation of DcR3/FasL complex could be responsible of the inhibition of FasL-mediated OC apoptosis. We showed that the treatment of MM OCs with anti-DcR3 antibody activated the apoptotic intracellular pathway leading to caspase-8 and caspase-3 cleavage. *Conclusion*. Our data demonstrated that T lymphocytes and malignant plasma cells from MM bone disease patients by producing DcR3 protect OCs from apoptosis and contribute to the progression of MM bone disease.

PO-311

BORTEZOMIB PLUS DEXAMETHASONE THERAPY INDUCES OSTEOBLAST ACTIVATION IN RESPONSIVE PATIENTS WITH MULTIPLE MYELOMA

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Multiple myeloma (MM) is a plasma cell malignancy characterized by devastating bone destruction due to enhanced bone resorption and suppressed bone formation. Bortezomib is a novel proteasome inhibitor that has shown marked clinical effects on MM cells as well as bone marrow environment including osteoblasts and osteoclasts, and is getting increasingly used worldwide. However, severe pulmonary complications occurred among Japanese patients when treated with bortezomib alone, which becomes a major concern for utilization of this agent. Alternatively, dexamethasone has been implicated in enhancement of anti-MM activity and prevention of such adverse events during bortezomib therapy. In this study, we evaluated the feasibility and efficacy of bortezomib plus dexamethasone (BD) therapy and assessed bone metabolism in patients with relapsed or refractory MM. Fourteen patients received bortezomib 1.3 mg/m^2 on days 1, 4, 8, and 11 along with dexamethasone 20 mg/dose on days 1, 2, 4, 5, 8, 9, 11, and 12 on a 21-day cycle. After 1-3 cycles of BD therapy, 9 patients (64%) achieved objective response (5 very good partial response and 4 partial response). Three patients were in stable disease, and 2 were in progressive disease. Notably, a rapid increase of serum alkaline phosphatase (ALP) was observed in 6 of treatment-responsive patients but not in non-responders. Bone formation markers such as serum bone-specific ALP and osteocalcin significantly increased in 5 and 2 responsive patients, respectively, around one month after the initiation of BD therapy. Computed tomography (CT) scans showed radiographic improvements of bone lesions in 2 patients with increased osteocalcin levels. These observations suggest that BD therapy leads to osteoblast activation exclusively in responders despite the inhibitory effects of dexamethasone on osteoblasts. In contrast, bone resorption markers such as urine deoxypyridinoline and serum C-terminal telopeptide of type I collagen did not decrease obviously after BD therapy. Adverse events included grade 1-3 thrombocytopenia, grade 1-2 peripheral neuropathy, and grade 3 ileus, but these were transient and manageable. Importantly, no patient developed pulmonary complications. Thus, our results suggest that BD therapy is safe and promising therapeutic approach for Japanese patients with MM

PO-312

AZD6244, A MEK1/2 INHIBITOR, INHIBITS OSTEOCLAST DIFFERENTIATION AND ACTIVITY IN MULTIPLE MYELOMA

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Introduction. Multiple myeloma (MM)-associated bone disease is caused by upregulation of osteoclast (OCL) activity and constitutive inhibition of osteoblast function. The extracellular signal-regulated kinase 1/2 (ERK1/2) MAPK pathway contributes to cytokine-induced OCL differentiation and maturation. We hypothesized that inhibition of ERK1/2 could prevent OCL differentiation and downregulate OCL function. We investigated the effects of AZD6244 (ARRY-142886), which blocks the ERK1/2 MAPK pathway via direct inhibition of MEK1/2, on OCL in MM. *Materials and Methods.* Peripheral blood mononuclear cells (PBMC) from healthy donors and MM patients were harvested and stimulated with RANKL (50 ng/mL) and M-CSF (25 ng/mL) for 3 weeks to induce OCL formation, with or without addition of AZD6244 at concentrations of 0.02 - 10 μM. *Results*. OCL characteristics measured by flow cytometric analysis showed loss of anti-alphaVbeta3 integrin expression. OCL differentiation was determined by measuring the number of TRAP positive cells following treatment with AZD6244. AZD6244 inhibited OCL differentiation in a dose-dependent manner, causing a marked loss of TRAP+ cells. OCLs were cultured with dentine discs in the presence or absence of AZD6244 to assess bone resorption activity. We found that AZD6244 inhibited bone resorption. In addition to p-ERK downregulation, we found inhibition of c-fos and nuclear factor of activated T cells c1 (NFATc1), whereas no influence on PU.1 was seen. Two major myeloma growth and survival factors produced by OCLs, B-cell activation factor (BAFF) and a proliferation-inducing ligand (APRIL), were measured in OCL culture supernatants by ELISA. AZD6244 significantly inhibited secretion of BAFF and APRIL. The levels of macrophage inflammatory protein (MIP-1alpha), an important OCL differentiation factor and MM survival factor, were also reduced. *Conclusion*. These results indicate that AZD6244 inhibits OCL differentiation, leading to reduced bone resorption activity. Moreover, AZD6244 downregulates MIP-1alpha and BAFF and APRIL secretion by OCL, which could inhibit MM cell survival in the bone marrow microenvironment. We have previously demonstrated that AZD6244 inhibits proliferation and survival of human MM cell lines and freshly isolated patient MM cells (Abstracts #3463 & #3467, ASH 2006). In conclusion, the present study provides a preclinical rationale for the evaluation of AZD6244 as a potential new therapy for MM patients.

PO-313

LENALIDOMIDE AND BORTEZOMIB IN MULTIPLE MYELOMA: INFLUENCE ON OSTEOCLAST

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Introduction. Osteolytic bone disease in Multiple Myeloma (MM) is caused by enhanced osteoclast (OCL) activation and inhibition of osteoblast function. The proteasome inhibitor bortezomib (PS341, Velcade) has potent anti-myeloma activity with impressive clinical responses. A recent study indicated that bortezomib has inhibitory effects on OCL. Lenalidomide (CC-5013, Revlimid) is an immunomodulatory derivative of thalidomide that has shown promising anti-MM effects in patients with relapsed or refractory MM (Richardson et. al, Blood Jul 06). Significantly, a phase I clinical trial showed that lenalidomide and bortezomib could achieve responses in the majority of patients with MM, refractory to either agent alone. However, the effect of lenalidomide on human OCL lineage is unknown. Here we investigated the effect of lenalidomide and bortezomib on human OCL. *Materials and Methods.* Peripheral blood mononuclear cells (PBMC) from MM patients and healthy donors were stimulated with receptor activator of NFkappa-B ligand (RANKL) (50 ng/mL) and M-CSF (25 ng/mL) for two weeks to induce OCL formation, in the presence or absence of lenalidomide or bortezomib. *Results*. OCL were identified by flow cytometric analysis using anti-alphaVbeta3 integrin. Lenalidomide and bortezomib inhibited alphaVbeta3 integrin expression in a dose-dependent manner. TRAP staining (tartrate-resistant acid phosphatase) was performed to identify OCL. Lenalidomide as well as bortezomib inhibited OCL in a dose-dependent manner, as evidenced by a marked decrease in TRAP+ cells. To assess bone resorption activity, OCL were cultured with dentine discs, in the presence or absence of lenalidomide and bortezomib, followed by light microscopic analysis and additional measurement of soluble collagen I fragments from the supernatant. Both lenalidomide and bortezomib inhibited bone resorption in a dose-dependent manner. In addition, OCL culture supernatants were collected, and two major MM growth and survival factors produced by OCL, B-cell activation factor (BAFF) and a proliferation-inducing ligand (APRIL), were measured by specific ELISA. Both lenalidomide and bortezomib inhibited secretion of BAFF and APRIL. *Conclusion*. These results indicate, that lenalidomide and bortezomib both inhibit OCL differentiation and activity, respectively, thereby directly preventing the development of new osteolytic lesions. Moreover, BAFF and APRIL secretion by OCL is downregulated, inhibiting MM cell survival in the bone marrow microenvironment.

PO-314

RESVERATROL AND ITS ANALOGS FOR TREATING MULTIPLE MYELOMA

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Introduction. Multiple myeloma is characterized by the accumulation of malignant plasma cells in the bone marrow, and is nearly always associated with bone destruction. The bone lesions result from increased osteoclastic (OC) bone resorption and impaired osteoblastic (OB) bone formation. Resveratrol (RSV, trans-3, 4, 5-trihydroxystilebene), a natural polyphenolic phytoalexin found in various food products, is considered as a promising cancer chemopreventive agent. RSV targets molecular pathways that cancer cells and OC have in common. Therefore it is of interest to investigate RSV and its structurally modified analogs on myeloma cells (MM), OC, and OB. *Methods*. RSV and 5 RSV analogs were screened *in vitro* for their potency towards MM cell lines, differentiation of OC from human monocytes, OC bone resorption, and induction of OB differentiation markers in a human mesenchymal stem cell line (MSC). *Results*. RSV reduced dose-dependently the growth of MM by a mechanism involving cell apoptosis, but none of its analogs affected MM proliferation or induced apoptosis. Both RSV and its analogs inhibited OC formation in a dose dependent manner, but did not show

a direct effect on the bone resorptive activity of OC. Two of the analogs were about 5000 times more potent than RSV. Finally, both RSV and its analogs promoted the expression of OB markers in MSC. These included alkaline phosphatase, osteoclacin and osteopontin. RSV and two analogs also upregulated bone morphogenic protein-2 in MSC, synergistically with vitamin D3. *Conclusions*. A single compound, RSV, prevented myeloma cell proliferation, inhibited bone resorption through prevention of OC differentiation, and promoted OB differentiation markers in MSC. Its analogs showed higly increased potency against OC bone resorption, and promoted OB differentiation, but did not antagonize MM. These differences in potency suggest differences in mechanisms of action depending on the cell type, and stress the potential of RSV analogs for treating myeloma bone disease.

PO-315

INTERMITTENT TRATMENT WIH BORTEZOMIB INHIBITS TRANSIENTLY OSTEOCLAST RESORPTIVE ACTIVITY

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Introduction. Multiple myeloma (MM)-induced bone disease is due to acute degradation of bone by osteoclasts (OC), and absence of repair by bone forming osteoblasts (OB). It is currently treated with bisphosphonates, strong bone resorption inhibitors, which do not stimulate bone formation and may have undesirable effects. There are indications that the proteasome inhibitor Bortezomib (V) used for treating MM patients stimulates bone formation. The present study aims at investigating if V inhibits OC activity. *Methods*. OC were differentiated from human $CD14^+$ monocytes and treated continuously for 6-7 days with V at various concentrations. As prolonged inhibition of proteasome activity is toxic for any cell type, and as V is eliminated from the vascular compartment 30min after intravenous injection, V was also given intermittently, to mimick the *in vivo* situation. OC activity was assessed by measuring Tartrate-Resistant Acid Phosphatase (TRACP) activity in the medium, and generation of resorption pits when culturing OC on bone slices. Cell viability was determined by measuring metabolic activity. To extend our observations to the clinical situation, serum levels of CTX-I, a bone resorption marker, were measured during the 3 days following V administration in a single patient. *Results*. A continuous treatment of cultures with V at 4 nM and higher concentrations was highly toxic for OC and monocytes. A 3-hour-pulse treatment with V followed by a 3-day culture in the absence of V, was not toxic neither to monocytes nor to OC, even at a concentration as high as 100 nM. This 3-hour pulse was however highly toxic for myeloma cells. Interestingly, a 3-hour pulse with 25 nM V induced a 50% inhibition of the resorptive activity of OC. The release of TRACP in the medium was inhibited to a similar extent within the first 24 hours post-pulse, but tended to return to the control level during the next 2 days. This 3-hour pulse with 25 nM V inhibited strongly RANKL-induced translocation of NF-κB in the OC nuclei, an event dependent on proteasome function and critical for OC activity. Serum CTX-I levels decreased during the first 48 hours after each V injection (n=3), and tended to increase again after 72 hours suggesting a partial recovery of OC activity between each administration. *Conclusions*. Our results suggest that Bortezomib temporarily inhibits OC activity *in vitro* and *in vivo*. This effect is linked to RANKL-induced translocation of NF-κB in the OC nuclei and proteasome function.

PO-316

SERIAL EVALUATION OF BONE REMODELING IN NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS TREATED WITH THALIDOMIDE-DEXAMETHASONE, DOUBLE AUTOLOGOUS STEM CELL TRANSPLANT AND ZOLEDRONIC ACID

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The interactions between myeloma (MM) clone and bone marrow microenvironment promote bone resorption and decrease bone formation; treatment of MM-related bone disease could thus take advantage of both reduction of tumor burden with cytotoxic chemotherapy and inhibition of osteoclast activity by bisphosphonates. However, it has not been clarified yet whether these two approaches should be combined and whether bisphosphonates should be continued upon completion of anti MM therapy. The aim of this study was to evaluate bone remodelling through the various phases of a high-dose therapy program and after conclusion of the whole therapy. The study included 38 newly diagnosed MM patients (median age = 54 yrs, M/F 23/15) enrolled in the *Bologna 2002* clinical trial. By study design, all patients received four months of primary therapy with thalidomide (200 mg/d) combined with high-dose dexamethasone (40mg/d on d 1-4, 9-12, 17-20 on odd cycles and on d 1-4 on even cycles) followed by double autologous transplantation with melphalan 200 mg/sqm. Daily thalidomide (200 mg/d) and monthly courses of dexamethasone were continued until the second autologous transplantation. Intravenous zoledronic acid 4 mg every 28 days was administered throughout the whole treatment period and continued thereafter. Quantification of bone resorption (urinary free-pyridinoline (PYD), deoxypyridinoline (DPYD) and C-terminal telopeptide (serum crosslaps) bone formation (bone alkaline phosphatase (BAP) and osteocalcin) markers was performed at study entry, after 4 months of thalidomide-dexamethasone therapy, and 3 months after first and second transplant, respectively. A significant decrease in serum crosslaps was observed after 4 months of thalidomide-dexamethasone as compared to pre-treatment values (1873pmol/L±208.SE vs 5997pmol/L ±792SE, p=0.000), at the same time, however, a significant reduction in bone formation parameters was also observed (BAp=12.25 IU/mL±1.40SE vs 17.29IU/mL±1.21SE, p=0.008; osteocalcin = 12.19 ng/mL±1.52SE vs 25.89ng/mL± 2.22SE, p=0.000). Three months after 1st transplant we detected a further significant reduction in serum crosslaps as compared to post induction values, while bone formation parameters remained stable. Three months after completion of the second autologous transplant, no change in bone resorption pattern was observed, however both BAP (13.59U/L±7.98SE vs 10.78±3.4SE) and osteocalcin (14.86ng/mL±5.46SE vs 12.65ng/mL±3.97SE) were significantly increased as compared to post 1st transplant values. Our results seem to suggest that therapy aimed at eradicating the myeloma clone plays a dominant role in reducing bone resorption in the initial phases of treatment. Bisphosphonates, however, appear to be useful to restore bone formation and should thus be continued after completion of anti-MM therapy.

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UP-REGULATION OF TACE IN MONOCYTES DISRUPTS MYELOMA CELL-INDUCED OSTEOCLASTOGENESIS AND INDUCES MYELOID DENDRITIC CELL DIFFERENTIATION

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Osteoclasts (OCs) and myeloid dendritic cells (DCs) are derived from the same monocytic precursor cells; induction of the differentiation into OC and DC lineages is reciprocally regulated in a mutually exclusive fashion. Multiple myeloma (MM) cells affect the reciprocal regulation of differentiation into OC and DC lineages towards OC lineage. M-CSF and RANK ligand induce osteoclastogenesis from monocytes, while GM-CSF and IL-4 in combination trigger the differentiation of myeloid DC. We found that addition of GM-CSF and IL-4 in combination together with M-CSF and soluble RANK ligand completely suppressed osteoclastogenesis from monocytes, while potently inducing myeloid DC formation. In the present study, we explored the mechanism by which GM-CSF and IL-4, inducers of myeloid DC differentiation, inhibit osteoclastogenesis from human monocytes and the effects of such cytokine combination on MM cell-induced osteoclastogenesis. GM-CSF and IL-4 potently down-regulated surface expression of M-CSFR on monocytes and concomitantly up-regulated soluble form of M-CSFR in their culture supernatants, suggesting ectodomain shedding of membrane-bound M-CSFR. Interestingly, GM-CSF and IL-4 potently enhanced the activity as well as mRNA expression of TNF-α converting enzyme (TACE). TAPI-0, a TACE inhibitor, restored cell-surface M-CSFR and reduced the levels of soluble M-CSFR in monocytes in the presence of GM-CSF and IL-4, suggesting TACE-dependent cleavage of cell-surface M-CSFR. Furthermore, TAPI-0 restored OC formation induced by M-CSF and RANK ligand in a soluble M-CSFR-inhibitable fashion even in the presence of GM-CSF and IL-4, and at the same time suppressed CD1a+ myeloid DC formation. Thus, GM-CSF and IL-4 inhibit M-CSF and RANK ligand-mediated OC formation and induce myeloid DC differentiation by enhancing TACE expression and activity. Importantly, the GM-CSF and IL-4 treatment also disrupted osteoclastogenesis enhanced by U266 MM cells and induced CD1a+ myeloid DC differentiation in a TACE-dependent fashion. These observations suggest that GM-CSF and IL-4 in combination or other alternatives to up-regulate endogenous TACE activity specifically in monocytic lineage cells may have a potential as a novel therapeutic maneuver to drive into DC lineage the monocytic differentiation deflected to OC lineage in MM.

PO-318

TGF-BETA INHIBITION RESTORES BONE FORMATION TO SUPPRESS TUMOR GROWTH IN MYELOMA

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Myeloma cells stimulate bone resorption by enhancing osteoclast formation and suppress bone formation by inhibiting stromal cell differentiation into osteoblasts (OB), which creates a microenvironment suitable for myeloma cell growth and survival (a myeloma niche). TGF-β, an inhibitor for OB differentiation and mineralization, is abundantly deposited in a bone matrix, and released from the bone through bone resorption. In myeloma bone lesions with enhanced bone resorption TGF-β is thought to be accumulated and impair stromal cell differentiation into OB. We, therefore, hypothesized that TGF-β inhibition may restore OB differentiation from stromal cells to disrupt a myeloma niche. In the present study, we aimed to clarify the impact of TGF-β inhibition on myeloma tumor expansion along with suppression of OB differentiation in myeloma. SB431542, a TGF-β type 1 receptor kinase inhibitor, facilitated OB differentiation of stromal cells as well as MC3T3-E1 pre-osteoblastic cells. Notably, SB431542 in combination with BMP-2 was able to resume OB differentiation suppressed by conditioned media from myeloma cells as well as excessive TGF-β added exogenously, suggesting restoration of bone formation in myeloma through blockade of TGF-β actions. Interestingly, MC3T3-E1 cells differentiated enough to form mineralized nodules potently induced apoptosis in 5TGM1 and RPMI8226 MM cells with G1 arrest in sharp contrast to untreated or TGF-β-treated undifferentiated MC3T3-E1 cells. Such anti- myeloma actions still remained in MC3T3-E1 cells differentiated in the absence of beta-glycerophosphate without forming mineralized nodules, suggesting a responsible role for OB-derived non-mineralized factors associated with terminal differentiation of OB. Interactions between MM cells and stromal cells eventually give rise to drug resistance; the development of novel therapeutic maneuver to overcome the drug resistance is a major clinical issue in the treatment for myeloma. In this regard, OB terminally differentiated from stromal cells potentiated the cytotoxic effects of doxorubicin on myeloma cells, suggesting that OB may reverse the susceptibility of myeloma cells to chemotherapeutic agents. Collectively, these results are consistent with a hypothesis that induction of OB differentiation by TGF-beta inhibition can disrupt a myeloma niche, thereby containing myeloma tumor expansion with amelioration of destructive bone lesions.

PO-319

A SOLUBLE ACTIVIN TYPE II RECEPTOR PREVENTS MYELOMA BONE DISEASE

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Multiple myeloma is associated with the development of bone disease characterised by increased osteoclast activity and a suppression of osteoblastic bone formation. Our understanding of the molecular mechanisms responsible for increased osteoclastic activity has improved; however, our knowledge of the mechanism responsible for inhibiting bone formation remains poor. Equally, the effect of targeting bone formation rather than resorption is unknown. Recently, an antagonist of activin, a soluble form of the extra-cellular domain of the murine activin type II receptor, fused to a murine IgG-Fc fragment, (RAP-011) was shown to reverse ovariectomy-induced bone loss *in vivo*; however, the effect of this antagonist on myeloma bone disease is unknown. In the present study we investigated whether decreased bone formation contributes to the bone disease and whether RAP-011 prevents bone disease in the 5T2MM model of myeloma. 5T2MM cells injected into C57Bl/KaLwRij mice promoted a significant increase in osteoclast surface, the formation of osteolytic lesions and caused a significant decrease in bone area. Bone disease was associated with a decrease in osteoblast number ($p<0.001$), osteoblast surface ($p<0.001$) and a reduction in mineralization ($p<0.01$). Mice bearing 5T2MM cells were then treated with RAP-011 (10mg/kg, i.p. twice weekly), or a vehicle, from the time of 5T2MM injection, for a total of 12 weeks. MicroCT analysis of the proximal tibia and lumbar vertebrae demonstrated a 39% and 21% reduction in cancellous bone volume ($p<0.001$ and $p<0.01$) and a 37% and 15% reduction in trabecular number ($p<0.01$ and $p<0.05$) in 5T2MM-bearing mice compared to naive mice. RAP-011 completely prevented 5T2MM-induced decreases in trabecular volume and number in both tibia ($p<0.001$ and $p<0.05$) and vertebrae ($p<0.01$ and $p<0.05$) when compared to vehicle treated mice. Bone volume was 19% higher in the tibia ($p=168$) and 12% higher in vertebrae ($p<0.05$) of RAP-011 treated mice than naive non tumour bearing mice. RAP-011 prevented the development of osteolytic bone lesions ($p<0.05$), but had no effect on serum paraprotein or myeloma burden. These data suggest that the soluble activin type II receptor construct, RAP-011, prevents the development of osteolytic disease and represents a novel therapeutic approach to treating myeloma bone disease.

PO-320

THE EFFECT OF BORTEZOMIB MONOTHERAPY AND BORTEZOMIB-BASED REGIMENS ON BONE METABOLISM IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA

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Introduction. Bortezomib is a proteasome inhibitor, which is currently indicated for the treatment of relapsed/refractory myeloma (MM). Recent reports suggest that bortezomib increases osteoblast function and normalizes impaired bone metabolism in MM. The aim of this study was to evaluate the effect of bortezomib monotherapy and bortezomib-based regimens on bone turnover in patients with relapsed/refractory

MM. *Patients and Methods.* We studied prospectively 30 patients who received bortezomib monotherapy at the standard dose (1.3 mg/m^2), 11 patients who received the combination of bortezomib with dexamethasone (BD) and 53 patients who received the VMDT combination (velcade at a dose of 1.0 mg/m^2, melphalan, dexamethasone and intermittent thalidomide). The following serum indices of bone metabolism were measured on day 1 of cycle 1, and then on day 21 of cycle 4 for bortezomib monotherapy and BD regimen and on day 28 of cycle 4 for VMDT regimen: (i) osteoblast inhibitor dickkopf-1 (DKK-1); (ii) osteoclast regulators: soluble RANKL (sRANKL) and osteoprotegerin (OPG); (iii) bone resorption markers: C-telopeptide of collagen type-I (CTX) and tartrate-resistant acid phosphatase type-5b (TRACP-5b); and (iv) bone formation markers: bone-specific ALP (bALP) and osteocalcin (OC). We also studied 33 healthy controls of similar gender and age. *Results*. The objective response rate after 4 cycles of therapy was 46% for bortezomib monotherapy, 54% for BD combination and 60% for VMDT regimen. Myeloma patients of all studied groups had increased values of DKK-1, sRANKL, sRANKL/OPG ratio, and both markers of bone resorption at baseline (p<0.01). In contrast, bone formation as assessed by serum bALP and OC was significantly reduced (p<0.01). The administration of bortezomib produced a significant reduction of DKK-1 (p<0.04), sRANKL (p=0.01), CTX and TRACP-5b (p<0.001) after 4 cycles in all studied groups. There were no significant differences in terms of % reduction of these parameters between the 3 studied groups, although the VMDT regimen showed a greater median reduction of sRANKL (26% compared with baseline values) than bortezomib monotherapy (10%). Bortezomib monotherapy produced a dramatic increase in both markers of bone formation after 4 cycles of therapy (p<0.01). This effect was milder in patients who received the combination of BD (p=0.03) and was not observed in patients who received the VMDT regimen. More specifically, in VMDT there was an 8% reduction of bALP after 4 cycles of therapy and a 9% increase of OC (p=NS). The alterations in the majority of bone remodelling markers were irrespective of response to therapy. No healing of the lytic lesions was observed even in CR patients. *Conclusion*. This study suggests that bortezomib, either alone or in combination with other anti-myeloma agents, reduces bone resorption in patients with relapsed/refractory myeloma. However, the beneficial effect of bortezomib on bone formation seems to be reduced or lost when it is combined with other anti-myeloma agents.

PO-321

IN VIVO EFFECTS OF ZOLEDRONIC ACID ON BONE REMODELLING

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Zoledronic acid (ZA) is an amino-bisphosphonate with very potent antiresorptive activity which is widely used in the treatment of myeloma bone disease. The increasing incidence of osteonecrosis of the jaw and its possible association with the prolonged use of amino-bisphosphonates such as ZA led us to study the effects of increasing doses of ZA on *in vivo* bone remodelling. Although ZA has been shown to inhibit osteoclastogenesis and increase bone mineralization, very little data exists on its effects on osteoblastic activity and bone vascularity. In order to study these effects, 5 week old C57black6 mice were treated weekly intraperitoneally with saline or ZA at increasing doses (0.05-0.5 or 5 mg/Kg; the highest dose recapitulating a life time dose of ZA over a 5 year period in an adult multiple myeloma patient). Blood was collected at baseline and weekly, prior to ZA treatment. Intraperitoneal calcein injections were administered in order to study bone formation rates. Animals were sacrificed at the end of the third week, and ELISA assays for quantification of osteocalcein and TRAP5B were performed. Bone mineral density and bone mineral content were evaluated with PIXImus bone densitometer. Our preliminary results demonstrate an increase in bone mineral content, corrected for surface and weight, as well as bone mineral density, induced by ZA treatment. This was associated with decreased osteocalcein (bone formation) and TRAP5B (bone resorption) levels in ZA treated animals. MicroCT analysis, histomorphometry and immunohistochemistry with specific focus on effects on both osteoclasts and osteoblasts lineage cells, as well as effects on bone vascularity and mineralization, will be presented.

PO-322

ALPHAVBETA3$^+$ (AVB3) ADHESION MOLECULE DRIVES THE OSTEOCLAST (OC)-LIKE FUNCTIONAL DIFFERENTIATION OF MYELOMA CELLS (MC)

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Introduction. Accelerated osteoclastogenesis is the major event promoting the skeletal impairment in multiple myeloma (MM). OC are directly activated by MC, although these cells themselves may undergo OC-like morphologic transformation and produce bone erosions *in vitro*. Since OCs exert their function through several adhesion molecules, including AvB3, we investigated the role of this integrin expressed by MCs in their transdifferentiation to OCs. *Methods*. Marrow MCs were purified from eight patients with severe skeletal disease and two patients without bone lesions. U266 and RPMI-8226 MC lines were the controls. Semi-nested PCR assessed the CDR3 immunoglobulin (Ig) gene rearrangement, whereas OC markers including TRAcP, cathepsin-k, calcitonin-receptor, carbonic anhydrase and vATPase were evaluated by RT-PCR. The cytoskeletal rearrangement of F-actin was analyzed by immunofluorescence. Av and B3 expression by MCs was evaluated by flow-cytometry, whereas bone erosion on calcium phosphate discs and number of pits was measured by dedicated software. The role of AvB3 in OC-like transdifferentiation was explored in MCs by siRNA silencing for both chains. *Results*. Ontogenetic derivation from the MC lineage was confirmed by the monoclonal CDR3 rearrangement, CD138/CD38 and cIg expression. Cells from patients with bone disease (BD) expressed OC markers, in contrast with patients without BD or U266 and RPMI-8226. Formation of the F-actin ring confirmed the differentiation of MCs toward the OC-like phenotype. Cells from patients with BD expressed Av and B3 (80+7% and 75+9%) similarly to U266 and RPMI-8226 (>90%), whereas a minimal expression was occasionally demonstrated in a control patient (Av:2%; B3:8%). AvB3+ cells produced a high number of erosive pits, at variance from AvB3- cells (35+8 vs. 4+1 pits/cm2). Finally, the silencing of Av and/or B3 expression inhibited erosion in AvB3$^+$ cells, resulting in a reduced number of pits (7+2 pits/cm2) similar to AvB3- cells. *Conclusions*. Since AvB3 is involved in adhesion of marrow macrophages undergoing OC differentiation, it is conceivable that AvB3$^+$ MCs transdifferentiate to OCs in response to integrin activation, induced by the contact with stromal cells within the marrow environment. In addition, our data suggest that MCs induce this effect by AvB3, since its silencing reduces the osteoclastogenesis *in vitro*.

PO-323

MLN3897 IMPAIRS OSTEOCLASTOGENESIS BY C-FOS INHIBITION

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Osteolytic lesions due to enhanced osteoclast (OC) activity are a hallmark of myeloma bone disease and other metastatic cancers. Chemokines play a critical role in enhancing OC formation and activity. Since CCR1 is one of the main chemokine receptors expressed on monocytes and OC, we here characterized the effects of MLN3897 (Millennium Pharmaceuticals), a novel specific antagonist of CCR1, on OC formation and function. In order to analyze the effects of MLN3897 on osteoclastogenesis, we generated OC from peripheral blood mononuclear cells of healthy donors stimulated with RANKL and M-CSF (50 ng/mL) for 3 weeks, in the absence or presence of MLN3897. Mature OC were multinucleated TRAP+ cells, and their functional activity was confirmed by pit formation and collagen release ELISA assays. CCR1 expression was induced early during OC formation, at one week 60% of the monocyte population expressed CCR1 by flow cytometric analysis. Macrophage inflammatory protein-1 α secretion was also stimulated, from 57 pg/mL at 24 hours to 156 pg/mL at one week. Treatment with MLN 3897 (10 nM) interfered with this autocrine loop, resulting in inhibition of both OC formation (by 40%) and function (by 70%) in a dose-dependent way (p<0.05). Our data demonstrate that MLN3897 induced a 60% reduction in the multinucleated cell number at one week (con-

trol 61±14 vs treated 35±9), without affecting cell viability. MLN3897 also inhibited the OC-specific protease cathepsin k activity and protein expression, suggesting an independent effect of CCR1 inhibition on OC formation and activity. We further characterized the mechanism of action of MLN3897, analyzing the expression levels of c-fos and ERK, important mediators of OC differentiation. MLN3897 (10 nM) resulted in reduced c-fos transcription levels and concomitant abrogation of ERK activation. Taken together, these data suggest that CCR1 inhibition by MLN3897 blocks ERK pathway activation and c-fos expression and results in impaired monocyte multinucleation process as well as cathepsin K expression. These studies delineate a novel mechanism of action of MLN3897 on osteoclastogenesis, and provide the therapeutic rationale for its clinical evaluation for treatment of osteolytic bone disease.

PO-324

MLN3897 DISRUPTS OSTEOCLAST-MYELOMA CELLS INTERACTION

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Multiple Myeloma (MM) is characterized by increased osteoclast (OC) activity leading to severe bone disease. Macrophage inflammatory protein-1 alpha (MIP-1α) and RANTES activate CCR1 resulting in increased OC number and activity. Here, we studied the effects of a specific CCR1 antagonist, MLN3897 (Millenium Pharmaceuticals), on mature OC obtained by stimulating peripheral blood mononuclear cells of healthy donors with RANKL and MCSF (50 ng/mL) for three weeks. OC expressed high levels of CCR1 and secreted both MIP-1α (1 ng/mL ±1.8) and RANTES (12 pg/mL±0.66), suggesting an autocrine effect of these chemokines. Analyzing nuclear integrity with Hoechst33258 staining, we observed that after 12 hours of cytokine-deprivation MLN3897 enhanced OC nuclear fragmentation and condensation, suggesting a role for CCR1 ligands in OC survival. We also reported impaired OC function by treatment with MLN3897 (10 nM) for 72h in the presence of RANKL and MCSF (20% of reduction in resorbed area quantified by pit formation assay). We next studied the interactions between OC and MM cells. CCR1 was highly expressed on RPMI8266 MM cells, whereas INA6 showed lower levels (86% and 5% respectively, by flow cytometry). No direct cytotoxicity or growth arrest of these cell lines was induced by MLN3897. OC potently stimulated migration of RPMI8226 MM cells (8-fold increase), which was almost completely blocked by treatment with MLN3897. In contrast, MIP-1α alone induced only a modest effect on migration (2-fold increase), and neutralizing MIP-1α antibody partially reversed OC migratory effect, suggesting that other factors may contribute to it. Since OC secrete IL6, we studied their ability to stimulate INA6 proliferation. In coculture experiments, OC induced high proliferation rates of INA6 cells, while addition of MLN3897 for 48h reduced both IL-6 secretion by OC and INA6 proliferation (by 70% and 60% respectively). Our data therefore demonstrate that CCR1 inhibition by MLN3897 impairs mature OC survival and activity and it affects MM-OC interactions inhibiting migration to OC sites and proliferation of MM cells. Ongoing studies are further characterizing the effects of CCR1 inhibition on MM-OC interactions in order to provide the framework for its clinical evaluation in MM.

PO-325

INCADRONATE CAN INDUCE APOPTOSIS IN MONOCYTIC CELLS

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Introductions. Since the introduction of bisphosphonate(BP)s, the skeletal events have significantly decreased in myeloma. Besides,possible direct and indirect anti-myeloma effects of BPs have been reported. However, infusions of BPs are occasionally associated with adverse events including inflammatory responses with fever and CRP elevation. Through these observations, we speculate that BP might exert anti-monocytic cell activity, possibly causing a release of inflammatory cytokines. This study was undertaken to elucidate the possible apoptosis-inducing effects of BPs on human monocytic cells such as monoblast, monocyte, macrophage, dendritic cell (DC)s, and osteoclast (OC)s. *Materials and Methods*. THP-1, human monoblastic cell line, myeloma cell lines (RPMI8226, ARH77), and human blood monocytes were used. THP-1 cells and blood monocytes were stimulated using GM-CSF, LPS, IL-4, M-CSF, RANKL and other factors to obtain macrophage, DC, and OC. Unstimutaled cells were used as monoblasts or monocytes. Incadronate, YM-175, a gift from Asteras Pharmaceutical Company, was mainly used as BP. Annexin-V, TUNEL method, and morphological analyses were used to evaluate apoptosis. Western blotting analyses of caspase-3, 4, 5, 8, 9, 10 and other molecules as well as specific inhibitors to caspase molecules, were used to analyze the apoptosis. MAP kinase pathways were also analyzed. *Results*. Induction of macrophage, DC and OC were confirmed by surface or cytochemical markers, including the positivity of CD83, 86, 206 and 209 for DCs by GM-CSF + IL-4 or GM-CSF + LPS stimulation, and CD1a, CD11b and CD14 positivity for macrophages by GM-CSF or M-CSF stimulation. BP induced apoptosis not only in all monocytic cells (monoblast, macrophage, DC and OC) obtained from THP-1 cells but in monocyte, macrophages, DCs obtained from blood monocyte, at significantly lower concentrations (50 micromolar) than the concentration which induced apoptosis in myeloma cells (300-400 micromolar). Apoptosis of THP-1 by BP was associated with activation of caspase 3 and 7. At 12 hours after BP, phosphorylation of JNK was observed. iv) *Conclusions*. Incadronate induced apoptosis in several monocytic cells at lower concentration. Cytokine release and altered antigen presentation must be kept in mind when we prescribe BP for myeloma.

PO-326

ZOLEDRONIC ACID AND SURVIVAL IN PATIENTS WITH HIGH BALP

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Introduction. Biochemical markers of bone metabolism such as bone-specific alkaline phosphatase (BALP) provide insight into the ongoing pathophysiology of bone lesions. Zoledronic acid reduces markers of bone metabolism and the risk of skeletal morbidity in patients with multiple myeloma (MM). Preclinical studies in mouse models of myeloma have demonstrated that zoledronic acid has antimyeloma effects. Therefore, zoledronic acid may not only prevent skeletal complications but also increase survival in patients with MM. *Materials and Methods.* A retrospective exploratory analysis of patients with bone lesions from MM in a large (n=353), randomized, controlled trial of zoledronic acid 4 mg versus pamidronate 90 mg was performed to determine the effect of zoledronic acid on survival based on baseline BALP levels. Study drug was administered every 3 or 4 weeks for up to 24 months, with a final assessment at 25 months. Risk of death was assessed using univariate and multivariate Cox regression models that included prior skeletal-related events and baseline ECOG performance status. *Results*. Among MM patients who had baseline BALP assessments (n=212), zoledronic acid significantly increased the 25-month overall survival compared with pamidronate (76% versus 63%; p=0.026) and significantly reduced the risk of death by approximately 42% compared with pamidronate (p=0.03 for both) in univariate and multivariate analyses. However, in the overall MM population there were no significant between-group differences in survival. Among patients with high baseline BALP (≥146 U/L; n=89), zoledronic acid significantly improved survival compared with pamidronate (82% versus 53%; p=0.041) and significantly reduced the risk of death by approximately 56% compared with pamidronate in both univariate and multivariate analyses (p<0.05 for both). Among patients who had low baseline BALP (<146 U/L; n=123), zoledronic acid reduced the risk of death by approximately 30% compared with pamidronate, although this did not reach statistical significance (p>0.2) due to small sample size. In this subset, 25-month survival was similar for both treatment groups. *Conclusions*. These exploratory analyses suggest a survival benefit in MM patients with high baseline BALP levels treated with zoledronic acid compared with pamidronate. Prospective trials are needed to investigate the possible survival benefits in the high-BALP subset.

PO-327

ZOLEDRONIC ACID IS COST-EFFECTIVE VS. GENERIC PAMIDRONATE IN PATIENTS WITH MULTIPLE MYELOMA AND HIGH LEVELS OF BIOCHEMICAL MARKERS OF BONE FORMATION: EXPLORATORY ANALYSIS IN GERMANY AND THE UNITED KINGDOM

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Introduction. A recent retrospective analysis of a 25-month randomized clinical trial revealed that zoledronic acid (ZA) improves survival v. pamidronate (PA) in a subset of patients with myeloma bone disease and a high baseline bone-specific alkaline phosphatase (BALP) level (Dimopoulos *et al.*, 2006). The present analysis explores, from German and UK payer perspectives, the cost-effectiveness of ZA v. generic PA in multiple myeloma (MM) patients with high BALP. *Materials and methods*. A literature-based decision-analytic model was developed to compare quality-adjusted life-years (QALY) and direct cost of ZA v. generic PA in this population. Survival, skeletal related events (SRE), and bisphosphonate utilization data were derived from a post-hoc analysis of the clinical trial mentioned above. Drug, drug administration, and SRE costs were estimated using published sources and national tariffs (*e.g.*, British National Formulary). The impact of SREs on quality of life was estimated using the literature. Consistent with previous similar economic analyses (*e.g.*, Hillner *et al.*, 2000), patients were assumed to experience quality of life decrements lasting 1 month for each SRE experienced. *Results*. Compared to PA, ZA was projected to increase average survival by 2.68 months (from 18.72 to 21.40 months). The cumulative number of SRE was projected to be 2.18 per ZA patient v. 2.89 per PA patient. Per-patient drug-related costs were higher for ZA than PA. These costs were largely off-set by reductions in SRE-related costs, with ZA patients incurring €1,404 and £912 less in SRE-related costs in Germany and the UK, respectively. Compared to PA, the total cost of ZA were slightly higher (+€277) in Germany and lower (-£569) in the UK. ZA increased QALYs by 0.128. Thus, relative to PA, ZA was very cost effective, with a cost per QALY gained of €2168 (i.e., well below the €50,000 threshold) in Germany and was even cost saving in the UK. In sensitivity analyses, the cost effectiveness of ZA relative to PA was acceptable under a wide range of scenarios. *Conclusions*. This exploratory analysis suggests that ZA is highly cost effective compared to generic PA in German and UK MM patients with elevated baseline BALP levels.

GROUP 4: Imaging

PO-401

MICRO-COMPUTER TOMOGRAPHY (MICROCT) ANALYSIS DEMONSTRATES EXTENSIVE BONE LOSS IN THE KMS-12-BM XENOGENEIC MURINE MODEL OF MYELOMA

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We have recently described a new xenogeneic model of MM using the KMS-12-BM cell line (Blood, 2006, 108(11) 505a). Tail vein injection of 1x107 cells into unconditioned β2 m NOD/SCID mice leads to tumour infiltration in the bone marrow over a 6 week peroid, without any significant extramedullary disease. Using histomorphometric analysis we have shown an increase in osteoclasts in the tibiae and vertebrae of diseased animals, with a loss of trabecular bone in the tibiae (see table below). *Aim*. To further characterise the bone disease in this model using micro-computer tomography (micro CT). *Methods*. 1×10^7 KMS-12-BM cells or PBS (n=6) were injected via the tail vein into β2m NOD/SCID mice and bones examined 6 weeks later. Tibia and the first lumbar vertebra were scanned using the Skyscan 1172 microtomograph. Regions of interest were defined as trabecular or cortical bone that were 0.2 mm from the growth plate and extending a further 1 mm. Skyscan CT Analyzer software was used to calculate bone parameters. Trabecular bone 0.2 mm from the growth plate, within an area of 0.525mm^2 was assessed by histomorphometry. *Results*.

Tibiae

	Histology		MicroCT			
	TBA (%)	*TBV (%)*	*Trabecular Bone density (HU)*	*Trabecular Thickness (microns)*	*Trabecular Number (1/mm)*	*Cortical Thickness (microns)*
Diseased	2.5±0.5	2.5±0.7	4719±84	44.4±3.8	0.6±0.1	78.6±2.9
Control	5.9±2.5	6.4±1.3	5059±21	61.5±3.6	1.0±0.2	88.6±3.3
	p=0.03	p=0.03	p<0.01	p=0.01	p=0.03	p=0.02

Vertebrae

	Histology		MicroCT		
	TBA (%)	*TBV (%)*	*Trabecular Bone density (HU)*	*Trabecular Thickness (microns)*	*Trabecular Number (1/mm)*
Diseased	10.3±0.9	9.8+0.8	4486±29	37.5±0.9	2.6±0.2
Control	13.6±2.4	12.7±0.4	4539±41	42.0±0.9	3.1±0.2
	NS	p=0.01	NS	p=0.01	p=0.04

TBA=Trabecular Bone Area *TBV=Trabecular Bone Volume* *HU = Hounsfield Units*

Conclusion. The use of micro CT allows for detailed and extensive analysis of bone loss in this xenogeneic model of medullary myeloma. Loss of trabecular bone in the vertebrae was not previously evident with histomorphometric analysis. Micro CT is potentially a useful tool in assessing new therapies targeted at bone disease *in vivo*.

PO-402

DYNAMIC CONTRAST-ENHANCED MAGNETIC RESONANCE IMAGING IN MULTIPLE MYELOMA: FREQUENT DETECTION OF MICROCIRCULATION CHANGES IN ASYMPTOMATIC MYELOMA AND MGUS

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Introduction. Dynamic contrast-enhanced MRI (DCE MRI) is a further development of standard MRI adding functional information regarding bone marrow microcirculation using a pharmacokinetic analysis after a pump-controlled infusion of Gadolinium DTPA. Distribution of microcirculation and changes induced by MM can rapidly be viewed by a color-coded signal map superimposed onto the conventional MRI. Micro-

circulation data correlate with bone marrow angiogenic index and degree of myeloma infiltration (Nosas *et al.* 2005) and have important prognostic relevance regarding local and systemic disease activity (Moehler *et al.* 2001; Hillengass *et al.* 2007). *Material and Methods.* microcirculation in the bone marrow of patients with plasma cell disease was investigated using DCE MRI in 42 patients with MGUS, 45 with asymptomatic MM [aMM] (MGUS and aMM were termed non-requiring-chemotherapy-group [NRC group]) and 42 untreated patients with symptomatic disease (sMM) within a clinical trial. *Results*. Changes in the microcirculation pattern were identified in 57% of MGUS, 73% of aMM patients (total of 66% in the NRC group) and 84% of MM patients (differences between MGUS and sMM n.s.). We further analysed the pattern of microcirculation distribution and found 18 MGUS patients (43%) displaying a normal, 22 (52%) a diffuse and 2 (5%) a focal pattern. For the NRC group in 30 (34%) patients a normal, in 52 (60%) a diffuse and in 5 (6%) a focal pattern was seen. 40 (95%) of patients with symptomatic MM had an abnormal microcirculation pattern with 2 (5%) MM patients showing a normal, 19 (45%) a diffuse and 21 (50%) a focal pattern. *Conclusions*. DCE MRI is a fast and reliable technique linking functional data about bone marrow microcirculation with basic prognostic parameters as EFS. We identify microcirculation changes in a significant portion of patients with asymptomatic and early plasma cell disease indicating changes in the vascular system and microcirculation before onset of symptomatic disease. A current study evaluates correlation of quantitative microcirculation parameters and disease activity in untreated MM patients. Our findings could be the basis for stratified treatment of patients with novel therapeutics targeting the vascular system.

PO-403

BONE INVOLVEMENT IN MULTIPLE MYELOMA PATIENTS CARRYING THE T (4;14) CHROMOSOMAL TRANSLOCATION

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Chromosomal translocations involving the immunoglobulin heavy chain switch region (IgH) are quite common in multiple myeloma (MM), and some of them can reliably predict disease outcome. In particular, t(4;14) chromosomal abnormality is one of the most adverse prognostic factors for response duration and survival after high dose therapy and autologous stem cell transplantation. Despite the dismal prognosis, however, in this subset of patients, bone involvement, as evaluated by spine MRI, is relatively infrequent, at variance to what has been observed in other MM subtypes according to TC classification. In the present study we aimed at further testing this hypothesis by analyzing the extent of whole bone involvement in patients showing t(4;14) chromosomal translocation as compared to negative patients. For this purpose, 50 newly diagnosed MM patients (32M, 18F, median age = 54 yrs) underwent evaluation of total skeletal X-ray, whole spine MRI and, at the same time, quantification of markers of bone resorption (urinary NTX, PYR and DPYR and serum crosslaps) and bone formation (bone alkaline phosphatase-BAP and osteocalcin) was performed. Using a real-time PCR assay to detect the presence of IgH/MMSET fusion gene as a surrogate marker for t(4;14), we found 15 patients carrying this chromosomal abnormality, 7 of whom (46%) were also positive for the deletion of chromosome 13, this abnormality was detected in 11/35 (31%) patients who proved negative for IgH/MMSET hybrid transcript. The two groups of patients did not differ significantly in terms of median age, distribution of M protein isotype and light chain, beta-2 microglobulin, bone marrow plasma cell infiltration and disease stage. Marrow involvement, as detected with spinal MRI was similar in the two groups of patients, however, vertebral lesions were observed in 12/15 (80%) t(4;14) positive patients as compared to 14/35 (45%) t(4;14) negative patients; all the same, whole skeletal involvement was more pronounced in t(4;14) positive patients (median skeletal score = 6.24, as compared to 3.58 in t(4;14) negative cases, p=0.00). These data were confirmed by the evaluation of bone resorption markers; specifically, serum crosslaps were significantly increased in patients with t(4;14) abnormality compared to negative individuals (7984 pmol/L±1682SE vs 5123pmol/L±783SE p=0.04). Conversely, no difference in bone formation markers was found in the two groups of patients. Our results indicate that, even though the pattern of spinal involvement at MRI is similar in t(4;14) positive and negative patients, newly diagnosed MM patients who are t(4;14) positive show a more advanced bone disease and a more pronounced bone resorption pattern, this in contrast to what has been reported so far, but in line with the aggressive features of the disease in these individuals.

PO-404

WHOLE BODY MAGNETIC RESONANCE IMAGING IN MULTIPLE MYELOMA

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Introduction. Bony disease in multiple myeloma is traditionally assessed by radiological skeletal survey (RSS). However this technique has limitations and newer imaging techniques may provide a better assessment of bony disease. We have studied the use of whole body magnetic resonance imaging (WBMRI) in multiple myeloma. *Methods.* 169 WBMRI studies were performed (34 newly diagnosed myeloma, 40 relapsed myeloma, 45 follow up studies post treatment, 43 monoclonal gammopathy of undetermined significance(MGUS), 7 other plasma cell dyscrasias). The WBMRIs were performed using 1.5 Tesla scanning with moving table top in a procedure lasting 8 minutes. Each WBMRI and corresponding RSS was evaluated using a novel scoring system as follows: the body was divided up into 10 areas (skull, ribs, cervical spine, thoracic spine, lumbar spine, pelvis, right arm, left arm, right leg, left leg). In each area the extent of myeloma bone disease was scored as follows: 0=normal; 1=1 focus of abnormality; 2=more than 1 focus of abnormality; 3=diffuse disease. The scores for each of the ten areas were combined, giving an overall score out of thirty for WBMRI and RSS. *Results*. In patients with active myeloma 80% of patients had a positive WBMRI compared to a 54% with a positive RSS. Using our scoring system the mean score for WBMRI at 14.6/30 was significantly higher than that for RSS at 4.9/30. In patients with newly diagnosed multiple myeloma 80% had positive WBMRIs (mean score of 15.6/30) compared to 50% with a positive RSS (mean score 3.8/30). In patients with relapsed multiple myeloma 81% had positive WBMRIs (mean score of 11.8/30) compared to 62% with a positive RSS (mean score 6.4/30). WBMRI was significantly superior to RSS in all of the areas assessed except the skull. In follow up studies post treatment the WBMRI correlated with clinical response in 88% of cases. In MGUS patients 8% of studies were positive at a low level. *Conclusions*. We conclude that WBMRI is superior to RSS in both the identification and evaluation of extent of bone involvement in multiple myeloma. It is also useful in the assessment of response to therapy.

PO-405

DIFFUSION WEIGHTED IMAGING IN MULTIPLE MYELOMA PATIENTS ON 3 T MR: A NEW TOOL FOR TREATMENT RESPONSE?

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Aims. The objective of this small study was to determine the feasibility of whole body diffusion weighted MR imaging (WB-DWI) for therapeutic monitoring of myeloma lesions. *Materials and Methods.* A WB-DWI was performed on a 3 Tesla MRI system before and after treatment in 5 patients (M/F=3/2 ; average age=49 y (34-60 y) with Stage III multiple myeloma (n=4 and plasma cell leukaemia (n=1)). Therapeutic regimens were: bortezomib -/+ dexamethasone (n=3) or mephalan + autologue stem cell support (n=2). The images were reviewed retrospectively. Head, neck, thorax, abdomen, pelvis and upper part of the femur were included in the scan. Diffusion weighted images were acquired using three different b-values (b=0, 600 and 1250 s/mm^2). The signal intensity (SI) and the apparent diffusion coefficient (ADC)-values were calculated for all lesions before and after treatment. As a control, the SI and ADC-value were measured also in normal bone marrow areas and in muscles. *Results*. The mean period between the baseline MR and the follow-up scan was 5 months (range 2 to 7 months). In 4 patients a significant decrease in SI was seen for b600 and b1250 (p<0.0001) in all analysed lesions, while the ADC-value showed a significant increase (p=0.02). There was a good correlation between the imaging parameters and the skeletal related events and paraprotein detection in serum and urine. The only patient where a stable situation was found on SI, had less than a partial response on laboratory analysis. Comparison of SI and ADC-values in both scans showed no significant difference for normal bone (p>0.1) or muscles (p>0.1). *Conclusions*. An additional WB-DWI examination shows promise as a new and highly sensitive method for monitoring treatment of myeloma. A larger data set is required to examine whether WB-DWI MRI can be used as an alternate or even a more sensitive monitoring procedure than the currently used imaging tools.

PO-406

WHOLE BODY DIFFUSION WEIGHTED IMAGING BY 3T MR IN MULTIPLE PATIENTS: INITIAL EXPERIENCE

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Aims. To establish the feasibility of whole body MR by diffusion weighted imaging (WB-DWI) in evaluating bone destruction in multiple myeloma. *Materials and Methods*. Whole body MR-scans and all CT-scans from 10 patients were retrospectively reviewed. Head, neck, thorax, abdomen, pelvis and upper part of the femur were scanned by MR on a 3 Tesla MRI. Diffusion weighted images were acquired using three different b-values (b=0, 600 and 1250 s/mm^2). The signal intensity (SI) and the apparent diffusion coefficient (ADC)-values were calculated for each of the examined lesions. CT-scan was performed without contrast material. Eight osteolytic bone lesions on the CT-scan suspected for myeloma were selected in each patient and correlated with the images performed by whole body MR. For comparison, a WB-DWI was performed in a control group of 5 healthy volunteers. A number of regions in the bone marrow of similar size as in the patients were delineated, from which the SI and the ADC-values were calculated. *Results Patient characteristics*. M/F=8/2, mean age=55.5 (range 34-73 y), 9/10 Salmon Durie Stage III, 1 plasma cell leukaemia with average duration of disease of 26 months (range 7 days to 6 years). On visual inspection, there was an excellent correlation (77/80) between the bone lesions on CT and MR. Interlesional variability was found to be smaller on ADC than on CT density. There was a slight negative correlation between the density of the bone lesions on CT and the signal intensity on native DWI images, probably due to the bony tissue in the myeloma lesion. The ADC-values and signal intensity were measured in the control group (mean age=37 years (range 22-51 y). The ADC-value and SI in all b-values of the osteolytic lesions was significantly higher compared to the normal bone marrow (p<0.001). *Conclusions*. WB-DWI as an additional imaging tool seems to be a new highly sensitive method for evaluation and characterization of myeloma bone lesions.

PO-407

WHOLLE-BODY FDG-PET/CT. A RETROSPECTIVE SURVEY IN 16 CASES OF PLSAMA CELL NEOPLASMS

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Introduction. Imaging studies in the evaluation of plasma cell neoplasms, including solitary plasmacytomas (SP) and multiple myeloma (MM), ideally should comprise squeletal and extramedullary disease sites at presentation and during follow-up in order to establish the extent of the disease, the response to therapy and the detection of complications or relapse. FDG PET/CT is an emerging technique in this setting. *Materials*. We retrospectively analyzed 18 whole-body PET/CT scans performed in 16 patients with plasma cell neoplasms from October 2003 to November 2006. *Results*. SP at diagnosis (8 exams): 3/6 patients with presumed SP of bone and 2/2 with extramedullary plasmacytomas had advanced bone disease, while the other 3/6 bone SP had normal scans. MM at diagnosis (3 exams): 3/3 patients, including a non-secretory MM, had more extensive bone disease than suspected by standard XR bone survey. MM at follow-up (7 exams): 4/7 scans were normal after treatment, 2/7 showed slight foci of uptake, and 1/7 a frank positivity indicative of focal relapse. *Conclusions*. Of interest, therapy decisions were taken in 9 patients based on PET/CT findings: 5 patients with presumed SP of bone or extramedullary plasmacytomas, who would have been otherwise treated with radiotherapy alone, received chemotherapy; other 4 patients could stop treatment after their residual lesions showed no activity. PET/CT is a whole-body technique useful to detect active disease and it is of particular interest to confirm presumed SP at diagnosis, and to evaluate plasma cell tumours when MRI still shows masses after treatment. It also allows enumeration of the sites of focal disease making it useful within the new Durie-Salmon PLUS MM staging system. PET/CT should be considered ideal in the evaluation of MM and related plasma cell disorders.

PO-408

ASSESSMENT OF BONE DISEASE WITH 18F-FLUORODEOXYGLUCOSE POSITRON EMISSION TOMOGRAPHY AT DIAGNOSIS AND AFTER HIGH-DOSE THERAPY IN PREVIOUSLY UNTREATED MULTIPLE MYELOMA PATIENTS

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Aims. The objective of this study is to define the role of 18F-fluorodeoxyglucose (FDG) integrated with computed tomography (PET-CT) in assessing the extent of active disease at the time of initial presentation and in evaluating treatment response in MM. *Material and Methods*. We studied 32 previously untreated MM patients at diagnosis, after induction therapy and 3 months after ASCT . The results of PET-CT scans were compared with whole body X-ray (WBXR) and whole spine Magnetic Resonance (MRI) at diagnosis and with clinical response to treatment at restaging . The patients underwent induction therapy consisting of thalidomide plus dexamethasone or VAD for 4 months, then proceeded to collection of PBSC mobilized by 4 g/m^2 Cyclophosphamide + G-CSF and and two courses of myeloablative treatment with 200 mg/m^2 Melphalan. *Results*. At diagnosis PET-CT showed concordant findings with WBXR in 14/32 (43%) and with MRI in 17/32 patients (53%). PET-CT scans detected more disease sites than WBXR in 12/32 cases (39%) and than MRI in 3/32 (8%). MRI showed a focal or diffuse pattern of bone marrow involvement of one or more vertebras in 12 patients (39%), who had negative PET-CT. Moreover, WBXR identified lytic lesions of subcentimeter size in the skull of 6/32 patients (18%), who had no increased uptake of FDG. Fifteen patients with positive PET-TAC at baseline had a restaging after induction chemotherapy (3) or after ASCT (12). In 13 patients (86%) PET -CT was completely negative (6 cases) or showed areas with a standard uptake volume (SUV) < 3 (7 cases); all these reached CR or VGPR after therapy. The 2 patients in PR after therapy showed a persistence of a multifocal uptake with SUV >3. *Conclusions*. We conclude that in our series of newly diagnosed MM PET-CT could identify additional sites of diseases to WBXR or MRI in 15/32 (46%) of the patients. However, MRI was superior over PET-CT in the assessment of bone marrow involvement of the spine and pelvis. PET -CT scans was useful to monitor response to therapy , since all patients in CR or VGPR after therapy showed the complete disappearance of the previous sites of abnormal uptake or the persistence of a few areas with a SUV <3 .

PO-409

FDG PET FOR STAGING AND THERAPEUTIC EVALUATION IN PATIENT WITH PLASMOCYTOMA

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Objective. Plasmocytoma is a rare disease which needs whole body imaging for staging and post-treatment evaluation. The utility of FDG-PET has not been well evaluated in such patients. The aim of this prospective study was to assess the benefits of FDG-PET in staging and therapeutic evaluation of plasmocytoma. *Methods*. Patients with pathologically documented plasmocytoma underwent whole body FDG-PET performed using a hybrid PET/CT system (Discovery LS, GE) 60 minutes after injection of 5 MBq/kg of FDG. FDG-PET results were compared to those obtained with MRI. Per-region analysis was performed. The gold standard was follow-up or histopathology. *Results*. Twenty one patients, median age 60 yr [35-78] were studied and 32 FDG-PET exams were performed, 20 for staging and 12 for therapeutic evaluation. For staging, a total of 400 regions was analysed by PET and 115 regions could be compared using both PET and MRI. The sensitivity (Se), specificity (Sp), predictive positive value (PPV) and predictive negative value (PNV) were calculated. Results are shown in Table 1.

For therapeutic evaluation a total of 240 regions was analysed in 12 PET/CT and compared with MRI for 71 regions. In 6 patients a complete response was observed, in 3, only a partial response and in 3 no response. Se, Sp, PPV and PNV are shown in Table 2.

Table 1 : PET and PET/MRI performance for staging

All regions analysed by PET		n=400	
Regions analysed by both PET and MRI		n=115	
PET/CT	PET/CT	MRI	
Se	98% (47/48)	96% (25/26)	92% (24/26)
Sp	99% (350/352)	99% (88/89)	94% (84/89)
PPV	96% (47/49)	96% (25/26)	83% (24/29)
NPV	100% (350/351)	99% (88/89)	98% (84/86)

Table 2 : PET and PET/MRI performance for therapeutic evaluation

All regions analysed by PET		n=240	
Regions analysed by both PET and MRI		n=63	
PET/CT	PET/CT	MRI	
Se	100% (17/17)	100% (9/9)	100% (9/9)
Sp	100% (223/223)	100% (54/54)	89% (48/54)
PPV	100% (17/17)	100% (9/9)	60% (9/15)
NPV	100% (223/223)	100% (54/54)	100% (48/48)

Conclusions. This study showed that FDG-PET has higher performance than MRI for evaluation of plasmocytoma especially in post-treatment evaluation.

PO-410

POSITRON EMISSION TOMOGRAPHY FOR THE PREDICTION OF PROGRESSION FREE SURVIVAL IN MULTIPLE MYELOMA

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Introduction. There is no optimal method to predict early progressive disease in multiple myeloma (MM). We evaluated the dynamic Fluorodesoxy-glucose positron emission tomography (FDG-PET) for prediction of progression as the metabolic changes of a tumour under influence of cytotoxic substances appear to have predictive value in other tumours. *Materials and Methods.* We investigated a group of 19 patients with multiple myeloma with 58 measurable lesions. All patients received an anthracycline-based chemotherapy. Three FDG-PET-studies were carried out (1. prior to the chemotherapy, 2. after the first course of chemo-therapy, 3. after the third course). The clinical follow-up data and the EBMT-criteria served as reference for the PET-data. The following parameters were retrieved from the dynamic PET studies: standard uptake value (SUV), fractal dimension (FD), two compartment model with computation of the kinetic parameters k1, k2, k3, k4 and the vessel density (VB). Furthermore, the FDG- influx according to Patlak was calculated using the rates of the two-compartment model and the formula (k1 x k3)/(k2 + k3). Pearson correlation and discriminant analysis was used for data analysis. For the discriminant analysis we dichotomized the patients in two groups (PFS < 6 months/> 6 months). *Results*. The best correlation between progression free survival (PFS) and PET was for the first PET by k3 and SUV and for the second PET by FD and SUV. Best parameters for the discrimination between PFS 6 months were the combination of SUV of the first and second PET with an overall classification rate (CCR) of 96%, followed by k3 and SUV of the first PET (CCR 91%) and SUV of the first PET (CCR 89%). *Conclusions*. These data of our continuing study show that PET maybe used for prediction of PFS. Our analysis revealed that a combination of parameters is superior to SUV.

GROUP 5: Novel treatment development (preclinical studies)

PO-501

BENDAMUSTINE INDUCES G2- ARREST BY ATM-CHK2-P53

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Introduction. Bendamustine is a bi-functional alkylating agent that has been proven to be effective and even superior to melphalan in the treatment of myeloma. There are no published preclinical data available regarding multiple myeloma. Methods and *Results*. Bendamustine induces apoptosis as measured by annexinV/PI in the four myeloma cell-lines NCI-H929, OPM-2, RPMI-8226 and U-266 with an IC50 of 30-60 µg/mL and cleavage of caspase-3. Incubation with 10-30 µg/mL results in G2-arrest most significant for NCI-H929 with an increase of 45% G2-cells. Higher concentrations of the drug overcome this checkpoint-arrest and increase the SubG1-fraction as a further sign of apoptosis. Western blotting detects the molecular correlate of the G2-arrest. The primary DNA-damage signalling kinases ATM and CHK2, but not ATR and CHK1, are activated. CDC2 is inhibited by inhibitory phosphorylation at Tyr162 and expression level of cyclin B increases. Further, no significant change in cdc25C status appears. P53 expression is augmented and its activation status is increased by phosphorylation of Ser15, the phosphorylation site for ATM. Bcl-2, bcl-XL, bax and bad levels remain unaltered. Targeting P38 MAPK by the selective inhibitor SB202190 potently increases the apoptotic effect of bendamustine. Co-incubation of bendamustine with non-toxic concentrations of the inhibitor doubles apoptosis (p=0,014). Additionally, SB202190 completely abrogates the G2-arrest. *Conclusions*. Bendamustine induces ATM-chk2-cdc2/P34-mediated G2-arrest and P53-mediated apoptosis. The selective inhibitor of P38-MAPK SB212090 increases apoptosis by twofold, completely abrogates G2-arrest and can therefore be considered as an additive drug for future combination regimens.

PO-502

THE *IN VITRO* AND *IN VIVO* ANTI-TUMOR EFFECTS OF JS-K IN MM

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Introduction. JS-K (O2-(2,4-dinitrophenyl) 1-[(4-ethoxycarbonyl)piperazin-1-yl]diazen-1-ium-1,2-diolate) is a diazeniumdiolate class of prodrug which is designed to release nitric oxide (NO•) on reaction with glutathione S-transferases (GST). GST has been shown to be overexpressed in a broad spectrum of tumor cells including multiple myeloma (MM). Therefore, JS-K can possibly turn GST overexpression to the tumor's disadvantage by generating high intracellular concentrations of cytotoxic NO•. In this study we investigated the cytotoxicity of JS-K in MM *in vitro* and *in vivo*. *Materials and Methods.* Cytotoxicity was detected by MTT; flow-cytometry, immunocytochemistry and western-blotting were used for mechanistic studies. *Results*. JS-K showed significant cytotoxicity in both conventional therapy-sensitive and -resistant MM cell lines, as well as patient MM cells (IC50: 0.3-2.5 uM). Importantly, no significant cytotoxic effects of JS-K at these doses were observed in normal peripheral blood mononuclear cells. JS-K treatment induced apoptosis in MM cells which was associated with PARP, caspase 8, and caspase 9 cleavage; increased cell surface expression of Fas/CD95; Mcl-1 cleavage; Bcl-2 phosphorylation; as well as mitochondrial cyt c, AIF, and EndoG release. Moreover, JS-K could overcome the survival and growth advantages conferred by exogenous IL-6 and IGF-1, or by adherence of MM cells to bone marrow stromal cells. Flow cytometry experiments revealed significant NO• generation in JS-K-treated MM cells. Since NO• is known to cause DNA double strand breaks (DSB), we hypothesized that JS-K induces DSB in MM cells and confirmed DSB formation by neu-

tral comet assay. We further showed that JS-K also activated DNA damage response pathways as evidenced by H2AX, Chk2 and p53 phosphorylation. In addition, JNK was also activated by JS-K treatment in MM cells, and inhibition of JNK significantly decreased JS-K-induced cytotoxicity, suggesting that JS-K induced apoptosis is mediated via JNK signaling. Finally, JS-K was also significantly effective in inhibiting tumor growth and prolonging median survival ($p<0.01$) in a human plasmacytoma xenograft mouse model. Analysis of tumors harvested from treated animals showed that JS-K induced apoptosis and decreased angiogenesis *in vivo*. *Conclusion*. Taken together, these data provide the preclinical rationale for the clinical evaluation of JS-K to improve patient outcome in MM.

PO-503

IRINOTECAN (CPT-11) FOR MULTIPLE MYELOMA

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Introduction. Multiple myeloma (MM) still remains an incurable disease, thus needs alternative agents. Since constitutive expression of topoisomerase I (TopoI) in MM cells and the efficacy of SN-38, an active metabolite of irinotecan (CPT-11), have been reported (Br J Haematol 83:68, 1993; Cancer Res 64: 8749, 2004), we investigated on the therapeutic potential of the CPT-11. *Materials and Methods*. Eight MM together with 5 lymphoma cell lines were examined for cellular proliferation and viability in the presence of various concentrations of CPT-11 or SN-38 (gift from Yakult Inc., Tokyo) by means of 3H-thymidine uptake and MTS assays. Total RNA prepared from these cell lines and purified plasma cells derived from MM patients was quantified for the mRNA levels of carboxylesterase-2 (CE-2). Stable U266 cells overexpressing various levels of CE-2 (U266/CE-2) were established and examined regarding sensitivity to CPT-11. To make sure *in vivo* effect, SCID mice subcutaneously inoculated with 1×10^7 RPMI8226 cells were treated by twice weekly intra-peritoneal injection with 25 mg/kg CPT-11 alone or in combination with intravenous injection of 1 mg/kg bortezomib. Tumor size and serum free-light-chain (FLC) level were measured. *Results*. CPT-11 and SN-38 inhibited DNA synthesis of most MM cells to <15% and to <10% with 2 μg/mL and 2 ng/mL, respectively. In 4/8 MM cells, IC50 was <2 μg/mL for CPT-11 and <2 ng/mL for SN-38. Such efficacy of CPT-11 was partly explained by expression level of CE-2, which plays a major role in catalyzing CPT-11 to SN-38, being higher in MM than lymphoma cells. IC50 for CPT-11 was 3.0 and 0.13 μg/mL for U266/mock and U266/CE-2 cells, respectively. In murine xenograft model, CPT-11 as a single agent significantly reduced the tumor volume at day 20 when compared to the controls from 7,408±710 to 1,182±473 mm^3 ($p<0.01$). To our surprise, the subcutaneous tumor completely disappeared in 5/5 mice treated by combination of CPT-11 with bortezomib. Serum kappa FLC produced by RPMI8226 cells was significantly lower in the treatment group as well. *Conclusion*. Efficacy of the CPT-11 for MM can be validated in the clinical trial setting.

PO-504

S-DIMETHYLARSINOGLUTATHIONE (ZIO-101) A NOVEL ARSENICAL WITH ACTIVITY IN MYELOMA

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Introduction. Arsenic trioxide (As2O3) is highly active in acute promyelocytic leukemia (APL) but less so in other cancers including myeloma. Therefore investigation of arsenics with different toxicity profiles and potential mechanisms of action is warranted. ZIO-101, a new less toxic organic arsenic is in phase-2 trials. *Materials and Methods.* ZIO-101 and As2O3 were compared in several assays including induction of apoptosis as assessed by Annexin V-FITC/Propidium iodide staining and flow cytometry, gene expression profiling (GEP) using the Affymetrix Human 133 plus 2 arrays and protein expression by Western blotting. Effects of dose, modulating of glutathione (GSH) and addition of cysteine were determined. *Results*. We compared the ability of ZIO-101 and As2O3 to kill 4 myeloma cell lines (RPMI 8226, U266, KMS11, MM.1s). Interestingly the most As_2O_3-insensitive line (RPMI 8226) was highly sensitive to ZIO-101. GSH is a critical regulator of As_2O_3-induced cell death. In contrast buthionine-(S,R)-sulfoximine (BSO)-mediated GSH depletion had little effect on ZIO-101. Moreover ascorbic acid protected cells from ZIO-101. Finally addition of exogenous GSH or cysteine inhibited ZIO-101 but not As_2O_3-induced cell death. We next used GEP to characterize these differences. Initially, we focused on genes whose expression was similarly regulated in the 4 cell lines. 320 genes were increased ≥1.5-fold at 6 h versus 58 at 24 h. 265 genes were decreased ≤1.5-fold at 6 h versus 12 at 24 h. The pattern of gene-expression seen with ZIO-101 differed from that seen with As_2O_3. For example, genes associated with metal responses (MT-1, ZnT-1) and oxidative stress (HO-1, NQO-1, malic enzyme, GSH synthesis pathway, ferritin) were unaffected or only transiently increased by ZIO-101 treatment whereas these genes are persistently increased by As_2O_3-treatment. In contrast, ZIO-101-treatment markedly increased expression of the pro-apoptotic gene Noxa compared to As_2O_3-treatment. *Conclusions*. These data indicate that while ZIO-101 is an arsenical it¢s activity is different than As_2O_3. These differences between ZIO-101 and As_2O_3 likely reflect different metabolism, mechanisms of action or both. Consequently, ZIO-101 is likely to be active in cells resistant to As_2O_3.

PO-505

INDUCTION OF NECROSIS TO HUMAN MYELOMA CELLS BY KIGAMICIN

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Introduction. Kigamicin (KM) is a new compound derived from Actinomycetes that was originally reported to induce necrosis in pancreatic cancer cells under nutrient-starved conditions via inhibition of PI3-kinase. Since the importance of PI3-kinase in myeloma cells has repeatedly been reported, we evaluated the anti-myeloma activity of KM toward myeloma cells. *Materials and Methods.* KMS-12-PE was provided from Dr. Ohtsuki (Kawasaki Medical School, Kurashiki, JAPAN). Fresh myeloma cells were purified by CD138-coated immune-magnetic beads. Kigamicin was obtained from Dr. Esumi (Investigative Treatment Division, National Cancer Center Research Institute East, Kashiwa, Chiba, JAPAN). Cytotoxicity was evaluated by tripan blue dye exclusion analysis. In some experiments, WST8 analysis (Dojindo Laboratories, Kumamoto, JAPAN) was performed according to manufacturer's protocol. Necrosis was evaluated with Annexin V/PI staining analyzed by Epics V flow-cytometer (Coulter, Miami, FL). *Results*. The CC50 of KM toward a myeloma cell line, 12PE, was approximately 100 nM after 24 hours of exposure. When whole mononuclear cells obtained from patient bone marrow were cultured with KM, normal lymphocytes were spared, but all the myeloma cells underwent necrosis, suggesting a relatively selective cytotoxicity of KM toward myeloma cells. Western blot analysis revealed a decrease in phosphorylated-AKT following treatment with KM, indicating that KM inhibits PI-3 kinase activity as previously reported. However, a pan-PI3-kinase inhibitor, LY294002, did not induce necrosis of myeloma cells, indicating that PI3-kinase inhibition may not be the major cytotoxic pathway of KM. Interestingly, western blot analysis revealed that KM completely abolished the expressions of both cyclin D1 and p21 in myeloma cells, indicating that it disrupts cell cycle regulation. Finally, a melphalan-resistant myeloma cell line showed significant cell death following KM treatment and this cell death was even more efficient than that induced in the melphalan-sensitive parental cell line. *Conclusion*. Since most anti-cancer reagents induce apoptosis in myeloma cells, the induction of necrosis by KM represents a unique mechanism that may allow drug resistance to be overcome. We believe that KM could be a promising candidate for future therapeutic use against multiple myeloma.

PO-506

CLARITHROMICIN INDUCES AUTOPHAGY IN MYELOMA CELLS

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Introduction. It is reported that combination of thalidomide with clarithromycin (CAM) augments anti-tumor activity to multiple myeloma cells. To determine the mechanism of CAM to myeloma cells, we analyzed direct effect of CAM to myeloma cells *in vitro*. *Materials and Meth-*

ods. Myeloma cell lines and primary myeloma cells purified by CD138-conjugated immune-magnetic beads were utilized. Clarithromycin was obtained from Taisho-Toyama pharmaceuticals (Tokyo, JAPAN). Morphology was analyzed either by May-Giemza staining or electron microscopy. Auto-lysozome was stained with Lysotracker (Invitrogen) and analyzed using fluorescent microscopy. Antibody to LC3 was obtained from Dr. T. Yoshimori (Osaka University). *Results*. Morphological examination revealed CAM induced vacuoles in the cytoplasm of both myeloma cell lines and primary myeloma cells. Electron microscopy revealed that those vacuoles morphologically resemble auto-lysozome. After the treatment with CAM, vacuoles which were stained with Lysotracker accumulated in the cytoplasm. Since initiation of autophagy depends on PI3-kinase, we investigated whether CAM induced AKT phospholyration. AKT phospholyration by CAM was readily observed, and moreover, the emergence of vacuoles stainable with Lysotracker was inhibited when the cells were pretreated with PI3-kinase inhibitors. To further confirm that autophagy is induced by CAM, the process of LC3-I to LC3-II, a hallmark of autophagy, was examined. We found that the induction of LC3-II by CAM occurred at a dose-dependent manner. Bafilomycin-A (BAF), which inhibits fusion of lysozome to auto-phagosome, inhibited auto-lysozome formation by CAM. Interestingly, either CAM or BAF alone equally induced cell death. *Conclusion*. CAM induces auto-lysozome accumulation through activating PI3-kinase. Therefore, we conclude that CAM induces autophagy and contributes cell death. As we found either CAM or BAF induced cell death, the manipulation of certain autophagy processes might represent a new therapeutic approach in the treatment of myeloma.

PO-507

C-JUN AND C-ABL IN HUMAN MULTIPLE MYELOMA CELL DEATH

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Introduction and Aims. The tyrophostin adaphostin achieves remarkable responses in patients with chronic myelocytic leukemia, including Bcr/Abl-positive, Bcr/Abl-negative, and Bcr-Abl T315I mutant tumor cells resistant to both imatinib mesylate and second-generation BMS354825 and AMN107. In addition, it demonstrates cytotoxicity against chronic lymphocytic leukemia and acute myelocytic leukemia cells. Several mechanisms have been proposed as a basis for its robust anti-tumor activity including generation and release of reactive oxygen species (ROS), cytochrome-c and apoptosis- inhibiting factor (AIF), caspase cleavage, JNK activation, as well as inactivation of Raf-1, Stat3, and Stat5. As these adaphostin- targeted pathways are relevant to MM pathogenesis, we here sought to determine the potential molecular sequelae and therapeutic promise of adaphostin in MM. *Materials and Methods.* Studies were performed in MM, erythroleukemia, and CML cell lines. Microarray and western blot analysis of MM cells were used to demonstrate adaphostin- induced c-Jun upregulation and c-Abl cleavage. The effect of specific knockdowns of c-Jun or c-Abl using siRNA, as well as transient overexpression of wild-type c-Jun, c-Abl, as well as c-Abl cleavage mutants and c-Abl- fragments, was assessed in proliferation and survival assays. iii) *Results*. After demonstrating the anti- MM cytotoxicity of adaphostin, we carried out expression profiling of adaphostin- treated MM cells to identify its molecular targets. Surprisingly, c-Jun was the most upregulated gene, even at the earliest point of analysis (2 hours). We also observed adaphostin- induced c-Abl cleavage in immunoblot analysis. Proteasome inhibitor bortezomib, but not melphalan or dexamethasone, induced similar effects, indicating agent-dependent mechanisms. Using caspase inhibitors as well as caspase-resistant mutants of c-Abl (TM-c-Abl and D565A-Abl), we confirmed that c-Abl cleavage in MM cells requires caspase activity. Importantly, knockdown of c-Jun and c-Abl expression by siRNA confirms that adaphostin- induced c-Jun upregulation triggers downstream caspase-mediated c-Abl cleavage, inhibition of MM cell growth, and induction of apoptosis. Finally, our data suggest that this mechanism may not be restricted to MM, but may also be important in a broad range of malignancies, including erythroleukemia and solid tumors. *Conclusions*. These data demonstrate a new mechanism of drug- induced growth- inhibition and apoptosis involving c-Jun upregulation and fragmentation of c-Abl.

PO-508

BH3- ONLY PROTEIN REGULATION BY As_2O_3 IN MYELOMA

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Introduction. Arsenic trioxide (As_2O_3) has emerged as a promising therapeutic agent in hematological malignancies. The use of As_2O_3) to treat MM is supported by preclinical studies in which this agent has been shown to inhibit growth and induce apoptosis in myeloma cell lines at concentrations that can be safely achieved in patients. Although some cellular effects have been identified, the precise mechanism(s) of action of As_2O_3) in myeloma are not completely elucidated. The purpose of the present study was to evaluate the response in four human MM cell lines (U266, MM.1s, 8226/S and KMS11) following exposure to As_2O_3) to gain further insight into the mechanism(s) of action of As_2O_3) in MM. *Materials and Methods.* MM cell lines were treated with 2 μM As_2O_3) for 6, 24 and 48 h. Annexin/PI staining was used to determine ATO-induced apoptosis. Total RNA was isolated for gene expression profiling and total protein lysates were used for westerns blot analysis. Silencing was performed by nucleofection of indicated siRNAs. Transfected cells were tested for As_2O_3)-induced apoptosis by Annexin/PI staining and gene silencing was determined by Real-Time PCR or protein expression by western blot. *Results*. As_2O_3) induced up-regulation of three pro-apoptotic BH3-only proteins (Bmf, Noxa and Puma) at both the mRNA and protein level, while two anti-apoptotic proteins, Bcl-x and Mcl-1 were down-regulated. Noxa expression was enhanced by GSH depletion and inhibited by increasing GSH levels in the cell. Silencing experiments, using siBmf and siNoxa, to partially inhibit the up-regulation of these two proteins, significantly protected cells As_2O_3)-induced apoptosis. Bim silencing also protected cells from As_2O_3) toxicity while knockdown of Puma and Bid had no effect. Additionally, ABT-737, a Bcl-2/Bcl-x inhibitor, synergized with As_2O_3) for MM.1s and KMS11 cell lines, while for U266 and 8226/S mainly additive affect was observed for As_2O_3) and ABT-737 co-treatment. *Conclusions*. These results are consistent with the latest models of Bcl-2 protein function where up-regulation of sensitizer BH3-only proteins (Noxa and Bmf) function to inhibit anti-apoptotic Bcl-2 proteins to allow Bim to activate Bax/Bak. Additionally the data suggest that further testing of As_2O_3) with BH3-mimetics is warranted.

PO-509

POLY(IC) MODIFY THE SURVIVAL OF MULTIPLE MYELOMA CELLS

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Introduction and aims. Multiple Myeloma is a fatal plasma-cell malignancy localized in the bone marrow. We have recently shown that myeloma cells express a large panel of Toll-like receptors (TLR). Upon triggering, TLR4, 7 and 9 promote myeloma survival and cell growth. Myeloma cells do also express TLR3 that recognizes double stranded RNA as well as Poly(IC). Poly(IC) has been used in several cancer clinical trials as an interferon-inducer. The efficiency of Poly(IC) in Multiple Myeloma was diverse and the mechanisms of action not understood. We therefore determined its mechanisms of action. *Material and Method*. The study was done on primary and myeloma cell lines. TLR3 expression was determined by RT-PCR. We tested the effect of Poly(IC) on proliferation and survival of myeloma cells in the presence or absence of specific signal transduction inhibitors. *Results*. We first determined TLR3 expression and function on primary myeloma cells. TLR3 mRNA is present in 69% (18/26) of patients. Overnight incubation of myeloma bone marrow cells with Poly(IC) induces a 50% to 96% decrease of tumor cells in two patients (2/6). Surprisingly, treatment of myeloma cell lines by Poly(IC) induces either proliferation (3/6), either apoptosis/ cell growth arrest (3/6). Apoptosis was TLR3 mediated as the effect was totally blocked by the incubation with chloroquine. The TLR3-induced apoptosis was mediated by an autocrine secretion of IFNa as 1) high secretion of IFNa was solely detected in supernatants from cells undergoing apoptosis, and 2) neutralization of IFNab blocked the apoptosis. In those cells, several signalling pathways were activated as we observed Erk, p38 Mapkinase, Akt and Stat3 phosphorylation, and the nuclear translocation of NFκB. P38 pathway controlled mainly the IFNa secretion as specific neutralization reduced it by a mean of 94%. The remain-

ing amount of IFNa acts through erk and p38 pathways as neutralization of both blocked the apoptosis induced by exogenous IFNa. *Conclusions*. The mixed effects of Poly(IC) treatment in Myeloma patients could be explained by different responses of TLR3-expressing Myeloma cells. The identification of the mechanisms responsible for this heterogeneity would be critical in the design of a Poly(IC)-based therapy.

PO-510

HIV PROTEASE INHIBITORS INDUCE APOPTOSIS OF MYELOMA CELLS

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Protease inhibitors induce apoptosis of tumor plasma cells and act synergistically with other drugs in part by reversion of the multidrug resistance gene product. However, their use is limited by toxic side effect and development of resistance. We have tested the effect of several HIV protease inhibitors (HIP), which are thought to exert an inhibitory effect on proteasome, on myeloma plasma cells and have investigated whether their effects are synergistic with drugs usually used Melphalan (MLP), Dexamethasone (Dex), Bortezomib (Bb) or new drugs as the HDAc inhibitors: Valproic acid (Va). The effects of the HIP (Nelfinavir, Saquinavir and Tipranavir) on the proliferation and survival of t(4;14) positive (LP1 and OPM2) and negative (RPMI) myeloma cell lines and plasma cells from 10 patients with myeloma (including 4 with a t(4;14)), were analyzed using 3HThymidine incorporation, WST1 at 48 h and Annexin/IP staining. We also examined the effect of the combination of HIP with MLP, Dex, Bb and Va. Nelfinavir but not Saquinavir nor Tipranavir inhibits the proliferation of myeloma cell lines in a dose dependant manner (2-10 μM). At pharmacological dosage (5 μM) the proliferation of LP1 cells and plasma cells from t(4;14)+ were inibited by 50 to 90%. Interestingly, this inhibitory effect was not reversed in the presence of IL6 (10 ng/mL), IGF1 (100 mg/mL) or FGF1 (10 ng/mL). Combination of Nelfinavir (2-5 μM) with Dex (50 microM) or MLP (30 microM) or Bb (10 nM) increase the inhibitory effect of HIP. The most synergistic effect was observed with the association of Nelfinavir (2 μM) and Va (1 mM) leading to 95% of growth inhibition of LP1 cells. The antiproliferative effect of Nelfinavir was associated with an increased level of plasma cell apoptosis. These results suggest that Nelfinavir, in combination with Dex, MLP, Bb and Va may be useful in the treatment myeloma, especially with t(4;14) which are characterized by a poor prognosis linked to early and chemoresistant relapses. Moreover, the synergistic effect of Nelfinavir and Bb suggest a that proteasome inhibition is not the only antitumoral effect of HIP. Studies are ongoing to determine the precise molecular mechanisms underlying this effect

PO-511

TARGETING CDK4/6 AND THE CELL CYCLE IN THE 5T MYELOMA MODELS

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Loss of cell cycle control in myeloma primarily stems from coordinated deregulation of Cdk4-cyclin D1 or Cdk6 (Cdk4)-cyclin D2, which predisposes bone marrow (BM) myeloma cells to proliferation *in vivo*. PD 0332991 is the first orally bioactive small molecule that potently and specifically inhibits Cdk4 and Cdk6. It represents a promising cell cycle-based therapy for myeloma owing to its ability to potently inhibit Cdk4/6 (IC50 ~ 60 nM) and induce G1 cell cycle arrest in both primary human myeloma cells in BM stromal cell co-cultures and in cycling myeloma cell lines. In addition, PD 0332991 prevents tumor growth in an aggressive disseminated NOD/SCID xenograft human myeloma model. These findings, together with the favorable outcome of a Phase I clinical trial, have facilitated the development of an impending randomized Phase I/II PD 0332991 clinical trial. To optimize the therapeutic targeting of Cdk4/6 with PD 0332991, we have now investigated the effectiveness of PD 0332991 in inhibiting Cdk4/6 and controlling myeloma tumor progression in the immune competent, bone migrating 5TMM models. By quantitative real-time PCR analyses, we found that as is the case with the majority of human primary myeloma cells, primary 5T2 and 5T33 tumor cells overexpress Cdk6, Cdk4 and cyclin D2, but not cyclin D1, when compared with normal mouse plasma cells. We then demonstrated that PD 0332991 rapidly inhibits the phosphorylation of Rb by Cdk4/6 in primary 5T2 myeloma tumor cells *ex vivo*. BrdU labeling studies further show that PD 0332991 inhibits cell cycle progression through G1 in the rapidly proliferating 5T33 tumor cells *in vivo* and *ex vivo*. Accordingly, treatment with PD 0332991 significantly prolongs the survival of tumor-induced 5T33 mice; 27 to >39 days in the PD 0332991-treated group (N=9) versus 22 to 29 days in the vehicle-treated group (N=9), as shown in the Kaplan Meier survival curves. Thus, PD 0332991 targets Cdk4/6 and inhibits cell cycle progression in mouse myeloma cells in an intact immune system. These findings complement the studies of the immune-deficient NOD/SCID human myeloma xenografts, and strongly support therapeutic targeting of Cdk4/6 with PD 0332991 in myeloma.

PO-512

COULD MITOGENS BE A NEW THERAPEUTIC INTERVENTION IN MULTIPLE MYELOMA?

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During cytogenetic analysis of multiple myeloma samples, attempts to improve metaphase acquisition by stimulating with the B cell specific mitogens, pokeweed mitogen (PWM) and Lipopolysaccharide (LPS) demonstrated to us that mitogenic stimulation actually reduced cell numbers consistently (data unpublished). Given the unusual results obtained, we were interested in exploring the use of mitogens as a novel therapeutic approach to MM, since MM is known to become refractory to conventional therapies. Both control B cell lines and MM cell lines have been cultured in the presence of mitogens phytohaemagglutinin (PHA-L), PWM, LPS, Conanavalin A (Con A) and Haemocyanin from Keyhole Limpets (KLH). These have also been cultured in combination with either one of the chemotherapy drugs vincristine, melphalan or thalidomide. The data has shown that culturing MM cell lines with mitogens alone reduces the overall cell population in the MM cell lines (RPMI 8226 with PWM p=0.0304) when compared to the control samples (AGLCL with PWM p=0.3123). PWM and PHA-L appeared more potent at decreasing the cell numbers when compared to LPS (for HS Sultan PHA p=0.0304, PWM p=0.0304 and LPS p=0.0606). When mitogens in combination with chemotherapeutics were added to the culture, the overall cell numbers greatly decreased in comparison to cultures with mitogens or chemotherapy drugs alone, suggesting that the mitogens may aid in the therapeutic effects of the chemotherapy. Indeed in RPMI 8226 cells, cell death was due to PHA alone and addition of thalidomide had no extra beneficial effect. It is currently unclear why or how the mitogens have this effect on MM cells, however literature and other researchers have observed this phenomenon in other haematological malignancies. It has been speculated that mitogens can detect abnormal cells and aid in their recognition and death via immune cells, thus the cell killing may well be more dramatic *in vivo*. Cell lines can be considered abnormal clones, however our data does not suggest this effect is cell line specific. We therefore intend to test this phenomenon in patient samples to ascertain if mitogens can be utilised for therapy, since PWM and PHA have previously been used safely in the clinic.

PO-513

GCS-100; A MECHANISTIC THERAPY FOR MYELOMA

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Introduction. Myeloma cells are activated by multiple signalling pathways mediated by marrow stromal cells secreting cytokines. This leads to upregulation of anti-apoptosis, drug resistance, cell cycle and metabolic pathways. Disruption of these networks can lead to myeloma cell death and are attractive targets for novel therapies. GCS-100, a modified citrus pectin, successfully completed Phase I studies for solid tumors and induces cell death in lymphoma / CLL by blocking of the galectin-3 / Bcl-2 dimerisation. The mechanism of action in myeloma is not clearly understood. We aimed to elucidate the mechanism of action for this novel agent to evaluate its potential for better myeloma therapy. *Results*. Myeloma cell lines RPMI8226, U266 and OPM-2 were studied. Time and dose dependent proliferation, apoptosis induction and mitochondrial membrane potential loss occurred with GCS-100. Apoptosis was associated with a marked reduction in mcl-1 and bcl-xL after 24 hours and no changes in protein levels of bcl-2 or galectin-3. Akt activation, associated with proliferation and cell cycle regulation, is reduced after GCS-100 treatment. Pre-treatment of cells with GCS-100 prior to stimulation

with IGF-1 prevents the increased activation normally observed. In addition GCS-100 treatment was associated with a reduction of constitutive activation of IκBα, p65NFκB and IKK and a reduction of IκBα activation associated with TNFα stimulation suggesting a role in NFκB pathway inhibition. Examination of cell cycle regulatory proteins revealed down regulation of cyclin D1, p16INK4A and CDK6 at 24hrs with no change in expression of CDK4 and p15INK4B. However, p21CIP1 was upregulated with a corresponding decrease in protein levels of cyclin E2 and CDK2 . p27KIP1 remained unaltered. Studies are currently ongoing for primary cells. *Conclusion*. GCS-100 is a novel complex carbohydrate that is effective for the induction of myeloma cell death. It reduces proliferation and induces apoptosis by down regulating crucial anti-apoptotic proteins, cell cycle regulators and signalling proteins. These effects may be mediated directly on the tumour cell or its microenvironment. Phase I studies in myeloma are underway.

PO-514

THE JAK2/STAT3 INHIBITOR CUCURBITACIN I (JSI-124) HAS POTENT ANTI-MYELOMA EFFECTS INDEPENDENT OF STAT3 INHIBITION

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Introduction. Multiple myeloma (MM) is a plasma cell proliferative disorder that results in considerable morbidity and mortality. JSI-124 is a plant natural product identified previously as cucurbitacin I, isolated from various plant families such as the Cucurbitaceae and Cruciferae and has been recently described as a specific inhibitor of signal transducer and activator of transcription-3 (STAT3). Based on the critical role of the IL-6/STAT3 pathway in MM we studied the effects of JSI-124 on different MM cell lines. *Materials and Methods.* Different human myeloma cell lines including MM1.S, IM9, OPM-2, RPMI-8226, ARH77 in addition to the murine 5TGM myeloma cell lines were incubated with increasing concentrations of JSI-124. The impact of JSI-124 on cell proliferation, cell cycle and induction of apoptosis was studied using [3H]-Thymidine incorporation, cell counting, flow cytometry and Annexin/PI staining and caspase 3 activation. *Results*. JSI-124 was able to inhibit proliferation and induce apoptosis in several MM cell lines, including MM1.S, IM9, OPM-2, RPMI-8226, ARH77, U266 and 5TGM in a dose- and time-dependent manner. Furthermore, JSI-124 could be shown to induce cell cycle arrest in the G2-M phase and we were able to confirm that JSI-124 treatment was able to abrogate IL-6 and bone marrow stroma (BMSC)-induced STAT3 activation in MM1.S cells. However, the growth inhibitory and apoptosis-inducing effects of JSI-124 were independent of JAK2/STAT3 inhibition as MM1.S and 5TGM cells were highly sensitive to JSI-124, but lacked constitutive STAT3 activation. *Conclusion*. Our results indicate that JSI-124 is a powerful direct inhibitor of myeloma cells independent of STAT3 activation and is also able to block BMSC-dependent STAT3 activation and might therefore serve as a potent novel anti-myeloma agent targeting both myeloma and bone marrow microenvironment. Further studies are warranted to identify the STAT3 independent pathways leading to inhibition of myeloma cell growth and induction of apoptosis.

PO-515

IN VITRO AND *IN VIVO* ACTIVITY OF VALPROIC ACID IN MYELOMA

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Introduction. Valproic acid (VPA) is a well-tolerated anticonvulsant that has been recently identified as a histone deacetylase (HDCA) inhibitor. VPA induces hyperacetylation of histone H3 and H4 and inhibits both class I and II HDCACs. It has recently been shown that VPA exerts *in vitro* and *in vivo* anti-tumor activity against solid tumors and some leukemias. The aim of this study was to evaluate the *in vitro* and *in vivo* effects of VPA on multiple myeloma (MM) cells. *Material and Methods.* We evaluated, by MTT assay, the *in vitro* activity of VPA on IL-6 independent (MM1s, OPM1, DOX-40) and dependent (INA-6) MM cell-lines. Induction of acetylation was assessed by Western Blot analysis. *In vivo* effects were studied using a xenograft animal model of human MM. A cohort of SCID mice bearing subcutaneous MM1s were treated i.p. daily with VPA (200 mg/kg, n=5) or vehicle alone (n=5) for 16 days. Tumors were measured every 2 days, and survival was calculated using the Kaplan Mayer curve. *Results*. We first demonstrated the *in vitro* activity of VPA against IL-6 independent and dependent MM cells. A time- and dose-dependent decrease in cell growth and survival of MM cell-lines was observed, with IC_{50} in the range of 1-3 mM. A preliminary Western Blot analysis of MM1s cells exposed to VPA showed an accumulation of acetylated histones in treated cells versus untreated controls. We next evaluated the *in vivo* activity of VPA in a xenograft MM model. Following VPA treatment, we found a significant (p=0.006) inhibition of tumor growth in mice treated with VPA compared to control, which translated into a significant (p=0.002) survival advantage in the VPA treated animals versus the control group. Effects induced *in vivo* by VPA on cell cycle and global gene expression, by DNA microarray profiling, are presently under investigation. *Conclusions*. Taken together, our data demonstrate the *in vitro* and *in vivo* anti-tumor activity of VPA and provide a preclinical rationale for its clinical evaluation, both as a single agent and in combination, to improve patient outcome in MM.

PO-516

EFFECT OF IL-6 ON JAK/STAT PATHWAY AND COMBINED TREATMENT OF BORTEZOMIB AND VALPROIC ACID ON MM

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Introduction. Interleukin-6 (IL-6) is a central growth factor for multiple myeloma (MM) and plays a pivotal role in the pathogenesis of MM through the activation of JAK/STAT pathway. Bortezomib is a novel proteasome inhibitor with established activities against MM. However, certain MM cells become resistant against bortezomib and an effort to find out new agents is urgent. Valproic acid (VPA) has an activity of histone deacetylase (HDAC) inhibitor and is known to induce the differentiation of myeloid blasts and stabilize the myelodysplastic syndrome. *Materials and Methods.* Human MM cell lines (U-266 and IM-9) cultured with serum-free media were stimulated with human recombinant IL-6 and MTT assay was performed. Effects of IL-6 on JAK/STAT pathway were examined by immunoblot assay. MTT assay, flow cytometric cell cycle analysis, and colorimetric caspase-3 activity assay were performed to investigate the effects of combined treatment of bortezomib and valproic acid on MM. IL-6 level of culture supernatants was measured by ELISA. Immunoblot assay was performed to evaluate the cell cycle arrest after treatment of both agents. *Results*. IL-6 stimulated the proliferation of MM cells and induced the phosphorylation of ERK, and these responses were more prominent in U-266. IL-6-stimulated activation of JAK/STAT pathway could be abrogated by JAK and NFkB inhibitors, respectively. Bortezomib activated caspase-3 and induced G1 cell cycle arrest in MM cells. While VPA alone could suppress the MM cell growth, combination of bortezomib and VPA had a tendency to suppress MM cell proliferation more effectively and dampen autocrine IL-6 secretion from MM cells compared to bortezomib alone. This combination activated caspase-3 and induced apoptosis more than bortezomib alone. In immunoblot, this combination further decreased the expression of cyclin A, cyclin D1, cyclin E, CDK2, CDK4, and CDK6, whereas up-regulated p21 and p27 expression than bortezomib alone. *Conclusions*. IL-6 stimulated cell growth was dependent on cell lines. Combined treatment of bortezomib and VPA more effectively suppressed the proliferation of MM cells and induced apoptosis and cell cycle arrest than bortezomib alone.

PO-517

MECHANISTIC INSIGHT INTO ANTI-TUMOR EFFECTS OF REVERSINE IN HUMAN MYELOMA AND LYMPHOMA CELL LINES

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Introduction. We recently reported that the adenosine analog reversine (2-(4-Morpholinoanilino)-6-cyclohexylaminopurine) triggers MM cell death at sub-uM concentrations. The structural homology of reversine with substrates for kinases prompted us to evaluate whether reversine

is a kinase inhibitor and, if so, whether it targets a unique array of kinases compared to other kinase inhibitors with anti-MM properties. *Materials/Methods/Results*. We studied reversine in an *in vitro* kinase activity screening against a panel of 56 kinases implicated in the pathophysiology of MM, other hematologic neoplasias and solid tumors. We also evaluated the effects of reversine on a panel of 60 cell lines by MTT assay, flow cytometry and immunoblotting. The kinase activity screening revealed that reversine triggers inhibition by >33% of 16/56 (29%) of kinases tested, including Aurora A, B and C, JAK2, Syk, PTK2 (FAK), TRKA, TRKB, kit, and c-met. In contrast, reversine caused no inhibition or <30% inhibition in the majority of tested kinases (40/56, 71%), including major cancer drug development targets, such as AKT1, -2 and -3, CK2, FGF-R3 (wild-type and mutant), IKK, IGF-1R, CHK1, pim-1 and -2, PDK1, Raf isoforms, SGK1/2 and p70S6K. The fact that these kinases are spared by reversine, yet the latter triggered cell death in our MM and lymphoma cell line panel (with most IC50s <1 ìM) not only indicates that reversine is not an unselective pan-kinase inhibitor, but also suggests that MM cell death can be triggered without direct targeting of many kinases currently considered critical for MM pathophysiology. This observation is supported by our data that constitutively active Akt overexpression did not protect MM-1S cells from reversine-induced cell death. The pattern of its effects on NF-κB activity, levels of Bcl-2 family members, inhibitors of apoptosis, or major heat shock proteins are distinct from those of other conventional or investigational anti-MM studied to date. *Conclusions*. Reversine is a pleiotropic, but not completely unselective, kinase inhibitor with sub-uM activity against MM and other tumor cells. Its intriguing pattern of activity against various kinases, and distinct features from other anti-MM agents, along with preliminary results from *in vivo* safety and efficacy studies in MM models suggest that this is a promising candidate for possible future clinical trials.

PO-518

AVICINS: A NOVEL CLASS OF ANTI-MYELOMA AGENTS

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Introduction. In our efforts to identify new compounds with anti-MM activity, we evaluated the chemical class of avicins, which are triterpenoid saponins that induce apoptosis of neoplastic cells by affecting mitochondrial function independently of membrane-bound death receptors. We have previously shown that mitochondria constitute key regulators of MM cell responsiveness to diverse anti-tumor agents, (*e.g.* bortezomib), we therefore evaluated the *in vitro* anti-MM effects of this class of compounds. *Materials/Methods/Results*. Using MTT survival assays, we observed that Avicin D and Avicin G, the main members of this class of compounds, are active against a broad panel of MM cell lines (n=37) and primary tumor cells, including MM cells resistant to conventional (*e.g.* Dex, alkylators, anthracyclines) or novel (*e.g.* Thal, immunomodulatory Thal derivatives, bortezomib, Apo2L/TRAIL) agents. Avicin D and G had highly concordant IC50 values (<250 nM for the overwhelming majority of MM cell lines tested) which were comparable with those among the most Avicin-sensitive tumor models tested so far. Minimal, if any, effect on the viability of normal hematopoietic cells or bone marrow stromal cells (BMSCs) were observed in these dose ranges of Avicins. Co-culture with BMSCs or transfection of MM cells with construct for constitutively active Akt or Bcl-2 did not protect them from Avicins, which also sensitized MM cells to cytotoxic chemotherapeutics. Hierarchical clustering and relevance network analyses showed that the patterns of MM cell sensitivity to Avicin D vs Avicin G are highly concordant, but clearly distinct from those for other, conventional or investigational, anti-MM drugs. This further supports the notion that the anti-MM properties of Avicins are mediated by molecular mechanisms distinct from those of currently available anti-MM drugs and that Avicins may have activity even against MM subgroups resistant to other novel therapies currently in clinical development. Our ongoing *in vivo* safety studies in SCID/NOD mice identified dose levels at which Avicins do not cause toxicity to vital organs. Upcoming efficacy studies will specifically address their *in vivo* anti-MM activity. *Conclusions*. Avicins represent a novel class of anti-MM agents that merit further consideration for possible future clinical trials to improve patient outcome in MM.

PO-519

NPI-1387 REGULATES IKKα AND NF-κB: A TARGET FOR MYELOMA

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Introduction. A link between cancer and inflammation has long been proposed through the action of the transcription factor NF-κB. As constitutive NF-κB activation is linked to growth and survival of multiple myeloma (MM) cells, targeting this survival pathway to eradicate MM is an attractive therapeutic approach. We describe NPI-1387, a potent inhibitor of NF-κB activation and its effects on MM cells, including those resistant to conventional agents dexamethasone, doxorubicin and bortezomib. *Methods*. Cell-based assays were used to screen a library of ~200 semi-synthetic analogs derived from the naturally derived pimarane diterpene, Acanthoic acid. Analog, NPI-1387 most potently inhibited LPS-induced TNF-α synthesis in murine RAW 264.7 cells and reduced TNF-α induced NF-κB activation in a human HEK293 NF-κB/luciferase reporter cell line. *Results*. To further explore the molecular target(s) of NPI-1387, we addressed its effects on the upstream kinase of IκBα. By immunoprecipitation of IKKα from NPI-1387 treated cancer cells, we demonstrated that NPI-1387 decreased IKKα kinase activity in a dose-dependent manner suggesting that NPI-1387 is either a direct IKKα kinase inhibitor or functions more upstream in the NF-κB pathway. Treatment with NPI-1387 induced a dose-dependent (p<0.004) decrease in viability in all six MM cell lines tested. NPI-1387 triggered significant apoptosis in these cells, as measured by Annexin V staining and a marked increase in nuclear condensation reflected by dense pattern of DAPI staining under phase contrast microscopy. In contrast, untreated control cells exhibited homogeneous and intact nuclei. NPI-1387 also triggered proteolytic cleavage of poly (ADP ribose) polymerase (PARP). Importantly NPI-1387 decreased the viability of cells from bortezomib-refractory MM patients. No significant toxicity of NPI-1387 was observed against peripheral blood mononuclear cells from healthy donors or the viability of CD138- cells or bone marrow derived stromal cells from MM patients. Apoptosis can proceed through an intrinsic pathway and caspase-9 activation and an extrinsic pathway and caspase-8 activation, both leading to downstream caspase-3 activation. Our results show that NPI-1387 induces activation of caspase-8, caspase-9 and caspase-3 cleavage. *Summary*. These findings provide the rationale for clinical evaluation of NPI-1387 to induce MM cell killing through regulating IKKα /NF-κB activities, overcome drug-resistance and improve patient outcome in MM.

PO-520

NF-κB ACTIVATOR INHIBITOR EFFECTIVELY INHIBITS GROWTH OF HUMAN U266 MYELOMA CELLS VIA BLOCKAGE OF IL-6 MEDIATED JAK-STAT CELL SIGNALING PATHWAYS

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Introduction. Interleukin-6 (IL-6) plays a pivotal role in the pathogenesis of multiple myeloma (MM) as a growth and survival factor for MM cells. But, little is known about its downstream pathways. These findings leaded us to investigate the effect of IL-6/ IL-6sR (IL-6 soluble receptor) on MM cells. Recent studies showed that a new proteasome inhibitor (bortezomib), via deregulation of NF-κB activation, showed marked activity in MM. So, inhibitors of NF-κB activation could be potent growth inhibitors in MM. *Materials and Methods*. MM cell lines, U266 and IM9 were treated with IL-6 and/or IL-6sR and growth rates and JAK/STAT as well as MAPK pathways were examined. Then the effect of JAK inhibitors and inhibitors of NF-κB activator were examined. *Results*. When U266 cells were treated with IL-6 and/or IL-6sR, their growth rate was increased and JAK/STAT as well as MAPK pathways were activated. However, the growth rate of IM9 cells was not affected by IL-6 and/or IL-6sR treatment. In addition, activation of JAK/STAT pathway (p-STAT3) and MAPK pathway (p-Erk) was not detected in IM9 cells. These results indicated that U266 is IL-6 and/or IL-6sR dependent but IM9 was

not. For this reason, we used U266 to further investigate the role of IL-6 and/or IL-6sR in MM. IL-6 increased phosphorylation of STAT-1, -3 including c-Myc and cyclinD1, which was one of downstream of p-STAT3. Three different types of JAK inhibitors decreased the activation of the above pathways. JAK inhibitor I most effectively blocked JAK/STAT cell signaling. We also found that JAK inhibitor I effectively reduced the activation of transcription factor nuclear factor-κB [NFκB (p65 and p-IκB-a)]. We showed that NF-κB activator inhibitor dramatically inhibited IL-6 induced p-STAT-1,-3, and p-Erk activation compared with other inhibitors including JAK inhibitor I. EMSA analysis also showed that NF-κB activator inhibitor prevented the activation of NF-κB. Subsequently, we also found that the growth of MM cells via JAK/STAT and MAPK pathways was inhibited effectively by combined treatment of JAK inhibitor I and NF-κB activator inhibitor. *Conclusion*. Blockage of JAK/STAT mediated NF-κB activation was useful to control the growth of MM cells and consequently, inhibitor of IL-6 mediated NF-κB cell signaling pathway may be a potential new modality to treat patients with MM.

PO-521

TARGETING PKC ISOFORMS IN MM BY ENZASTAURIN (LY317615.HCL)

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Introduction. Protein kinase C (PKC) overexpression has been reported in multiple myeloma (MM) cells, including the adverse prognostic patient group with t (4;14) translocation. Therefore, we here investigated the novel, orally available PKC inhibitor Enzastaurin for its anti-MM activity. *Material and Methods.* Effects of Enzastaurin on MM cell-lines and primary patient cells were analyzed by DNA-synthesis and MTT-assays, cell-fractionation, western-blotting, transwell migration and adhesion, as well as *in vitro* angiogenesis assays. PKC-isoform activity was assessed by *in vitro* kinase assay. A xenograft model in beige-nude Xid mice was used for *in vivo* studies. *Results*. Enzastaurin, at a low micromolar range equivalent to the concentrations achieved in patient plasma during clinical trials, inhibits both proliferation and survival of MM cell lines and tumor cells isolated from patients with multidrug- resistant MM; as well as overcomes MM cell growth triggered by tumor cell binding to bone marrow stromal (BMSC) and endothelial cells . Importantly, synergistic cytotoxicity is observed when Enzastaurin is combined with bortezomib. Besides proliferation and survival, Enzastaurin inhibits MM cell adhesion to BMSCs, as well as VEGF- and IGF-1-triggered MM cell migration. Enzastaurin specifically inhibits membrane, cytosolic, and nuclear phosphorylation of homologous PKC isoform residues, as well as associated kinase activity induced by the major PKC activator TPA. In MM, Enzastaurin inhibits PKC activation triggered by growth factors and cytokines secreted by BMSCs; extracellular matrix protein fibronectin and VEGF or IL-6; as well as MM patient serum. Phosphorylation of downstream signaling molecules was also abrogated, including cytoplasmic and nuclear MARCKS, ERK, JNK, ribosomal protein S6, and GSK3β; as well as nuclear cMyc. Enzastaurin also blocks VEGF- triggered signaling pathways in endothelial cells, thereby inhibiting tubule formation and angiogenesis. Gene expression profiling demonstrated that PKC inhibition modulates Wnt-signaling and activates ER-stress response. Finally, tumor growth, survival, and angiogenesis are abrogated by Enzastaurin in an *in vivo* xenograft model of human MM. *Conclusion*. Our results demonstrate *in vitro* and *in vivo* efficacy of the orally available PKC inhibitor Enzastaurin in MM, strongly supporting its clinical evaluation, alone or in combination therapies, to improve patient outcome in MM.

PO-522

TARGETING CYCLIN D1 IN THE TREATMENT OF MULTIPLE MYELOMA

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Either an overexpression or dysregulation of cyclin D1, D2, or D3, has been reported in the majority of multiple myeloma (MM) tumors, suggesting a possible early unifying event in MM pathogenesis. This proposed critical role of cyclin D dysregulation in myeloma pathogenesis makes the cyclins, specifically cyclin D1, an attractive therapeutic target. We have evaluated a specific small molecule cyclin D1 inhibitor, P276-00 in MM. We have observed both time and dose dependent *in vitro* activity against a broad range of MM cells sensitive and resistant to conventional agents like dexamethasone, doxorubicin, and melphalan with IC50 ranging from 400-800 nM. Importantly, it has demonstrated activity in primary patient derived tumor cells. Cell cycle analysis confirmed that P276-00 induced either growth arrest or apoptosis in MM cells depending on the cell line. Apoptosis was in part caspase dependent suggested by partial reversal of cytotoxicity by Z-VAD Fmk. P276-00 inhibited Rb-1 phosphorylation as early as 6 hours in most of the MM cell lines tested associated with a decrease in cdk4 suggesting a regulatory role of P276-00 in cell cycle progression. These changes preceeded growth arrest and apoptosis of MM cells on cell cycle analysis. As cyclin D1 dysregulation or overexpression can render MM cells more susceptible to proliferative stimuli such as IL-6, IGF-1, and the bone marrow microenvironment, we tested the effects of P276-00 in the presence of these cytokines and bone marrow stromal cells (BMSCs). Our data confirms that P276-00 was able to overcome these proliferative signals and induce apoptosis in MM cells. Next we evaluated *in vivo* efficacy of P276-00 in NOD-SCID mice bearing GFP+ MM xenografts. Our data confirms *in vivo* anti-tumor activity of P276-00 as suggested by a significant decrease in biluminesence of GFP+ MM cells ($p<0.05$) and a decrease in tumor volume and in improvement in survival. *In vitro* combination studies with bortezomib have been completed suggesting strong synergism. These studies confirm cyclin D1 to be an important therapeutic target in MM and form the basis of a phase I/II study of P276-00 alone and in combination in the treatment of MM.

PO-523

PRO-APOPTOTIC ACTIVITY OF THE BCL-2 INHIBITOR ABT-737 ON PRIMARY MULTIPLE MYELOMA SAMPLES

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The bcl-2 family proteins are key regulators of cell survival and are frequently found aberrantly expressed in lymphoid malignancies and in multiple myeloma (MM). Bcl-2 overexpression, particularly, has been widely described in MM cell lines and primary samples, and is linked to tumorigenesis and chemoresistance. Here, we investigated the cell cycle and apoptotic effects of ABT-737 (kindly provided by Abbott Laboratories), a Bcl-2 (BH3) inhibitor, on MM cell lines and on primary $CD138^+$ malignant plasma cells. The KMS18 MM cell line was exposed to increasing concentrations of ABT-737 (from 0.1 to 1 μM) for 24, 48 and 72 hours, and a dose- and time-dependent cell growth inhibition was documented (IC50s 0.286 μM at 72 hours). These effects were paralleled by a significant increase of apoptotic cells as demonstrated by the increment of Annexin V+ cells, at 24 hours, from 17.3%±4.4 in DMSO to 31.7%±12.9, 45.8%±9.1 ($p=0.032$), 49.8%±7.8 ($p=0.017$) and 60.8%±5.8 ($p=0.0026$) in the presence of ABT-737 at 0.1, 0.25, 0.5 and 1ìM, respectively. These data were confirmed by measuring the subG0/1 peak (Acridine-Orange). A cell cycle perturbation, particularly a significant ($p=0.021$) G1-phase depletion (from 54.6%±4.5 to 26.9%±1.4) was found after 72 hours at 1 μM. The effects of ABT-737 were then examined on primary cells from untreated patients: 6 MM and 1 plasma cell leukemia (PCL). Bone marrow aspirate cells, following CD138 enrichment (>80% of purity), were cultured *in vitro* with ABT-737 (at scalar concentrations from 0.1 to 1 μM) up to 72 hours. At very low concentrations (0.1 μM), ABT-737 significantly ($p=0.01$) increased $CD138^+$ apoptotic cells from 19.8%±9.0 to 52.9%±17.2 (24 hours) in MM samples. Effective apoptosis was observed in all 6 MM samples. Similarly, the PCL sample was strikingly sensitive to ABT-737 (a 3- and 5-fold increase of apoptotic cells at 0.1 μM and 0.5 μM). In conclusion, ABT-737 shows potent *in vitro* growth-inhibitory and pro-apoptotic activity at nanomolar concentrations in MM cells, indicating that it may appropriately target survival mechanisms in this disease. A further pre-clinical/clinical development of this compound is warranted.

PO-524

THE GREEN TEA POLYPHENOL EPPIGALLOCATECHIN GALLATE (EGCG) INHIBITS GROWTH AND IL-6 SIGNALLING PATHWAYS IN MULTIPLE MYELOMA CELLS

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Introduction. Epigallocatechin gallate (EGCG) is the predominant polyphenolic constituent of green tea leaves that possesses antitumor, antiinflammatory, and antioxidant activity. EGCG exerts its effects through potentially multiple mechanisms including inhibition of growth factor receptor signalling. The compound is currently under investigation in a phase I/II clinical trial for treatment of patients with early stage chronic lymphocytic leukemia at Mayo Clinic. The goal of our study was to examine the *in vitro* effects of EGCG in multiple myeloma (MM). *Materials and Methods.* Human myeloma cell lines (n=6) including IL-6 dependent INA-6 cells were cultured for three days in the absence or presence of EGCG. Cell growth and viability was determined in a colorimetric tetrazolium (MTS) based assay and by trypanblue exclusion. For signalling experiments, INA-6 cells were IL-6 and serum starved and then treated with EGCG for two hours before IL-6 was added. Whole cell lysates were prepared and subjected to SDS-PAGE and Western blot analysis. *Results*. EGCG inhibited the *in vitro* growth of human myeloma cell lines by inducing cell death in a time and dose-dependent manner. IC50 concentrations were between 12,5 μM and 50 μM. Pretreatment of INA-6 cells with EGCG resulted in a dose-dependent inhibition of IL-6 induced STAT3 tyrosine phosphorylation. Phosphorylation of p44/p42 MAPK, which is constitutively activated in INA-6 cells, was not affected. Neither the addition of excess amounts of IL-6 nor overexpression of Mcl-1 could protect from EGCG induced cytotoxicity. However, INA-6 cells ectopically expressing high amounts of Bcl-xL were less sensitive. *Conclusions*. EGCG has growth inhibitory activity on myeloma cells. Specific inhibition of signalling pathways that regulate expression of anti-apoptotic proteins could be one mechanism how EGCG exerts its activity. Our work provides the rationale for further studies to evaluate the effect of EGCG not only in B-CLL, but also in plasma cell tumors.

PO-525

DEFIBROTIDE INDUCES DOWNREGULATION OF HEPARANASE EXPRESSION IN MULTIPLE MYELOMA CELL LINES: A NOVEL MECHANISM OF ACTION

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Introduction. Elevated heparanase (HPSE) expression in humans has been correlated with advanced progression and metastasis of many tumor types, including multiple myeloma (MM), HPSE activity in MM is associated with altered gene expression that may promote an aggressive tumor phenotype with high microvessel density. These findings indicate an important role of HPSE in regulating the growth and progression of MM. Defibrotide (DF) is a polydisperse oligonucleotide with anti-thrombotic, thrombolytic, anti-ischemic, and anti-adhesive properties. Recently, DF has been shown to increase *in vivo* sensitivity of MM cells to conventional chemotherapy by modulating interactions of MM cells with their local microenvironment. Here we examine whether DF regulates the expression and activity of HPSE in two MM cell lines, U266 and RPMI 8226. *Methods.* Expression of HPSE was evaluated in the two MM cell lines treated for 24h, with or without DF, by flow cytometric analysis (FACS) using a monoclonal antibody against heparanase. HPSE gene expression was assessed by real time polymerase chain reaction using cDNA from those cells. HPSE activity was measured on MM extracts with a heparan-degrading enzymatic assay (Takara Biomedicals, Japan). Size exclusion high performance liquid chromatography was performed to identify the degradation product of DF incubated for 24h in 10% serum. *Results*. FACS analysis revealed that DF downregulates the HPSE expression in the two lines of MM in a dose dependent manner (at concentration of 10, 50, 100 e 150 μg/mL). DF also significantly decreased of HPSE gene expression ($p<0.01$) in those MM cells after 24h of treatment. In addition, we demonstrated a significant reduction of heparanase activity by DF ($p<0.01$) in MM cells. Furthermore, the degradation product of DF did not show any biological activity. *Conclusion*. DF downregulates HPSE expression and activity in MM cell lines. These preclinical data support clinical trials of DF in combination with other tumor therapeutics to improve patient outcome in MM.

PO-526

BASIS OF 8-NH2-ADENOSINE DECREASED PHOSPHORYLATION OF P38

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We have previously characterized 8-Amino-adenosine (8-NH2-Ado) as a novel purine analog which is cytotoxic in myeloma cell lines. This drug acts by decreasing RNA synthesis, decreasing the cellular bioenergetics and also by decreasing the phosphorylation of key growth related kinases. In addition we observed that the serine/threonine specific phosphatase, PP2A is involved, but is not the only mechanism leading to the decreased phosphorylation. To more fully elucidate the mechanisms of 8-NH2-Ado actions, we have investigated whether tyrosine specific kinases are likewise affected by 8-NH2-Ado treatment. By immunoblotting, we did not detect a decrease in phophorylation of Fyn, Pyk2, Lyn, or VEGFR2 indicating specificity of 8-NH2-Ado activity towards serine/theronine kinases. We focused on characterizing the potential mechanisms for the 8-NH2-Ado induced decrease in phosphorylation of p38, a serine/threonine kinase critical for the growth of myeloma cells. We examined whether 8-NH2-Ado treatment selectively targets phosphorylated p38 for degradation resulting in the observed overall decrease in phosphorylation. Using the proteosome inhibitor bortezomib, we measured the amount of phospho-p38 in the MM.1S myeloma cell line following a time course of treatment with 8-NH2-Ado. We observed a similar decrease in phosphorylation regardless of the presence of bortezomib indicating that increased protein degradation is not a mechanism for the decreased phosphorylation. The myeloma growth factors IGF1 and IL-6 stimulate p38 activity and could potentially inhibit the actions of 8-NH2-Ado. Immunoblotting of phospho-p38 after treatment with 8-NH2-Ado in the presence of IGF1 or IL-6 demonstrated no decrease in p38 phosphorylation indicating these myeloma growth factors do not inhibit 8-NH2-Ado activity. We have previously reported 8-NH2-Ado treatment of MM.1S cells results in increased 8-NH2-ATP and decreased endogenous ATP. To examine if the 8-NH2-ATP could inhibit the kinase activity of p38, we utilized *in vitro* p38 kinase assays in the presence of increasing 8-NH2-ATP and measured the amount of p38 activity. We observed that p38 kinase activity is inhibited by 8-NH2-ATP. In conclusion, 8-NH2-Ado induced decrease in phosphorylation is specific for serine/threonine kinases. The mechanism of decreased p38 phosphorylation is not only through phosphatase activity, but also through the ability of the metabolite, 8-NH2-ATP, to decrease kinase activity.

PO-527

TARGETING METABOLIC PATHWAYS IN MULTIPLE MYELOMA

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Background. We have previously demonstrated that 8-amino-adenosine (8-NH2-Ado) is rapidly taken up by multiple myeloma MM.1S and bone marrow Hs5 cells where it is converted to 8-NH2-ATP with concomitant loss of endogenous ATP. This equivalent accumulation leads to apoptosis and de-phosphorylation of Akt, Gsk3β and p38 in MM.1S cells but not in the Hs5 cells. With recent interest in the Warburg effect i.e. the utilization of aerobic glycolysis vs. the more efficient oxidative phosphorylation to generate ATP, the objective of these studies was to utilize the differential signal transduction of 8-NH2-Ado in MM.1S and Hs5 cells to define molecular targets controlling glycolysis and/or cellular respiration. *Results*. To determine if Akt de-phosphorylation with 8-NH2-Ado modulates glycolysis in MM.1S cells we investigated uptake of glucose, one of the early rate limiting steps of the glycolytic pathway. We measured [3H] 2-DG uptake in MM.1S and Hs5 cells treated with 8-NH2-Ado for 5 hrs. Interestingly MM.1S cells show a 50% reduction in [3H] 2-DG uptake while there is no decrease in the Hs5 cells. This reduction in [3H] 2-DG uptake is specific to 8-NH2-Ado and not seen with the congener analogue 2-NH2-Ado. By immunoblot analysis we detect no regulation of Glut1 glucose transporter expression. Cellular viability MTS assays on MM.1S cells treated with glycolytic pathway inhibitor 2-DG, demonstrated MM.1S cells to be sensitive to glycolytic pathway inhibition. In preliminary studies we have determined oxygen consumption in untreated MM.1S cells to be low suggestive of glycol-

ysis being a potential alternative source for ATP generation. *Conclusions*. We have investigated the relevance of glycolysis and targeting of glucose utilization in MM.1S cells treated with 8-NH2-Ado. We have preliminary data showing low cellular baseline O2 consumption in MM.1S cells, sensitivity to the glycolytic pathway inhibitor 2-DG and reduction in [3H] 2-DG uptake upon treatment with 8-NH2-Ado. Further characterization of cellular respiration and glycolysis in MM could provide insight into novel therapeutic strategies targeting a metabolic pathway.

PO-528

INHIBITION OF EXTRACELLULAR SIGNAL-REGULATED KINASE1/2 MITOGEN-ACTIVATED PROTEIN KINASE (MAPK/ERK1/2) ACTIVITY BY MEK1/2 INHIBITOR AZD6244 (ARRY-142886) INDUCES HUMAN MULTIPLE MYELOMA (MM) CELL APOPTOSIS AND INHIBITS OSTEOCLASTS WITHIN THE BONE MARROW (BM) MICROENVIRONMENT

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Activation of the extracellular signal-regulated kinase1/2 (ERK1/2) signaling pathway mediates human multiple myeloma (MM) growth and survival conferred by cytokines and adhesion to bone marrow stromal cells (BMSCs). In this study, we examined the effect of AZD6244 (ARRY-142886), a novel and specific MEK1/2 inhibitor, on human MM cells. AZD6244 inhibits constitutive and cytokine-stimulated ERK1/2, but not AKT phosphorylation. It inhibits the proliferation and survival of human MM cell lines, regardless of sensitivity to conventional chemotherapy, as well as freshly isolated patient MM cells. Significantly, AZD6244 induces apoptosis in patient MM cells even in the presence of BMSCs, as evidenced by caspase 3 activity and PARP cleavage at concentrations as low as 20 nM. AZD6244 suppresses MM cell survival/growth signaling pathways and upregulates proapoptotic cascades. It reduces adhesion molecule expression in MM cells, associated with decreased MM adhesion to BMSCs. These pleiotropic effects of AZD6244 upon apoptosis, survival, adhesion and cytokine secretion abrogate BMSC-derived protection of MM cells, thereby sensitizing them to both conventional (dexamethasone) and novel (perifosine, lenalidomide, and bortezomib) therapies. In contrast, AZD6244 has minimal cytotoxicity in BMSCs and does not inhibit DNA synthesis in CD40 ligand-stimulated CD19-expressing B cells derived from normal donors at concentrations toxic to MM cells (0.02-2 μM). In an *in vivo* xenograft model of human MM, significant inhibition of tumor growth and survival was observed in AZD6244-treated mice Moreover, AZD6244 downregulates the expression/secretion of osteoclast (OC)-activating factors from MM cells and inhibits OC differentiation induced by RANKL and M-CSF from PBMCs of MM patients. Together, these results provide the preclinical basis for clinical trials with AZD6244 in MM.

PO-529

MELAN-A PEPTIDE TRIGGERS ANTI-MYELOMA CD8+ T CELLS THROUGH CROSSREACTIVITY WITH HM1.24

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Introduction. The Melan-Aaa26-35 (EAAGIGILTV) peptide is a HLA-A2-restricted T-cell epitope within the Melan-A (MART-1) tumor antigen expressed on malignant melanoma cells. Melan-A and Melan-A analogue (ELAGIGILTV, Melan-Aaa26-35*A27L) specific T cells can be expanded reliably for immunotherapeutic approaches *in vitro*. We study the ability of Melan-A analogue specific T-cells to recognise the HM1.24aa22-30 (LLLGIGILV) peptide that is presented by HLA-A2+ malignant plasma cells. HM1.24 is a type-II transmembrane-protein which is expressed on normal and malignant plasma cells. *Methods.* Peripheral blood mononuclear cells (PBMC) from HLA-A2+ healthy donors (HD) as well as HLA-A2+ MM patients were stimulated with Melan-A analogue peptide-loaded autologous dendritic cells, and expanded *in vitro*. T-cell activation was assessed by IFN-gamma specific ELISpot and cytotoxicity by 51Cr-release-assays. T2 cells pulsed with irrelevant peptide and the Melan-A-/HM1.24-/HLA-A2+ breast-carcinoma cell-line MCF-7 were used as controls. The frequency of Melan-Aaa26-35*A27L-specific CD8+ T-cells was determined by tetramere technique. *Results*. Relevant amounts (up to 17% of all CD8+ T-cells) of Melan-A analogue specific T-cells were expanded from PBMC from MM patients. Furthermore Melan-A analogue specific T-cells from HLA-A2+ HD and HLA-A2+ MM patients showed a specific IFN-gamma secretion mediated by HM1.24 peptide-pulsed T2 cells and lysed the HLA-A2+ U266, and XG-1 myeloma cell lines and the lymphoblastoid cell line IM-9. Melan-A analogue specific T-cells from a MM patient specifically lysed autologous MM cells. *Conclusion*. The current data demonstrate that Melan-A analogue specific T-cells cross react with HM1.24aa22-30 and might be a tool for a future use in immunotherapy against MM.

PO-530

ANTI-MYELOMA ACTIVITY OF HULUC63 ALONE AND IN COMBINATION WITH BORTEZOMIB

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Introduction. HuLuc63 is a humanized monoclonal antibody (mAb) that targets the cell surface glycoprotein CS1 (CD2 subset 1, CRACC, SLAMF7, CD319). We have shown that CS1 is expressed on normal plasma cells, a subset of lymphocytes and at high levels on myeloma cells from multiple myeloma (MM) patients. HuLuc63 treatment of mice with MM xenograft tumors resulted in significant *in vivo* anti-tumor activity. HuLuc63 mediates anti-MM activity via Fc interaction with natural killer (NK) cells, suggesting that antibody-dependent cellular cytotoxicity (ADCC) is the main mechanism of action. Bortezomib has been observed to down-modulate myeloma surface expression of MHC class I, an inhibitor of NK function. The purpose of this study was to examine whether using HuLuc63 in combination with bortezomib (Velcade(r)) provided therapeutic benefit. *Methods.* The effect of HuLuc63 and bortezomib treatment on expression of CS1 in MM cell lines and mouse xenograft tumors was examined by Flow cytometry and immunohistochemistry respectively. The combination of HuLuc63 with bortezomib was tested for anti-MM activity *in vitro* using ADCC assays and *in vivo* for anti-tumor activity in mouse models. *Results*. CS1 protein expression was examined on L363 and OPM2 multiple myeloma cell lines with no significant change in CS1 expression observed pre- or post-treatment with HuLuc63, bortezomib or with both agents. Pre-treatment with bortezomib significantly enhanced HuLuc63-mediated ADCC towards MM cell lines using NK effector cells from healthy donors. In the autologous setting, *in vitro* pre-treatment with bortezomib enhanced HuLuc63-mediated killing when using purified NK cells from a MM patient to target the patient's own myeloma cells. *In vivo* anti-tumor activity of HuLuc63 was also enhanced by co-treatment with bortezomib. Using sub-optimal doses of HuLuc63 (1 mg/kg given twice a week), combination treatment with bortezomib enhanced the anti-tumor effects in both L363 and OPM2 models by 40-50%. These effects are likely due to the combined anti-tumor effects of HuLuc63 and bortezomib. *Conclusions*. These preclinical studies continue to support HuLuc63 as a new therapeutic for the treatment of MM and suggest that bortezomib co-treatment may add to the anti-myeloma activity of HuLuc63. HuLuc63 is currently being evaluated in a phase I clinical study as monotherapy for the treatment of relapsed/refractory multiple myeloma.

PO-531

LACK OF TLR LIGAND-MEDIATED INNATE RESPONSES IN MYELOMA

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Although current vaccine strategies have achieved anti-myeloma immune responses, meaningful clinical responses have not been observed. This may be related with the significant immune dysfunction observed in myeloma that may interfere with the development of effective anti-myeloma immune responses. In order to overcome such dysfunction, signaling through Toll-like receptors (TLRs) can induce adjuvant

effects by increasing local production of chemokines, and proinflammatory cytokines, and by enhancing antigen-presentation by APCs. Here, we have evaluated the effects of TLR ligands in myeloma. Proliferation of T cells, depleted of Treg cells, with anti-CD3 antibody was significantly lower in MGUS (n=9, SI=12±2) and MM (n=9, SI=28±8) compared with normal donors (n=9, SI=74±9, $p<0.01$). As expected addition of Treg cells was able to suppress T cell proliferation in normal donors (v31%), however, their effects were less suppressive in MGUS (v26%), and in fact, increased T cell proliferation in MM (^29%). Additionally, LPS which functions through Toll-like receptor 4 (TLR4), is able to overcome suppression of T cell proliferation by Treg cells in normal controls (110%), however, it had limited influence in MGUS (49%) and MM (24%). Expression profile of myeloma cells (n=15) showed up-regulation of TLR4 (2.4 folds) and its related gene MD2 (4.5 folds) in all the MM cells tested compared to normal plasma cells indicating a possible modulation of immune responses by myeloma cells through TLR4 or its soluble agent. Proliferation of PBMC from normal donors was reduced by 75% and 50% following pre-incubation with serum from MGUS (N=4) and from MM (N=4) patients respectively. PBMC from myeloma patients (N=3) showed 4 to 10-fold less T cell proliferative responses in the presence of other TLR ligands including TLR2, TLR3, TLR5, TLR7 and TLR9 compared with PBMC isolated from normal donors. In addition, MM cell lines (N=6) showed proliferative responses to ligands of TLR3 and TLR7 while did not responded to ligands of TLR2, TLR4, and TLR5. Understanding of the molecular basis for lack of TLR ligand-mediated responses in myeloma may eventually render potential targets to improve immunotherapeutic strategies in myeloma.

PO-532

GENE-MODIFIED T CELLS TARGETING LEWIS POSITIVE MYELOMA

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Aim/Background. Haematological neoplasias including multiple myeloma (MM) are considered suitable targets for immunotherapy as evidenced by the success of allogeneic stem cell transplant and other immunotherapy approaches. Recently, adoptive T cell based immunotherapy with tumour infiltrating lymphocytes (TIL) or gene modified T cells has shown clinical activity in solid tumours. We have been examining the expression of the carbohydrate antigen LewisY (LeY) on MM with the aim of testing its suitability as a target for T cell mediated immunotherapy with T cells expressing a chimeric anti-LeY receptor. *Methods and Results*. We developed a construct of a chimeric T-cell receptor which recognises the LeY antigen in an MHC-independent manner, activates T-cells and confers additional co-stimulatory signals. We have shown efficient retroviral transduction of this construct into human T-cells with a transduction efficacy of up to 65% in a GMP-conform protocol. Functional analysis of transduced T-cells showed specific interferon gamma secretion in response to a co-culture with LeYpos target cells. Further, we demonstrated cytotoxicity of transduced T-cells against target cells with up to 89% specific lysis. Finally, *in-vivo* activity of gene-modified T cells was demonstrated in delaying tumour growth of myeloma xenografts in NOD/Scid mice. In three independent experiments NOD/Scid mice were challenged with s.c. deposits of a LeY pos. MM cell line. Mice receiving T cells transduced with the LeY vector (Le) had significant advantages regarding disease free survival (DFS) as well as overall survival (OS) compared with mice receiving primary unmanipulated T cells (T): Up to 71% of Le mice were tumour-free on day 56 while all T mice revealed plasmacytoma at the injection site (exp. 1). Similarly, 80% of mice having received anti LeYpos T cells were still alive at day 43 when all control mice had succumbed to disease (exp. 2). *Conclusion*. LeY is present in a subset of MM with high expression levels in individual patients. Preclinical studies with transduced T-cells targeting LeY are very promising. Consequently, we are soon to undertake a clinical phase I study using this transduction system for modification of autologous T-cells from patients with LeY positive MM.

PO-533

TARGETING CS1, A NEWLY IDENTIFIED HUMAN MULTIPLE MYELOMA ANTIGEN, BY A NOVEL HUMANIZED MONOCLONAL ANTIBODY HULUC63, BLOCKS MYELOMA CELL ADHESION IN THE BONE MARROW MICROENVIRONMENT AND INDUCES POTENT CYTOTOXICITY AGAINST MYELOMA CELLS EVEN RESISTANT TO CONVENTIONAL AND NOVEL THERAPIES

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Monoclonal antibody (mAb) therapies are currently unavailable for human multiple myeloma (MM) due to narrow target expression across MM patient samples. A preferred strategy would be to develop cytotoxic human mAbs against novel antigens that are highly expressed in MM cells yet have limited expression in other cell types. We here first characterized cell surface CS-1 (CD2 subset 1, CRACC, SLAMF7), a member of the CD2 family of cell surface glycoproteins, as a novel MM antigen and further investigated the potential therapeutic utility of HuLuc63, a novel humanized anti-CS1 mAb, for treating human MM. CS-1 mRNA and protein is highly expressed in CD138-purified primary tumor cells from the majority of MM patients (>97%). HuLuc63 induces antibody-dependent cellular cytotoxicity (ADCC) against CD138[+] MM cells expressing CS1 in a dose-dependent manner. Importantly, it triggers autologous ADCC against CS1-expressing primary MM cells from resistant to conventional or novel therapies including bortezomib (Velcade®) and HSP90 inhibitor; and pretreatment with conventional or novel anti-MM drugs markedly enhances HuLuc63-induced MM cell lysis. HuLuc63 induces significant ADCC even against MM cells adherent to bone marrow stromal cells (BMSCs), which confers resistance to conventional therapies. CS1 is expressed at adhesion-promoting uropod membranes of polarized MM cells, and short interfering RNA (siRNA) targeted to CS1 inhibits MM cell adhesion to BMSCs. Finally, circulating CS1 is only detected in MM patient sera, but not in normal donors. These results both define the functional significance of CS1 in MM and provide the preclinical rationale for clinical trials of HuLuc63, either alone or in combination, to improve patient outcome in MM.

PO-534

THE COMBINATION OF THE MTOR INHIBITOR RAPAMYCIN AND PROTEASOME INHIBITOR BORTEZOMIB IS SYNERGISTIC *IN VITRO* IN MULTIPLE MYELOMA

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Background. The PI3K and NFk-B/proteasome pathways are major regulators of survival in Multiple Myeloma (MM). Previous studies have demonstrated clinical efficacy of bortezomib in MM; however, not all patients responded to this agent. mTOR inhibitors have demonstrated significant *in vitro* and *in vivo* activity in MM, specifically clinical trials with the mTOR inhibitor CCI-779 (Wyeth) in MM. Therefore, we examined whether inhibition of the PI3K pathway by mTOR and proteasome inhibitors may lead to synergistic activity in MM. *Methods.* MM cell lines (MM.1S, RPMI, U266, OPM2) were treated with rapamycin 1-5 nM (Sigma Aldrich), bortezomib 2.5-10 nM (Millenium, MA), or the combination. Cytotoxicity was measured by the MTT assay at 48 hrs; DNA synthesis was measured using thymidine uptake assay; apoptosis was studied using Apo2.7 by flow cytometry, and cell cycle regulation was determined using flow cytometry. To determine whether these agents can overcome the growth advantage conferred by bone marrow stromal cells (BMSCs), we co-cultured cell lines with stromal cells. Normal peripheral blood mononuclear cells (PBMCs) were obtained from healthy volunteers. Determination of the additive or synergistic effect of the combination was calculated using the CalcuSyn software (Biosoft, MO) based on the Chou-Talalay method, with synergistic activity determined as a combination index (CI) of <1.0. *Results*. Rapamycin induced dose-dependent cytotoxicity from 0.1nM to 1nM, with an IC_{50} of 5 nM in MM.1S and OPM2. Interestingly, higher doses did not induce further cytotoxicity, confirming that low doses of rapamycin are as effective as higher doses. RPMI and U266 MM cell lines were less sensitive to rapamycin, with 5nM inducing 40% and 20%

decrease in survival, respectively. Bortezomib induced significant inhibition of survival in all MM cell lines with an IC50 of 2.5 nM, as previously reported. The combination of agents induced significant inhibition of proliferation as compared to each agent alone, specifically with the combination of 5 nM rapamycin with 5 nM of bortezomib. In the DNA synthesis assay, the combination of bortezomib and rapamycin was significantly cytotoxic compared to each agent alone, specifically at the dose of 5 nM rapamycin and bortezomib 2.5 nM. The combination of rapamycin 1 to 5 nM and bortezomib 5 to 10 nM were synergistic with a CI index less than 1.0, as in RPMI (CI=0.4) and U266 (CI=0.2) cell lines. The combination of rapamycin and bortezomib at serial concentrations did not trigger cytotoxicity in PBMCs from normal volunteers, indicating significant cytotoxicity in malignant cells, with lack of toxicity in normal PBMCs and suggesting a therapeutic index. The combination of bortezomib and rapamycin demonstrated a significant inhibitory effect on the growth of MM cell lines even in coculture with stromal cells. Cell cycle analysis demonstrated G1 arrest at 24 and 48 hrs in MM.1S cells. Similar results were obtained using primary CD138[+] myeloma cells from patients. *Conclusion*. The combination of rapamycin and bortezomib resulted in synergistic *in vitro* cytotoxicity in MM cells. These results provide the framework for clinical trials evaluating the combination of CCI-779 and bortezomib in MM.

PO-535

THE MTOR INHIBITOR RAD001 (EVEROLIMUS) INHIBITS MYELOMA TUMOR GROWTH *IN VIVO*

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Introduction. The mammalian target of rapamycin (mTOR) is an intracellular protein that acts as a central regulator of multiple signaling pathways (IGF, EGF, PDGF, VEGF, amino acids) that mediate abnormal growth, proliferation, survival and angiogenesis in cancer. mTOR is a critical component of the PI3K/Akt pathway, a key cell survival pathway that is dysregulated in many cancers. mTOR is an important therapeutic target because it is a *rate-limiting* bottleneck in the key signaling pathway that regulates cell survival, proliferation, and angiogenesis. RAD001 (everolimus) is a novel oral mTOR pathway inhibitor. Recent data suggests that RAD001 has direct effects on tumor cell proliferation and may have antiangiogenic activity due to inhibition of tumoral VEGF production and effects on vascular endothelial and smooth muscle cell biology. In this study, we investigated the anti-myeloma effects of RAD001 as a single agent in mice bearing multiple myeloma (MM) tumors. *Materials and Methods.* To evaluate the effects of RAD001 *in vivo*, we used our previously established melphalan resistant murine SCID-hu xenograft LAGlambda-1 model. Each immunodeficient (SCID) mouse was implanted with a fragment (2 - 4 mm^3) of the LAGlambda-1 tumor into the left hind limb muscle. The tumors were allowed to grow for 14 days at which time human IgG levels were detectable in the mouse serum, and mice were blindly assigned into one of four treatment groups. Tumor-bearing mice received RAD001 at 3, 10, or 30 mg/kg once daily five times per week (M-F) via oral gavage or a placebo on the same schedule. *Results*. Mice receiving RAD001 therapy showed marked inhibition of tumor growth at all doses ($p<0.0001$) and reduction of paraprotein levels ($p<0.0001$) compared to mice receiving placebo. Treatment with RAD001 was not associated with any observed toxicity. On day 28, LAGlambda-1-bearing mice receiving RAD001 showed an average 60% reduction in human paraprotein levels and an average 70% decrease in tumor volume compared to the placebo-treated animals. *Conclusions*. Preliminary results are encouraging with single agent RAD001 and additional studies are being performed to further optimize the clinical development of RAD001 treatment as a single agent and in combination with other anti-MM treatments for patients with relapsing or refractory MM.

PO-536

INHIBITION OF MTOR INDUCES APOPTOSIS INDEPENDENTLY OF P53 AND PROLONGS SURVIVAL IN THE INA-6 PLASMACYTOMA SCID MOUSE MODEL

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Introduction. The mammalian target of rapamycin (mToR) plays a crucial role in cell growth due to its role as a nutrient dependent regulator of important cytokine signalling pathways. Recently, mToR was also linked to p53 and the control of apoptosis. In multiple myeloma, mToR is involved in the AKT pathway which can be activated by the loss of the tumor suppressor PTEN or by growth and survival factors such as IL-6 and IGF-1. mToR can be blocked by the use of rapamycin or everolimus, both clinically approved for immunosuppression. *Materials and Methods.* We evaluated the *in vitro* activity of rapamycin and everolimus in various human myeloma cell lines using the MTS cell growth assay. For cell cycle analysis, cells were stained with propidium iodide and subjected to flow analysis. Protein expression was determined by immunoblotting. Rapamycin was also tested in the INA-6 SCID mouse model (two weeks of treatment). Plasmacytoma bearing mice received rapamycin for three consecutive days and the tumor was explanted to elucidate the mechanism of action. Histological staining was performed using an antibody specific for the human cleaved form of PARP. *Results*. Both drugs induced a dose-dependent growth inhibition of plasma cell lines. In the majority of cell lines, growth inhibition was mediated by G1 cell cycle arrest coupled with an induction of apoptosis. In contrast, the INA-6 cell line established in our laboratory and harbouring a p53 mutation, underwent apoptosis without cell cycle arrest. Western blot analysis of INA-6 cells revealed cleavage of PARP but no activation of the p53 dependent p21WAF1. Rapamycin treated mice had a significant survival benefit compared to control animals ($p=0.0004$). Tumors of rapamycin treated animals contained increased numbers of apoptotic cells compared to controls. *Conclusions*. Effective drugs targeting mToR are already available for clinical use. Rapamycin is highly active in the INA-6 SCID myeloma model and induces apoptosis *in vitro* as well as *in vivo*. The mechanism of action is independent of p53 indicating a more complex role for mToR in apoptosis control. Thus, the inhibition of mToR represents an attractive new therapeutic concept in multiple myeloma.

PO-537

COMBINATION OF THE AKT INHIBITOR PERIFOSINE WITH THE HSP90 INHIBITOR 17-(DIMETHYLAMINOETHYLAMINO)-17-DEMETHOXYGELDANAMYCIN (17-DMAG) HAS SYNERGISTIC ACTIVITY IN TUMOR CELL AND ITS MICROENVIRONMENT IN MULTIPLE MYELOMA (MM)

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Background. The PI3K/AKT and Heat Shock Protein (HSP) pathways interact at the level of AKT. We hypothesized that the combination of an AKT inhibitor, perifosine (Keryx, NY), and HSP90 inhibitor, 17-DMAG (supplied by NCI) will lead to synergistic cytotoxic activity in MM as well as on the bone marrow microenvironment (BMM), namely on angiogenesis and osteoclast (OCL) activity. *Methods.* MM cell lines with high level of AKT activity (OPM2) and lower AKT activity (MM.1S, U266, RPMI) were exposed to perifosine 5 and 20 mM and 17-DMAG 50-100 nM alone or in combination. Cytotoxicity was measured using the MTT survival assay, growth inhibition using thymidine uptake, and apoptosis and cell cycle analysis by flow cytometry. In addition, we tested the effect of perifosine and 17-DMAG on endothelial cells (HUVEC cell line, Cambrex, NY) and OCL. *Results*. Low doses of perifosine (5 to 10mM, 48h) induced 40% cytotoxicity that was synergistically enhanced to 50% and 65% in combination with 17-DMAG, 50 nM and 100nM respectively. Perifosine-induced inhibition of DNA synthesis, apoptosis and cell cycle arrest were significantly enhanced by 50 nM 17-DMAG. Similar results were observed in primary CD138[+] patient cells. Interestingly, sequential treatment with 17-DMAG for 12 to 24 hours before the addition of perifosine sensitized the cells to the cytotoxicity of the com-

bination. The mechanism of apoptosis induced by perifosine and 17-DMAG combination was through activation of SAPK/JNK pathway, followed by caspase -8, -9, then -3 and PARP cleavage and decreased expression of BCL-XL and MCL-1. In parallel, we also observed significant decrease in AKT but not MAPK pathways. Perifosine and 17-DMAG combination showed strong efficacy in co-culture with stromal cells. Similar results were observed with OCL activity. Finally, 17-DMAG and perifosine combination significantly triggered cytotoxicity in HUVEC endothelial cells cultured alone or with MM cells. This combination also strongly reduced vessels formation and induced caspases and PARP cleavage in endothelial cells. Importantly, the 2 drugs did not induce cytotoxicity in healthy peripheral blood mononuclear cells, indicating lack of toxicity on normal cells. *Conclusion*. Targeting both the AKT and HSP pathways represents an attractive approach to future therapeutic options in MM.

PO-538

IN VITRO AND N VIVO ACTIVITY OF THE VEGF INHIBITOR PAZOPANIB

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Introduction and Aims. Vascular endothelial growth factor (VEGF) and its receptors play an important role in the pathogenesis of multiple myeloma (MM). VEGF present in the MM bone marrow microenvironment induces neovascularization; triggers tumor cell growth, survival and migration; inhibits dendritic cell maturation; and promotes osteoclastogenesis. VEGF therefore provides a potential therapeutic target in MM. Here we investigated a novel and potent VEGF receptor inhibitor, pazopanib. *Materials and Methods*. Effects of pazopanib on MM and endothelial cell growth, survival, and migration were evaluated using western blot analysis, 3H-thymidine uptake, Boyden- modified chamber, tubule formation assays, FACS analysis as well as microarray analysis, RT- PCR, and specific cMyc knockdown by siRNA. *In vivo* effects of pazopanib were evaluated using a MM xenograft mouse model. *Results. in vitro*, pazopanib inhibits VEGF-triggered VEGF receptor phosphorylation and activation of downstream signaling molecules including Src kinase in MM cells and blocks MM cell migration, growth, and survival. Moreover, gene expression and signaling network analysis in pazopanib- treated cells demonstrate transcriptional changes of several signaling pathways, including marked downregulation of cMyc. siRNA targeting cMyc blocked VEGF production and secretion in MM cell lines. In addition, pazopanib reduced VEGF in the microenvironment and directly inhibited endothelial cell growth, migration, and vessel formation. Furthermore, pazopanib inhibited VEGF- induced upregulation of adhesion proteins on both endothelial and MM cells, thereby abrogating endothelial cell -MM cell adhesion and associated tumor cell proliferation. Pazopanib also strongly sensitized tumor cells bound to endothelial cells to DNA- damaging chemotherapeutic agents (i.e. melphalan), immunomodulatory drugs, and bortezomib. Similar activity of pazopanib was demonstrated *in vivo* using a MM xenograft mouse model. iv) *Conclusions*. In summary, this is the first report showing anti- MM activity of an anti-VEGF compound in both *in vitro* and *in vivo*, strongly supporting its clinical evaluation either as a single agent or in combination with other therapies.

PO-539

ANTI-TUMOR AND ANTI-ANGIOGENIC EFFECTS OF APLIDIN IN THE 5T33MM, SYNGENEIC, MODEL OF MULTIPLE MYELOMA

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Aplidin is an anti-tumor compound, undergoing Phase II evaluation in multiple myeloma and other solid tumors. Chemically, it is a cyclic depsipeptide, originally isolated from the marine tunicate Aplidium albicans and currently obtained by synthesis. In the current study, we analyzed the anti-myeloma effects of Aplidin in the syngeneic 5T33MM model. *in vitro*, DNA synthesis was measured by 3H-thymidine incorporation. Apoptosis was quantified using FACS analysis of AnnexinV, cytochrome C and cleaved caspase-9 and caspase-3. To proof the contribution of caspase activation to apoptosis induction, a caspase inhibitor Boc-D-FMK was used. Cell cycle progression was analyzed by FACS, using propidium iodide. To study the different cell cycle regulators, Western-Blot was performed. For the *in vivo* experiment, 30 5T33MM injected C57BlKa-LwRij mice were intraperitoneally treated with vehicle or Aplidin (60 and 90 µg/kg daily) and 10 naive mice were used as negative controls. After 4 weeks, when the vehicle mice were terminally diseased, all mice were sacrificed. Tumor load was assessed by determining plasmacytosis in the BM and paraprotein concentrations. Angiogenesis was determined by quantifying microvessel density after CD31 staining of BM sections. Aplidin inhibited 5T33MMvv DNA-synthesis with an IC50 of 3,77 nM. On cell cycle progression, the drug induced an arrest in transition from G0/G1 to S phase while Western Blot showed a decreased cyclin D1 and CDK4 expression. Furthermore, Aplidin induced apoptosis which could be blocked using a caspase inhibitor. Aplidin lowered the mitochondrial membrane potential, induced the release of cytochrome C and activated caspase-9 and caspase-3. *In vivo*, chronic treatment with Aplidin was well tolerated and reduced serum paraprotein concentration with 42% (p<0,001) while BM invasion with myeloma cells was decreased with 35% (p<0,001). Next to directly affect myeloma cells, Aplidin also reduced the myeloma associated neo-vascularization to basal values. This anti-angiogenic effect was confirmed *in vitro* using a BM endothelial cell line and a Rat Aortic Ring Assay in which Aplidin inhibited cell proliferation and vessel formation in MM induced neovascularization. These data indicate that Aplidin is well tolerated *in vivo* and its anti-tumor and anti-angiogenic effects support the use of the drug in chronic dosing schedules of multiple myeloma.

PO-540

INHIBITING PLEIOTROPHIN REDUCES MM AND ENDOTHELIAL GROWTH

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Aims. We characterized the effects of inhibition of pleiotrophin (PTN) with PTN siRNA, PTN antisense or anti-PTN antibody on both tumor cell proliferation and endothelial cell formation in multiple myeloma (MM) *in vitro* and *in vivo*. *Material and Methods.* We blocked expression with the introduction of PTN siRNA or antisense in the MM cell lines U266 and MM-1S. The MM cell lines were treated with either PTN antisense constructs or PTN siRNA. The purified monocytes were cultured on collagen-I and were co-cultured with or without treated MM cells. Next, we injected cells from the THP-1 monocyte cell line that stably expressed the green fluorescent protein (THP-1/GFP) with LAG lambda-1 cells, which highly express PTN, into SCID mice. *Results*. PTN protein was reduced and the growth of the cells was inhibited by PTN siRNA. Myeloma cells transduced with antisense PTN showed decreased proliferation compared to cells transduced with sense or vector alone. Inhibition of PTN with an anti-PTN antibody reduced the proliferation of MM cell lines and freshly isolated MM BMMCs containing >90% plasma cells. Importantly, the anti-PTN antibody also suppressed the growth of the human MM LAG lambda-1 in SCID mice. When co-cultured with MM cells, tube-like structures that expressed endothelial cell markers appeared after one week of culture but were blocked with PTN siRNA or with the PTN antisense construct. As determined by RT-PCR and Western blot analyses, endothelial gene expression (Tie-2, Flk-1, and VWF) was detected in monocytes exposed to the MM cells but was blocked with the introduction of either PTN siRNA or the antisense construct. Tumors contained GFP-expressing cells that also expressed human endothelial cell markers in their blood vessels whereas following treatment with anti-PTN antibody, a marked reduction in human endothelial cell marker expression was observed in the tumors. *Conclusions*. These studies provide further evidence that pleiotrophin both directly inhibits MM growth and induces monocyte transdifferentiation into endothelial cells, and have important implications for the pathogenesis of angiogenesis in human tumors. Since the expression of this protein is very limited in normal adult tissues, PTN may represent a new target for the treatment of MM.

PO-541

LBH589: EFFICACY AND PROTECTION OF BONE INTEGRITY IN MULTIPLE MYELOMA AS DEMONSTRATED IN CELL LINES AND *IN VIVO* MOUSE MODEL

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Despite recent advances in therapy, multiple myeloma (MM) remains an incurable disease, and osteolytic bone destruction and its complications are still sources of morbidity and mortality. Recent studies demonstrated that ubiquitinated proteins are degraded not only by proteasomes, but also by aggresomes, suggesting that the efficient clearance of cytotoxic misfolded protein aggregates by the protein machinery is critical for myeloma cell survival. The microtubule-associated deacetylase HDAC6 is a component of aggresomes, and recruits misfolded proteins for transport to aggresomes. HDAC inhibitors have therefore emerged as a treatment strategy for MM. LBH589 is a highly potent oral DAC inhibitor that targets both class I and II DACs, and is undergoing clinical development in hematological and solid malignancies. Here we report the ability of LBH589 to induce growth inhibition and apoptosis in MM cells, and potentiate the action of other drugs, such as bortezomib (BZ), *in vitro*. Furthermore, LBH589 produced significant anti-tumor activity with protection from bone damage in an *in vivo* mouse model of MM. For assessment of *in vitro* effects, MTT assays, thymidine uptake, and annexin staining were utilized. For *in vivo* pharmacology, a disseminated luciferized MM.1S MM xenograft mouse model was used. Effect on tumor burden (bioluminescence) was observed weekly using Xenogen IVIS200 Living Image software. At study end, animals were imaged with a high-resolution microCT scanner to evaluate bone integrity in the bilateral femur and tibia. LBH589 inhibited growth of MM cells sensitive or resistant to standard steroid or chemotherapeutic treatment as well as plasma cells isolated from MM patients. In particular, significant synergistic cytotoxicity was observed with LBH589 in combination with BZ without additional toxicity to normal bone marrow stromal cells. Comparable single-agent anti-tumor activity, evidenced by reduced tumor burden and delayed onset of clinical symptoms (defined as various degrees of gait change up to severe paralysis with spinal curvature) was observed with LBH589, BZ, and melphalan *in vivo*. Importantly, LBH589 alone appeared to demonstrate preservation of bone integrity in this model. These data confirm findings from earlier studies demonstrating that LBH589, as a single agent or in combination with BZ, is a promising therapy for MM.

PO-542

LBH589 SYNERGIZES WITH BOTH NOVEL AND CONVENTIONAL AGENTS TO INHIBIT TUMOR GROWTH AND TO INDUCE APOPTOSIS AND CELL CYCLE ARREST OF MULTIPLE MYELOMA CELLS: BASIS FOR CLINICAL TRIALS

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Multiple myeloma (MM) remains an incurable disease for which development of new strategies of treatment is required. LBH589, is a histone deacetylase inhibitor that neutralizes the charge of histones and generate a more open DNA conformation, promoting the expression of the corresponding genes. Here we report the *in vitro* anti-Myeloma activity and the mechanism of action of LBH589 as well as the analysis of potential synergism with both conventional and novel agent, in order to elucidate optimal double or triple combinations to be translated into the clinic. LBH589 showed potent antimyeloma activity (IC50 <40 nM) on both cell lines and fresh cells from MM patients, including cells resistant to conventional chemotherapeutic agents. Using gene array, quantitative PCR, and Western analyses, we observed that LBH589 affected a large number of genes involved in cell cycle and cell death pathways. LBH589 blocked cell cycle progression, and this was accompanied by p21, p53 and p57 up-regulation. LBH589 induced cell death through an increase in the mitochondrial outer membrane permeability. LBH589 favoured apoptosome formation by inducing Cytochrome C release, Apaf-1 up-regulation, and Caspase-9 cleavage. In addition, LBH589 stimulated a caspase-independent pathway, through the release of AIF from the mitochondria. LBH589 down regulated Bcl-2, and particularly Bcl-X. In addition, LBH589 was able to overcome the protective effect that confers IL-6, IGF-1 and BMSCs to myeloma cells in a dose dependent manner. Interestingly, LBH589 potentiated the action of drugs such as bortezomib, dexamethasone or melphalan. Moreover, the triple combination of LBH589, dexamethasone and bortezomib resulted in a significant increase in growth inhibition, cell cycle arrest and apoptosis of MM cells as compared to that seen with the drugs used either alone or in dual combinations. Other efficient combinations, that deserve clinical investigation are: LBH589, dexamethasone and melphalan as well as LBH589, dexamethasone and lenalidomide. Nevertheless, *in vitro* they are less active than the first one. All these data indicate that LBH589 could be a useful drug for the treatment of MM patients, particularly in combination with other novel agents such as bortezomib together with dexamethasone.

PO-543

IN VIVO EFFECTS OF VORINOSTAT IN COMBINATION WITH BORTEZOMIB

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Introduction. Histone deacetylase (HDAC) inhibitors represent a new mechanistic class of anti-cancer therapeutics that inhibit HDAC enzymes and have been shown to have anti-proliferative effects in cancer cells (including drug resistance subtypes), induce apoptosis, inhibit angiogenesis, and sensitize cancer cells when combined with other available anticancer therapies. Vorinostat (suberoylanilide hydroxamic acid) is a novel investigational small molecule drug that selectively inhibits HDAC enzymes resulting in acetylation of nucleosomal histones and activates gene transcription. *Materials and Methods.* To evaluate the effects of vorinostat *in vivo*, we used our previously established murine SCID-hu xenograft LAGlambda-1 model (Campbell *et al.*, International Journal of Oncology 2006). Each immunodeficient (SCID) mouse was implanted with a fragment (2-4 mm^3) of the LAGlambda-1 tumor into the left hind limb muscle. The tumors were allowed to grow for 14 days at which time human IgG levels were detectable in the mouse serum, and mice were blindly assigned into one of eight treatment groups. Tumor-bearing mice were treated in groups consisting of vehicle only, vorinostat alone (3, 10, and 30 mg/kg), bortezomib alone (0.5 mg/kg), or vorinostat (3, 10, or 30 mg/kg) + bortezomib (0.5 mg/kg). Mice receiving single agent vorinostat were injected once daily for five consecutive days weekly (M-F) via intraperitoneal injection. Mice received bortezomib were injected twice weekly (T, Th) via intravenous injection. Mice receiving the combination of vorinostat and bortezomib received vorinostat injections each morning (M-F) and bortezomib each afternoon (T, Th) for the duration of the study. *Results*. Mice receiving vorinostat/bortezomib combination therapy showed slight inhibition of tumor growth at the highest dose of vorinostat and reduction of paraprotein levels compared to mice receiving placebo or single agent therapy. Combination treatment with vorinostat and bortezomib was not associated with any observed toxicity. *Conclusions*. Preliminary results are encouraging when tumor-bearing mice are treated with the combination vorinostat/bortezomib and additional studies are being performed to further optimize the dose and schedule of a vorinostat-based combination therapeutic regimen. We are currently investigating vorinostat in combination with other anti-MM treatments for patients with relapsing or refractory MM.

PO-544

LENALIDOMIDE SYNERGIZES WITH SIMVASTATIN IN MYELOMA

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Introduction. Multiple myeloma is an incurable B-cell malignancy. Although improvements have been made in prolonging event free and overall survival, multi-drug resistance is still a major problem, and the search for new drug combinations continues. We have previously described that simvastatin, a HMG-CoA reductase inhibitor, induces apoptosis and inhibits proliferation in myeloma cell lines and drug-resistant patient myeloma cells via inhibition of geranylgeranylation. We also showed synergistic activity between simvastatin and dexamethasone and doxorubicin. Lenalidomide is a rising star in the treatment of multiple myeloma patients, with high response rates, oral intake, and less side effects than thalidomide. In this study, we investigated the poten-

tial efficacy of simvastatin and lenalidomide on myeloma cell viability. *Materials and Methods*. Myeloma cell lines and patient myeloma cells were incubated with different concentrations lenalidomide and simvastatin in a fixed ratio. After 2 and 4 days, cell viability, proliferation and apoptosis, (in)activation of signaling cascades, and caspase cleavage were determined. *Results*. Lenalidomide tested alone reduced plasma cell viability in different myeloma cell lines, mainly by inhibition of proliferation and hardly by induction of apoptosis. Myeloma cell kill by simvastatin alone was caused by inhibition of proliferation and induction of apoptosis. When both drugs were combined, a remarkable synergistic reduction of cell viability was observed in myeloma cell lines and in patient plasma cells (eg. 98% cell viability with simvastatin 1µm alone, 81% with lenalidomide 2 µm, 45% viability with the combination). This synergistic effect was both due to induction of apoptosis and inhibition of proliferation. Addition of mevalonate and GGOH abrogated the potentiating effect between lenalidomide and simvastatin. Furthermore, we observed an inactivation of pSTAT3 and an increase in caspase-8-cleavage in the combination, suggesting involvement of STAT3 in resistance to caspase-8-induced apoptosis by lenalidomide. *Conclusions*. Our study is further proof that the mevalonate pathway, and more specifically, geranylgeranylated proteins are important in multi-drug resistance in myeloma. This study provides a rationale for the clinical evaluation of the orally and directly available combination of lenalidomide with simvastatin in patients with multiple myeloma.

PO-545

CELL SPECIFIC PROTEASOME INHIBITION PROFILES BY NPI-0052

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Introduction. NPI-0052 is a novel, small molecule that regulates the chymotrypsin-like (CT-L), trypsin-like (T-L) and caspase-like (C-L) activities of the 20S proteasome with different kinetic and inhibition profiles versus bortezomib (Chauhan *et al.* Cancer Cell, 2005). Evaluating the inhibition and recovery of proteasome functions after administration of NPI-0052 can assist in the design of IND safety studies. *Materials*. Initial studies in rodents and primates defined the effects of NPI-0052 on proteasome activities in packed whole blood (PWB) lysates. Since PWB contains mainly non-nucleated red blood cells that cannot generate new proteasomes, the effect of NPI-0052 on proteasome activity was evaluated in nucleated peripheral blood mononuclear cells (PBMCs). *Results*. A single intravenous or oral treatment with NPI-0052 in mice inhibited the CT-L, T-L and C-L activities of PWB lysates in a dose-dependent manner which recovered significantly by day seven. In subsequent studies, the effects of repeated (weekly for three weeks) intravenous treatments with NPI-0052 on proteasome activities were evaluated in rats and primates. A dose-dependent inhibition was observed for all three proteasome activities in PWB lysates, with the most potent effects observed one day post-dosing which recovered partially by day seven. CT-L activity appeared to be most sensitive to inhibition by NPI-0052. A similar trend was observed after the second and third weekly treatments. The effects of NPI-0052 and bortezomib on proteasome activity in rodent derived PBMC lysates demonstrated a different inhibition profile of CT-L activity as compared to PWB. The inhibition in PBMCs markedly recovered within two-three days (versus seven for PWB) after NPI-0052 treatment and within one day for bortezomib. Importantly, the degree of inhibition in PWB versus PBMC were similar after bortezomib and NPI-0052 treatments, although at lower doses for NPI-0052. *Conclusion*. PBMC and PWB lysates show a different proteasome inhibition profile upon NPI-0052 or bortezomib administration (likely secondary to the ability of PBMCs to synthesize new proteasomes and their different half-lives in blood) and support assessing proteasome activities in multiple cell types during clinic trials. NPI-0052 is being evaluated in Phase I trials in which the proteasome inhibition profiles in multiple cell types will be studied.

PO-546

BSC2118, A NOVEL PROTEASOME INHIBITOR WITH *IN VITRO* ANTI-TUMOR ACTIVITY IN MULTIPLE MYELOMA

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Introduction. The ubiquitin-proteasome pathway was recently identified as a new therapeutic target in cancer treatment. The proteasome inhibitor bortezomib has been approved for the treatment of refractory multiple myeloma (MM). Recently we described the novel tripeptide compound BSc2118, which has inhibitory activity against all three proteolytic activities of the 20S proteasome (Cancer Res 2006;66:7598-605). *Methods.* We investigated the *in vitro* effects of BSc2118 in MM cell lines and in primary CD138-positive bone marrow MM cells by MTT cell viability and AnnexinV apoptosis-assays. The chymotrypsin-like proteasome activity and NF-κB activity were measured in MM cells by detection of proteasome-degraded peptides or NF-κB p65 subunit. Furthermore the effect of the proteasome inhibitor on the expression of the cyclin-dependent kinase (CDK) inhibitors p21 and p27 were investigated on protein level. *Results*. In MM cell lines OPM-2, RPMI-S and U266 we could show a significant dose-dependent reduction of cell viability by BSc2118 with an IC50 at 48hrs of 52nM, 65nM and 287nM, respectively. In primary MM cells from patients with active MM we found also a dose-dependent growth inhibition with an IC50 of 280nM. An induction of apoptosis by BSc2118 was shown after 48hrs incubation in all cell lines. Additionally, we detected a significant inhibition of intracellular proteasome activity in MM cell lines and in primary MM cells and an inhibition of TNFα-induced NF-κB activation in the OPM-2 and RPMI cell lines. We found that BSc2118 enhanced the expression of p21 in OPM-2, RPMI-S and U266, and of p27 in OPM-2. *Conclusions*. We could demonstrate the anti-tumor effects of the novel proteasome inhibitor BSc2118 in MM cells. The compound effectively inhibits cell proliferation and shows a high pro-apoptotic activity both in MM cell lines and primary MM cells. Mechanisms of action are the inhibition of proteasome and NF-κB activity and upregulation of the CDK inhibitor proteins p21 and p27. These preclinical data support the idea to consider BSc2118 as a promising new agent in anti-tumor drug development.

POSTER SESSION II

GROUP 6: Phase 1, 2, 3 clinical trials for advanced disease

PO-601

A PHASE I/II STUDY OF ATACICEPT, AN INHIBITOR OF APRIL AND BLYS, IN MULTIPLE MYELOMA (MM) AND WALDENSTROM'S MACROGLOBULINEMIA (WM)

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Introduction and aims. Atacicept (formerly known as TACI-Ig), a soluble receptor fusion protein comprised of the extracellular domain of the TNF-R family member TACI (Transmembrane Activator and CAML interactor) and the Fc portion of a human IgG, neutralizes essential myeloma cell survival factors, APRIL and BLyS. This trial was aimed at determining the tolerability, PK, PD and biological activity of atacicept in MM and WM patients. *Methods.* Open-label, dose-escalation trial to determine the MTD and the optimal biological dose in patients with refractory or relapsed MM or active, progressive WM. Eligible patients were enrolled in sequential cohorts for one cycle of five weekly subcutaneous injections of atacicept at 2, 4, 7 or 10 mg/kg. Patients who demonstrated at least stable disease after a 4 week follow up period were allowed to continue an extension phase consisting either of two additional cycles separated by a 4-week wash-out period or 15 weekly injections at a dose of 10 mg/kg. PK, safety, including anti-atacicept antibodies were assessed. The biological activity assessment included M-protein, β2-microglobulin, soluble syndecan-1, lymphocyte subpopulation counts, polyclonal immunoglobulins, free light chains, CRP and BCMA, as well as TACI and BAFF-Receptor expression. Response was assessed using modified Blade criteria. *Results*. 16 patients (12 MM and 4 WM) entered the trial. Fifteen patients completed the study; one patient was withdrawn for progressive disease. No DLT and no SAE related to study drug were observed. Five MM and 3 WM patients had stable disease after the first treatment cycle. Out of eight patients who entered the extension phase, 4 received two additional cycles and 4 received 15 weekly injections of atacicept; 5 had stable disease (4 MM and 1 WM), 2 progressive disease and 1 WM a minimal response. A marked decrease of polyclonal immunoglobulins and plasmocytes were observed. A sustained decrease in the M component and syndecan-1 occurred in several patients. *Conclusions*. Treatment with atacicept was well tolerated. No DLT was reported. Biological responses in accordance with the expected atacicept mode of action were observed in this heavily treated refractory population. Prolonged disease stabilization was seen in several patients and one WM patient achieved a minimal response.

PO-602

VEGF-R INHIBITION WITH PAZOPANIB (GW786034) IS INEFFECTIVE IN PRETREATED MYELOMA

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Introduction. Vascular endothelial growth factor (VEGF) stimulates Multiple Myeloma (MM) cell proliferation and migration *in vitro*. Pre-clinical studies have demonstrated that these effects can be blocked by inhibition of VEGF receptors (VEGFR) in MM cell lines that are sensitive or resistant to conventional therapy. Thus, specifically inhibiting the VEGFR has been considered an attractive therapeutic approach for MM. Pazopanib (GW786034) a potent, small molecule inhibitor of VEGFR-1, 2, and 3, PDGFR-alpha and beta, and c-kit which has demonstrated clinical activity in renal cell carcinoma, ovarian cancer, sarcoma and other solid tumors was evaluated in MM. *Methods.* This was an open-label, single arm phase II trial in patients (pts) with relapsed/refractory MM. The primary objective was response rate, and secondary objectives were time to tumor progression, pharmacokinetics (PK) and toxicity. A 2-stage Green-Dahlberg design was utilized with provision for early termination if < 3 responses (Blade criteria) were reported in Stage 1 (n=20). Pts received 800 mg pazopanib p.o. daily. *Results*. 21 pts with MM were treated: median age 59 yrs (range 29-74); SWOG stage 1/2 (71%). All pts were heavily pretreated, including 86% with more than 4 prior chemotherapy regimens and 71% with prior stem cell transplantation. Duration of treatment ranged from 14 to 279 days (mean = 69 days). No clinical responses were observed in 19 evaluable pts. In all patients, 18 (86%) were discontinued from study due to disease progression; two (10%) were discontinued due to toxicity (Gr 2 fatigue; Gr 3 nausea/vomiting) and one pt was a protocol violation completing one dose of pazopanib. The most common drug-related toxicities (n=21 pts) were nausea (38%), fatigue (29%), hypertension (24%), epistaxis (19%), headache (19%), and hair color changes (19%). There were no episodes of venous thromboembolism. No adrenal dysfunction was apparent. PK data paralleled the data observed in other pazopanib trials where responses in RCC, OvCa, sarcoma and other solid tumors have been observed. *Conclusion*. Despite using a dose that has demonstrated activity in various solid tumors, and achieving similar PK profiles, with demonstrable clinical *biomarker* activity (hair color change, hypertension), pazopanib did not demonstrate clinical activity in treatment of MM.

PO-603

PHASE II TRIAL WITH PLITIDEPSIN (APLIDIN) ALONE AND IN COMBINATION WITH DEXAMETHASONE (DEX) IN PATIENTS WITH RELAPSED/REFRACTORY MULTIPLE MYELOMA: PRELIMINARY RESULTS

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Introduction. Plitidepsin is a cyclic depsipeptide isolated from the marine tunicate, Aplidium albicans. *in vitro* studies have shown a potent activity against multiple myeloma (MM) cell lines and fresh cells obtained from MM patients. Plitidepsin was also active against cells resistant to conventional anti-MM agents and novel drugs (including bortezomib, thalidomide). A Phase I study explored 4 different schedules of administration. Muscle and liver (transaminases and/or alkaline phosphatese elevations) toxicities were the main DLTs. Hematologic toxicity was not observed at the recommended Phase II dose of 5 mg/m^2. Methods The aim of this non-randomized two-stage Phase II, multicenter, clinical and pharmacokinetic trial was to explore the activity of plitidepsin in refractory/relapsed MM patients. Plitidepisn (Aplidin - APL) was used at a dose of 5 mg/m^2 (administered over 3 hours by intravenous infusion) every 2 weeks. In the first stage, 16 evaluable patients (pts) were included. At least one response was required in order to proceed with the second stage, in which a total of 37 pts will be included. Response was assessed by Blade criteria and toxicities assessed by NCI-CTC, v3.0. *Results*. Between June'04 and December'06, 31 relapsed/refractory MM pts were enrolled. Median age at time of inclusion was 65 years (range: 47-82). The median number of prior lines of therapy were 4 (range: 1-9): a high proportion (60%) had received prior autologous stem cell transplant, thalidomide-based therapy (58%), and bortezomib (48%). Among the 26 pts evaluable for response (with 5 pts inevaluable because they did not receive three or more doses of APL), two have achieved PR (8%), and three pts (12%) achieved MR; in addition, disease stabilization was observed in 8 pts (31%). Thirteeen pts progressed during APL treatment. In august 2005 the protocol was amended and the addition of Dexamethasone (20 mg on days 1-4) was permitted in pts progressing after three cycles or with stable disease after four cycles. Accordingly, Dex was added in five pts including one with SD and three due to PD; among the four pts evaluable for response with the combination, the pt with SD remained in SD with a time to progression of 4,1 months; in the other three patients receiving the combination due to PD, one achieved PR, one MR and other SD. Accordingly, the overall response rate increased to 23% (8% PR and 15% MR). With a median follow-up of 14 months (range: 6,8-16,3), the time to progression in responding pts (PR/MR) was 5,8 months (4,9-7,6). The most common G3-4 adverse events included fatigue in 2 pts (7%), serum creatine phosphokinase increase in 2 pts (7%), muscle toxicity (weakness, myopathy) in 3 pts (10%) and hepatic toxicity in 3 pts (10%). No significant APL-related hematologic toxicity or neuropathy was observed. *Conclusion*. APL as monotherapy and in combination with

Dex has activity in heavily pretreated relapsed/refractory MM with 23% of pts achieving PR/MR and 27% disease/ stabilization. APL was generally well tolerated, although caution in pts with hepatic and muscle toxicity is warranted. Further combination studies are planned.

PO-604

TYROSINE KINASE INHIBITOR AS TREATMENT OF T(4;14) MYELOMA

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The t(4;14)(p16.3;q32), found in 15% of patients with multiple myeloma (MM) indicates a poor prognosis. Plasma cells with t(4;14) ectopically express the Fibroblast Growth Factor receptor 3 (FGFR3), which has proven transforming activity and may represent a therapeutic target. *in vitro*, the proliferation of myeloma cell lines is inhibited by AB1010 (1,5-5 microM), an inhibitor of FGFR3 tyrosine kinase activity. We have studied the efficacy and toxicity of AB1010 in 24 patients with relapsing/refractory t(4;14) MM. AB1010 (9 mg/kg/d) was given orally twice a day. Dexamethasone (Dex) (40 mg/d X4 days/ a month) was added in case of progression. In case of *explosive* relapse (deep cytopenias, renal failure), a chemotherapy was given followed by a *wash out* period of 1 months before start of AB1010. The response rate, TTP and toxicities were analysed. Among the 24 patients (M 33%/F 67%; median age 55 years) enrolled, 19 were evaluable. The main toxicities were gastrointestinal (nausea 63,5 grade I/II, diarrea 25% grade I, anorexia 25% grade II) and oedema (face 50% grade I/II and legs 24% grade I). A resolutive grade III neutropenia was noted. Dex was added in all cases. Six patients had explosive relapse and received chemotherapy (Thalidomide/Velcade/Dex or high dose melphalan 200 mg/m^2) before AB1010. No response was observed with AB1010/Dex in these cases. However, of the 11 patients (5 in first relapse, 4 in 2nd relapse, 2 >2nd relapse), one near CR, one PR and 2 minor responses were observed. The CR and PR were reached in patients in first relapse, and the TTP was 17 and 11 months respectively. The two remaining patients, included at a plateau with response < 75% to the first line, are still in stable disease. These results suggest that AB1010, in combination with Dex may be useful in the treatment of t(4;14) MM in early relapse. A second study is on going to evaluate the safety and efficacy of the combination of AB1010/Dex with novel drugs (Velcade). Moreover, further studies are needed to evaluate the interest of FGFR3 inhibitors in maintenance therapy to delay the rapid and chemoresistant relapse observed in these patients

PO-605

OPTIMIZING INTERLEUKIN-6 (IL-6) TARGETING IN MULTIPLE MYELOMA (MM)

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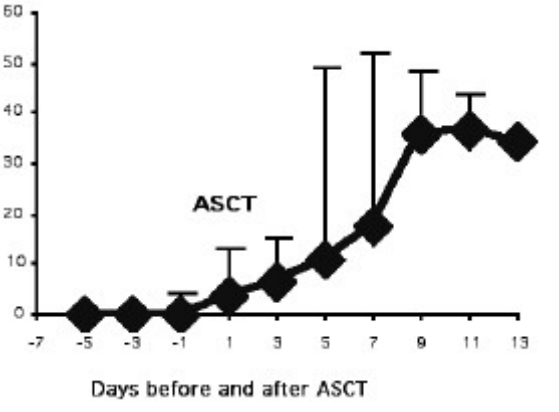
Among the 10 MM cell (C) growth factor families identified to date, IL-6, the first described, has a major role in controlling MMC survival. This high number of MMC factors impairs both the understanding of their respective roles in the *in vivo* emergence of the tumor clone and the place of inhibitory therapies. We present our experience for anti-IL-6 therapies, and the basis for future clinical trials. *Material and methods*. Murine anti-IL-6 monoclonal antibodies (MoAbs) have been used since 10 years in our group, including 48 patients treated by B-E8. 2 patients received a combination of 2 or 3 MoAbs.

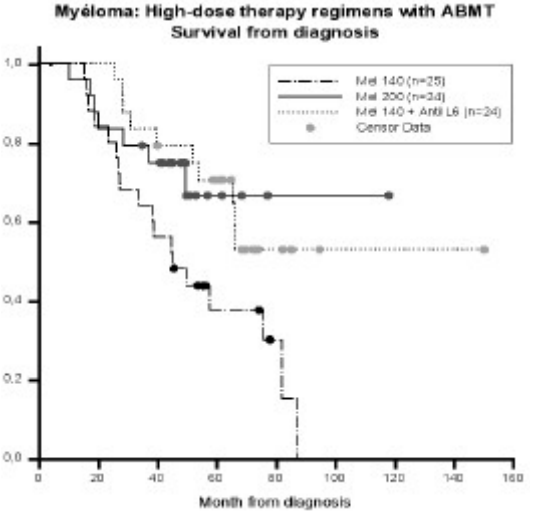
Table 1.

Patients population	
Plasma cell leukemia (PCL):	8 patients (5 secondary/2 primary)
Refractory myeloma:	2
Fulminating myeloma	2
B-E8 + high dose therapy (Mel140)	34
1st line therapy post-VAD	24
relapse (2nd autologous transplantation)	10

Calculation of the daily production of IL-6 and peaks of CRP/IL-6 serum levels along myeloma disease.

Results. Blocking IL-6 was associated with tumor mass reduction, particularly the proliferative compartment, as shown with PCL. The major clinical effect was observed in patients treated by B-E8 and high dose therapy. In an historical comparison, the association of Mel140+BE-8 was as effective as Mel200 and well tolerated including no delay of haematological recovery. Treatment was prolonged for 21 days and covers the peak of CRP/IL-6 observed at day 11.





This IL-6 production was due to an endogenous production of IL-6 linked to mucositis and infection. No biological efficacy was observed for murine MoAbs when the daily production of IL-6 was superior to 17mg/24h. In addition, we and other demonstrated *in vitro* synergistic effect with other inhibitors, particularly pan-ERB-B inhibitors and bortezomib. We demonstrated *in vitro* that MMC from 23 primary refractory patients to VAD responded *in vitro* to dexamethasone plus anti-IL-6 MoAb. Data concerning micro-array analysis will be presented. *Discussion*. Targeting IL-6 in multiple myeloma, particularly when IL-6/CRP is present, needs to be prolonged, and/or associated to chemotherapy. Association with high dose therapy or bortezomib is of major interest. CNTO328 (Centocor) offers a better biological efficacy as observed in Phase I studies (myeloma and metastatic renal cell carcinoma).

PO-606

A MULTICENTER PHASE I TRIAL OF PERIFOSINE (KRX-0401) IN COMBINATION WITH LENALIDOMIDE AND DEXAMETHASONE IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA: PRELIMINARY RESULTS. MULTIPLE MYELOMA RESEARCH CONSORTIUM (MMRC) TRIAL

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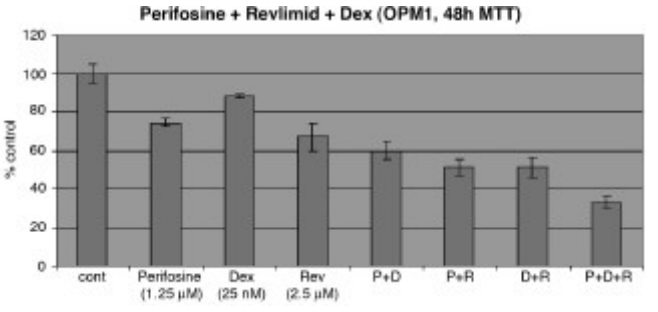
Introduction. Perifosine (Peri) a novel, oral signal transduction modulator with multiple effects including inhibition of Akt and activation of JNK, has demonstrated clinical activity when combined with dexamethasone (Dex) in patients (pts) with relapsed/refractory multiple myeloma (MM). Lenalidomide (Revlimid, Rev) a novel, oral immunomodulatory drug (IMiD) has single-agent activity against MM and additive effects when combined with Dex. Pre-clinical studies demonstrate increased cytotoxicity against MM cells when Peri was combined with Rev/Dex compared to each drug alone or a combination of two:



The addition of perifosine to Rev/Dex may enhance its clinical activity and provides further rationale for this trial. This phase I study aims to determine MTD and activity of Peri + Rev + Dex, the three oral drug combination therapy in pts with relapsed or refractory (2nd or 3rd line) MM. *Methods.* Four cohorts (≥6 pts each) are planned, dosing Peri at 50 or 100mg (daily), Rev 15 or 25mg (d 1-21) and Dex 20mg (d 1-4, 9-12 and 17-20 for 4 cycles) in 28-d cycles. Toxicity assessment uses NCI CTCAE v3.0; DLT is defined as grade (G) 3 non-hematologic toxicity, G4 neutropenia for 5 d and/or neutropenic fever, or platelets <25,000/mm^3 on >1 occasion despite transfusion. Response is assessed by modified EBMT criteria. *Results*. 3 pts have been enrolled in cohort 1 (Peri 50mg, Rev 15mg, Dex 20mg); one female, age 65 with 2 prior lines of tx; and 2 males, ages 59 and 60 both with 1 prior line of tx. To date, no DLT's have been reported. Of 2/3 pts evaluable for response, 1 pt is in MR and 1 SD. One pt is too early. *Conclusions*. Although early, all 3 pts to date have tolerated the combination of Peri + Rev + Dex well with no significant toxicities to report, and clinical activity noted within the first cycle of treatment. Accrual via the MMRC is ongoing and additional pts results in this phase I will be updated at the meeting.

PO-607

PERIFOSINE (KRX-0401) + LOW DOSE DEXAMETHASONE IS ACTIVE IN PATIENTS WITH RELAPSED AND REFRACTORY MULTIPLE MYELOMA (MM): PERIFOSINE MM INVESTIGATOR GROUP PHASE II MULTICENTER STUDY UPDATE

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Introduction. Perifosine (peri), an oral, synthetic alkylphospholipid, affects signal transduction pathways including inhibition of Akt and activation of JNK. *In vitro* peri induces significant cytotoxicity in resistant MM cell lines and patient MM cells, augmenting dexamethasone (dex), doxorubicin, melphalan and bortezomib-induced MM cytotoxicity: dex is active but side effects are dose-limiting. *Methods.* Patients (pts) began treatment on 150 mg with peri daily for a 21 day (d) cycle, assessed by serum- and/or urine-electrophoresis. Eligible pts had symptomatic relapsed or relapsed / refractory MM with measurable disease. Concurrent bisphosphonates were permitted, but pts with concomitant steroids (prednisone > 10 mg/d), serum creatinine of > 3.0 mg/dL and hemoglobin < 8.0 g/dL within 14 d of enrollment were excluded. Pts progressing on peri alone, documented on 2 occasions at least one week apart, had dex 20 mg twice weekly added. Response was assessed by EBMT criteria and toxicities by NCI-CTCAE, v3.0. *Results*. 35 pts (24 men and 11 women, median age 61y, range 49-78) with relapsed, refractory MM have received peri-dex to date. Pts received a median of 4 lines of prior treatment (range 1-9). Prior therapy included dex (97%), thalidomide (91%), bortezomib (69%), lenalidomide (20%) and SCT (83%). Currently 27 pts are evaluable for response.

Table 1.

Perifosine + Dex	*N (%)*	*Duration (wks)*
PR	4 (15%)	18, 18, 21+, 25+
MR	3 (11%)	15, 31+, 35
SD	15 (56%)	7 – 30+ (median 17)*

**5 pts ongoing at 16, 19, 21, 27 and 30 wks*

Most common adverse events (G1-2) included nausea (63%); vomiting (40%); diarrhea (57%); fatigue (43%) and increased creatinine (20%). Peri dose reduction was required in 13 pts and 3 pts came off study due to adverse events. Attributable toxicities proved manageable with appropriate supportive care and peri-dex was generally well tolerated, with no peripheral neuropathy, myelosuppression or DVT seen. *Conclusion*. Peri in combination with low-dose dex demonstrates activity in pts with advanced, relapsed/refractory MM, achieving PR, MR and/or stabilization of disease in 81% of evaluable pts to date. Peri + dex has been generally well tolerated. Studies with peri in combination with bortezomib and with lenalidomide ±dex are ongoing.

PO-608

A MULTICENTER PHASE I/II TRIAL OF PERIFOSINE (KRX-0401) + BORTEZOMIB IN RELAPSED AND REFRACTORY MULTIPLE MYELOMA PATIENTS PREVIOUSLY TREATED WTH BORTEZOMIB:PRELIMINARY RESULTS

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[1]Dana-Farber Cancer Inst., Boston, MA; [2]Univ. of Michigan Cancer Ctr., Ann Arbor, MI; [3]Alta Bates Cancer Center, Berkeley, CA; [4]City of Hope Nat l Medical Center, Duarte, CA; [5]Emory Winship Cancer Inst., Atlanta, GA: [6]Hematology, Huriez Hospital, Lille, France: [7]Keryx Biopharmaceuticals, Inc, NY, NY

Introduction. Perifosine (peri) is an oral, signal transduction modulator with multiple effects including inhibition of Akt and activation of JNK. *in vitro*, peri + bortezomib (Velcade®, Vel) shows additive cytotoxicity against MM cells with peri inhibiting Vel induced Akt activation. Peri combined with dexamethasone (dex) showed activity with 69% of evaluable advanced, relapsed/refractory MM patients (pts) achieving response and/or stabilization of disease (ASH 2006 #3582). This phase I/II study aimed to determine MTD and activity of peri plus Vel ± dex therapy in pts with relapsed/refractory MM. *Methods.* Four cohorts (≥3 pts each) were planned, with dosing of peri 50 or 100 mg (daily) and Vel 1.0 or 1.3 mg/m^2 (d 1, 4, 8, 11) in 21-d cycles. Dex 20 mg (on day of and day after each Vel dose) could be added in pts with progressive disease (PD). NCI CTCAE v3.0 was used for toxicity assessment; DLT was defined as grade (G) 3 non-hematologic toxicity, G4 neutropenia for 5 d and/or neutropenic fever, or platelets <10,000/mm^3 on >1 occasion despite transfusion. Response was assessed by modified EBMT criteria. *Results*. 6 pts have been enrolled to date; 3 pts in cohort 1 (peri 50mg, Vel 1.0 mg/m^2) and 3 pts in cohort 2 (peri 100 mg, Vel 1.0 mg/m^2). Among 3 men/3 women, median age was 64 (range: 42-86), and median no. of prior therapies was 5 (range: 3-7). To date, no DLT's have been reported. No G3 fatigue, nausea, diarrhea, or peripheral neuropathy has been seen. One G3 thrombocytopenia event was reported. Of 4/6 pts evaluable for response, results are as follows:

Table 1.

Cohort	*Response*	*Cycle as of 2/5/07*	*Prior Lines of Tx*
1	PR	8	4 (2 Vel-based)
	SD (dex added cycle 3)	9	3 (2 Vel-based)
2	MR (dex added cycle 4)	6	5 (1 Vel-based)
	PD	Off Tx	7 (3 Vel-based)

One pt (cohort 1) withdrew after cycle 1 due to personal reasons and one pt in cohort 2 is too early. *Conclusions*. Encouraging anti-MM activ-

ity has been observed in the cohorts completed to date, with no unexpected toxicity interactions seen. The phase II portion of the study will follow the phase I and results will be updated at the meeting.

PO-609

PHASE-1/-2 TRIALS OF DOSE AND SCHEDULE OF ZIO-101 (S-DIMETHYLARSINO-GLUTATHIONE) IN MULTIPLE MYELOMA

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Phase-1/-2 trials of dose and schedule of ZIO-101 (S-dimethylarsinoglutathione) in Multiple Myeloma JR Berenson1, S Jagannath2, R Boccia2, R Sobecks4, A Belch5, B Schwartz6, RP Gale6, M Hussein7. 1Institution for Myeloma and Bone Cancer Research, West Hollywood, CA, 2St. Vincent's Comprehensive Cancer Center, New York, NY, 3Center for Cancer and Blood Disorders, Bethesda, MD, 4The Cleveland Clinic Cancer Center, Cleveland, OH, 5Cross Cancer Center, Edmonton, AB, CANADA, 6ZIOPHARM Oncology, Charlestown, MA, 7H. Lee Moffitt Cancer Center, Tampa, FL. ABSTRACT *Background*. ZIO-101 (S-dimethylarsino-glutathione), a novel organic arsenic, is active against human myeloma cell lines and in immune-deficient mice with human myeloma xenografts. Anti-myeloma activity is mediated by disrupted mitochondrial function, increased reactive oxygen species (ROS) production, modified signal transduction and anti-angiogenesis. Objectives: (1) Estimate maximum tolerated dose (MTD), dose-limiting toxicity (DLT), safety profile and activity at a schedule of once daily for 5 consecutive d every 4 w; (2) compare activity at MTD of this schedule and a schedule 2X/w for 3 w every 4 w. *Results*. (1) Phase-1/2: 24 patients; median age, 61 y (range, 41-84 y); median N prior therapies, 7 (range, 4-10). Prior autotransplant (N=11). Estimated MTD is 420 mg/m^2/d. DLT is transient confusion/ataxia. No clinically-important biochemical, bone marrow, or heart toxicities were seen and there was neither neuropathy nor QTc-prolongation. 6 of 14 evaluable patients had stable disease (SD), 3 patients >5 mos. Comparison of activity estimates for both schedules will be presented *Conclusions*. The MTD for ZIO-101 in the daily for 5 consecutive d every 4 w schedule is 420 mg/m^2/d and DLT, transient confusion /ataxia. There was SD in 6 patients. There was no bone marrow toxicity or neuropathy making ZIO-101 potentially useful in advanced, extensively-treated myeloma. Estimated activity after different schedules will be reported.

PO-610

TANESPIMYCIN (T) + BORTEZOMIB (BZ) IN MULTIPLE MYELOMA (MM): PHARMACOLOGY, SAFETY AND ACTIVITY IN RELAPSED/REFRACTORY PATIENTS

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Introduction. Tanespimycin (17-AAG/KOS 953) disrupts Hsp90, a molecular chaperone of client proteins including IL-6 and IGF-1R key to MM growth, survival and drug resistance. Single agent T is well tolerated with modest anti- MM activity in Phase 1; preclinical studies suggest synergy with BZ. *Methods.* Patients (pts) received BZ followed by 1-hr infusion of T on D1,4,8,11 q 21d. 49 pts were enrolled in 7 cohorts (T 100-340 mg/m^2; BZ 0.7-1.3 mg/m^2). *Results*. PK of T was similar with or without BZ. Inhibition of 20S proteasome with T+BZ was similar to BZ single agent data. PBLs showed induction of HSP70 4h post-infusion with maintenance of induction prior to T infusion across the dosing interval; pAKT plus total AKT were also reduced 4 and 72h following infusion. CD138 but not CD4 or CD8 cells from serial BM aspirates showed induction of apoptosis by flow cytometry. Decreased expression of IGR-1R and IL-6R client proteins was seen after treatment. In Cohort 7, 19 pts received T 340 / BZ 1.3 mg/m^2 (the recommended dose for future study). Common all-grade (G) drug-related toxicity in pts included diarrhea (42%), nausea (32%), vomiting (26%), AST/ALT (26%/21%), myalgias (16%), and dizziness (16%). G3 thrombocytopenia was noted in 16%; no other G3 toxicity was observed in more than 1 pt. DLT: G3 myalgias/cramps, increased AST, pancreatitis (reversible in all cases). Responses were seen across dose levels in BZ-naïve, pre-treated and refractory pts (ie no response to or disease progression within 60d of last dose of BZ-containing regimen). For BZ-refractory pts (n=10): 1 pt with 3 prior regimens had confirmed PR after 2 cycles and continues in Cycle 9 (M-spike v92%); 2nd pt with 2 prior regimens achieved PR after 2 cycles and continues in Cycle 8; a 3rd pt with 7 prior regimens with confirmed PR after 3 cycles continues in Cycle 7. *Conclusions*. Treatment with T/BZ combination has manageable toxicity and is active in MM, with durable HSP90 inhibition and proteasome inhibition (vs single-agent BZ) seen. Importantly, anti-MM activity in BZ-refractory pts was observed; phase 3 trials of T/BZ in relapsed MM are planned.

PO-611

CLINICAL RESPONSES IN MULTIPLE MYELOMA AND WALDENSTROM'S MACROGLOBULINEMIA WITH THE PROTEASOME INHIBITOR CARFILZOMIB (PR-171)

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Introduction. Carfilzomib (PR-171) is an irreversible tetrapeptide inhibitor of the 26S proteasome. Intensive daily dosing with carfilzomib was well tolerated in preclinical animal studies. *Materials and Methods.* Two different dose schedules were tested in the Phase 1 clinical trials. In PX-171-001, carfilzomib was administered on a two week cycle, QDx5 with nine days rest; in PX-171-002, carfilzomib was administered on a four week cycle, QDx2 weekly for three weeks with 12 days rest. Eligible patients recruited to these studies had multiple myeloma (MM), Non-Hodgkin's Lymphoma (NHL), Hodgkin's Disease (HD), or Waldenstrom's Macroglobulinemia (WM). *Results*. Thus far, a total of 56 subjects have been enrolled. Seven responses have been reported thus far on these two protocols. Of three plasma cell dyscrasia patients treated on the QDx5 protocol at or above the minimal effective dose (MED) of 11 mg/m^2, two responses have been observed thus far: a Partial Response (PR) one MM subject and a Minimal Response (MR) in a subject with WM. Of nine MM patients treated on the QDx2 protocol at or above the MED of 15 mg/m^2, three patients have had PRs and two patients had a MR thus far. One DLT occurred in the QDx5 protocol, febrile neutropenia at 20 mg/m^2. In the QDx2 protocol, two DLTs occurred at 27 mg/m^2, one was grade 4 anemia and the other was grade 3 hypoxia; both patients are still receiving carfilzomib. Six additional patients have had Stable Disease lasting longer than 6 months and symptomatic improvement (recovery from anemia, thrombocytopenia, neutropenia, and loss of kidney function) has been seen in patients on both protocols. 14 subjects remain on study with stable disease or better. Proteasome inhibition in whole blood at the highest dose levels exceeded 80% one hour after the first dose. There have been no observations of painful peripheral neuropathy on either study. Responses were observed in MM patients who were fully refractory to bortezomib and in patients who were intolerant to bortezomib due to the development of painful peripheral neuropathy. *Conclusions*. Thus far, intensive dosing with carfilzomib is well-tolerated despite extensive proteasome inhibition. Seven responses have been observed, and several subjects have achieved long lasting SD and/or symptomatic improvement.

PO-612

BORTEZOMIB IN COMBINATION WITH CONVENTIONAL CHEMOTHERAPEUTIC AGENTS FOR MULTIPLE MYELOMA COMPARED WITH BORTEZOMIB ALONE

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Background. Recent studies have demonstrated synergy between bortezomib and a number of conventional cytotoxic agents. This study examined whether or not the speed of the response, progression and safety from a combination treatment of bortezomib with common chemotherapeutic drugs is superior to bortezomib monotherapy. *Patients and methods.* Sixty patients with multiple myeloma (MM) who had received at least 2 cycles of treatment including bortezomib were enrolled in this study. The median age was 57 (35-79) years and 50% were male. Thirty patients were treated with bortezomib alone and 30 were treated with bortezomib combined with chemotherapeutic agents including thalidomide. The monoclonal immunoglobulin (mIg) or free

light chain (FLC) concentrations were determined in the sera before and after 2 cycles of bortezomib treatment. The adverse events were assessed and graded according to the NCI Common Toxicity Criteria (version 2.0). *Results*. Thirty-seven of the 60 patients (62%) showed an early objective response (EOR) after the second bortezomib treatment according to a ≥50% decrease in the serum mIg or FLC concentration. Improvements in the response were observed when common chemotherapeutic agents were added to bortezomib monotherapy. In patients who received bortezomib combined with chemotherapeutic agents, 25 out of 30 patients (83%) showed an EOR, whereas 12 out of 30 patients (40%) given bortezomib monotherapy achieved an EOR after the second cycle of bortezomib treatment (p=0.001); the median decrease from the baseline in the paraprotein level was 74.6±5.9% and 43.7±4.2%, respectively (p<0.001). With the combination treatment, peripheral neuropathy of ≥grade III occurred in 13 patients (43%) compared with 6 (20%) in those given bortezomib alone (p=0.05). The median time to the progression of disease was similar in the two groups. The multivariate Cox regression model showed that post-bortezomib stem cell transplant, a high serum albumin and the absence of bone lesions are favorable factors for the progression-free survival following bortezomib treatment. *Conclusion*. Bortezomib in combination with common chemotherapeutic agents is more active in the treatment of MM than with bortezomib alone. However, more effective post-bortezomib treatment including stem cell transplantation is needed to reduce the rate of disease progression particularly in patients with advanced disease.

PO-613

EFFICACY AND SAFETY OF BORTEZOMIB IN PATIENTS WITH REFRACTORY AND RELAPSED MULTIPLE MYELOMA (MM) OUTSIDE CLINICAL TRIALS: RESULTS FROM THE CATALAN MYELOMA/AMYLOID STUDY GROUP (GEMMAC)

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Background. Bortezomib (Velcade®) has recently been approved for the treatment of refractory and relapsed MM. A response rate ranging from 35 to 50% has been reported in patients included in prospective clinical trials. However, the data on the efficacy and safety of bortezomib outside the context of clinical trials are limited. *Aim*. To analyze the efficacy and safety of bortezomib therapy in refractory or relapsed MM patients treated in community practice. Patients and *Methods*. Between August 2003 and February 2006, 120 patients (64M/56F, median age: 62 years) with refractory or relapsed MM were treated with bortezomib outside the context of clinical trials in 17 centres in the area of Catalonia. Forty-nine (49%) patients had untreated relapse, 31% refractory relapse and 20% primary refractory disease. The median number of previous lines of therapy was 2 (range: 1-6). Patients received bortezomib 1.3 mg/m^2 i.v. on days 1, 4, 8, and 11 every 21 days. The median number of cycles administered was 4 (range: 1-13). Responses were evaluated according to the EBMT criteria. *Results*. The response rate to bortezomib was 52% (62/120), with 15 (13%) complete, 37 (31%) partial and 10 (8%) minimal responses. The remaining 58 patients showed no response: no change (16), progressive disease (23), early death within the first two months from bortezomib onset (17) and non-evaluable (2). The median time to best response was 7 months (range: 0.5-13.6). Grade 3 or 4 adverse events, which occurred in 42% of patients mainly consisted in thrombocytopenia (32 cases), asthenia (13) and peripheral neuropathy (9). The drug was discontinued because of side effects in 17 (16%) patients. After a median follow-up of 12.8 months (range: 0.2-34), 28 of the 62 (45%) responding patients had relapsed (45%). The median time to progression was 12.3 months (range: 1.7-23) and the median duration of response was 6.7 months (range: 0.3-12.8) *Conclusion*. In this observational study, the response rate to bortezomib in patients with relapsed and refractory MM treated in community hospitals as well as the time to progression and duration of response were comparable to that achieved in the recently reported prospective clinical studies.

PO-614

EFFECT OF BORTEZOMIB IN MELPHALAN RESISTANT MYELOMA

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Introduction. Multiple Myeloma (MM) is characterized by accumulation of plasma cells in the bone marrow. High-dose melphalan (MEL) and stem cell support is the standard treatment for MM. Patients refractory to MEL often show cross resistance to conventional drugs. Bortezomib (BOR), a proteasome inhibitor, inhibits degradation of ubuiqinitated proteins like the NFkB inhibiting protein IkB, reducing the transcriptional activity of NFkB, resulting in apoptosis. BOR has anti myeloma activity *in vivo* and *in vitro*. *Methods.* A MEL resistant MM cell line (MM1MEL) was generated from the MM1S cell line by exposing MM1S cells to increasing concentrations of MEL. The relative sensitivity to MEL, BOR, LAQ824 and to doxorubicine (DOX) and dexamethasone (DEX) or combinations was determined by IC50 values. Shifts in cell proliferative activity was measured by flowcytometry. Using westernblots the expression of DNA damage checkpoint proteins CHK1 and CHK2, the cyclins E, E2, B1, B2, the mitotic progression proteins CDC25a and CDC25c, the MAP kinases P38, ERK1, JNK, AKT, STAT3 and the DNA damage response proteins P53 and NFkB and IkB were measured. Affymetrix arrays were used to detect the gene expression difference between both cell lines. *Result*. The MM1MEL cell line is 8 fold resistant to MEL compared to MM1S. with a minor cross resistance to BOR or LAQ824. The most striking changes in the MM1MEL vs MM1S were base line activation of caspase 3, reduced phosphorylation of P38, increased P53 and cyclin B2, a 200- fold increase of fibronectin and a reduced expression of CD44. The ratios of IC50 values of MM1MEL vs MM1S for DEX and DOX were 900 (p<10-7) and 3,2 (p<0,07). IC_{50} ratios for BOR and LAQ824 were not increased. Synergy of BOR was observed with LAQ824, not with DEX or DOX in both cell lines. BOR induced a cell cycle G2M transition block in MM1MEL cells and an increased activation of cytosolic NFkBp65. Stromal cell adhesion dependance increased IC50 for DOX and LAQ824, but not BOR. *Discussion*. BOR may overcome resistance to MEL and other drugs by multiple pathways, in which adhesion and homing and also P53 may be involved.

PO-615

COULD VELCADE SHOW THE BENEFIT OVER THE AUTOLOGOUS RETRANSPLANTATION IN RELAPSING MULTIPLE MYELOMA?

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Background. Therapy of relapsing multiple myeloma (MM) is still considered to be experimental. Velcade continues to show benefit in relapsing/refractory myeloma treatment. Design of study: We tested prospectively Velcade in the 1st relaps of MM in comparison with the 2nd autologous transplantation (AT2) or Velcade used after AT2 continued by maintenance therapy. Results were compared using intra-individual analyses [the comparison of time to progression I (TTP I) (after AT1), TTP II (after Velcade to 2nd relaps or after AT2 continued by maintenance therapy) and TTP III (after Velcade used in T2 model) in one patient], therefore inter-individual differences are excluded (T2 model). *Patients and Methods.* Between April 2004 and October 2006, 11 patients with relapsing/progressing MM after AT1 were treated with Velcade, 6 patients in the 2nd relaps after AT2 continued with maintenance therapy (Thalidomid, CED, IL-2 activated graft, pamidronate or interferon-α). A control group consisted of 53 patients treated with repeated AT continued with the above mentioned maintenance therapy (T2 model). Median follow-up in the control group, in the 1st group (Velcade in the 1st relaps) and in the 2nd group (Velcade after T2 model) was 73.9 months, 61.3 months and 93.3 months respectively. *Results*. The overall response rate (CR+PR) to Velcade used after AT2 continued with maintenace therapy was 34%, 55% in a group Velcade instead of AT 2 and 88% to AT 2 in T2model. We performed 3 comparisons: 1)TTP I was 20.7 months, TTP II has not been achieved yet in a group of patients treated with Velcade in the 1st relaps (p=0. 656). But in 3/11 (27%) have been TTP II prolonged over TTP I and there are still 5 patiets who have not achieved the 2nd relaps yet. Therefore theese patients can still influence the results and may indicate strong benefit of Velcade used in the 1st relaps. The difference did not reach the statistical signifficance. 2)Median TTP II (to 2nd

relaps after AT2 with maintenance therapy) was 5 months, median TTP III (to 3rd relaps after Velcade) in the same group was 6.4 months (p=0.489). In 4/6 (67%) was TTP III longer than TTP II. 3)TTP II (Velcade in 1st relaps) versus TTP II (in T2 model-a control group) - in 4/6 (67%) TTP II was longer than control group TTP II. The difference approaches statistical significance (p=0.078). *Conclusions*. Velcade has shown benefit in relapsing myeloma post auto transplant and our preliminary data indicate possible benefit of Velcade over AT2. Further prospective follow-up is needed to confirm our prelimiary results.

PO-616

HIGH RESPONSE RATE WITH BCD COMBINATION FOR RELAPSING MM

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Introduction. Optimum chemotherapy for multiple myeloma has induced a high primary response rate (80-90%), with complete remission (CR) rate of 40% when followed by intensive therapy supported by autologous stem cells. All patients with responsive myeloma require periodic recontrol of relapsing disease in order to prolong survival. *In vitro* synergy against myeloma appears higher when bortezomib is combined with an alkylating agent than with any other drug. *Methods*. Between 12/04 and 12/06, we assessed retrospectively, bortezomib 1.3 mg/m^2 IV twice weekly for 4 infusions, cyclophosphamide 70 mg/m^2 p.o. twice daily for 4 days, dexamethasone 20 mg/m^2 p.o. on days 1-4, 9-12, 17-20 in 35 patients with myeloma relapsing despite maintenance with thalidomide and other programs. The median number of prior therapies was 3, including intensive therapy for 25 patients, and none were resistant to prior bortezomib. The median age was 60, median Hgb 11.0 gms/dL, median B2M 3.4 mg/L, and median time after initial treatment 4.1 years. A second and final course was usually given 4 weeks later before maintenance with other programs. Partial remission was defined by >50% reduction of serum myeloma protein and/or >90% reduction of Bence Jones protein. *Results*. Among 35 patients, PR was observed in 25 patients (71%), a frequency double that of single agent bortezomib and similar to that observed with combinations of bortezomib with glucocorticoid, doxil or melphalan; remissions occurred in 14 of 23 patients with myeloma relapsing despite thalidomide. Grade 1 or 2 side effects were common but grade 3 or 4 toxicity occurred in 7 patients. The median remission time of 25 patients with responsive disease was 6 months, and median survival of all patients was projected at 18 months. *Conclusion*. The *in vitro* synergy of bortezomib and cyclophosphamide, the limited prior exposure to an alkylating agent or high-dose glucocorticoid, and the excellent tolerance, support the value of this combination for patients with myeloma that has relapsed on prior maintenance therapies. The short duration of control justifies further study of continued maintenance with BCD combination and/or intensive consolidation that may induce longer remission and survival.

PO-617

PROSPECTIVE COMPARISON OF SUBCUTANEOUS VS INTRAVENOUS ADMINISTRATION OF BORTEZOMIB IN PATIENTS WITH MULTIPLE MYELOMA : PHARMACOKINETICS, EFFICACY AND TOXICITY

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Bortezomib (Bz) is a novel proteasome inhibitor that is registered for treatment of relapsed or refractory MM as an IV bolus injection. Animal toxicology studies have indicated good local tolerability and an acceptable PK profile of SC injection of Bz supporting together with preliminary clinical data the exploration of this route of administration in humans. We conducted a randomized trial to compare pharmacokinetics/pharmacodynamics (PK/PD), toxicity and efficacy of SC and IV injections of Bz, both at standard dose of 1.3 mg/m^2 twice weekly for 2 wks (day 1-4-8-11), with 1 wk rest, for up to 8 cycles. From 01/06 to 10/06, 24 pts with relapsed/refractory MM were randomized to receive Bz either IV (arm A, n=12) or SC (arm B, n=12); Patients received a median number of 5 cycles in both groups. The local tolerance of SC injections was good. Response rates were similar in both groups (CR+VGPR/PR/MR: 3/2/1 in arm A, vs 3/2/2 patients in arm B. Grade 3 / 4 adverse events occurred in 7 cases in arm A, vs 5 in arm B, leading to study discontinuation in 5 and 3 cases, respectively. PK analysis : average plasma Cmax values were lower for SC as compared to IV (15 ng/mL at day 1, and 23 at day 11, vs 130 and 127, p<0.001), but overall systemic availability as measured by plasma AUC values were comparable between SC and IV administration for both day 1 & 11 (145 and 388 ng.h/mL, vs 133 and 386, p=0.7 & 0.98, respectively). Average Tmax values were less than 1 hour for both routes. PD analysis (20S proteasome inhibition): average area under the curve for effect (AUE) values were comparable between SC and IV administration both for day 1 & 11 (761 and 1324%.h, vs 1089 and 1110, p=0.29 & 0.54, respectively), while average Emax values were lower for SC administration (Emax day 1 & day 11 SC, 58 and 56%, vs 72 and 69% IV, p=0.003 & 0.01 respectively). The overall systemic availability of Bz was comparable between SC and IV administration for both day 1 and 11. Similarly, the average AUE results were comparable between SC and IV, while average Emax was lower for SC administration. Response rates were identical in the 2 groups of patients, with similar toxicity profile. Given the good local tolerance of SC injection, this route of administration could be an alternative option to IV injection.

PO-618

VTD REGIMEN (VELCADE + THALIDOMIDE + DEXAMETHASONE) - EFFECTIVE AND FEASIBLE THERAPY OF REFRACTORY / RELAPSED MULTIPLE MYELOMA

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Background. Velcade and Thalidomide have been shown to be effective as single agents in patients with relapsing and/or refractory multiple myeloma (R/R MM). Neuropathy is the most common complication of both Velcade/Thalidomide therapy. Synergic effect of Dexamethasone treatment in combination with Velcade/or Thalidomid has been proved. *Aim*. The aim of our work was to verify effect and toxicity of VTD regimen in R/R MM. *Patients and Methods.* A group of 11 patients (4 female, 7 male) with R/R MM, received VTD therapy. Their median age was 56 years (range 43-69 years). Clinical stage:IA-2x, IIA-3x, IIIA-6x. Indication for VTD therapy was: second relapse-5x, 3st-4x,4th-2x. 73% pts. (8/11) was previously treated with Thalidomide, one of them had initially signs of neuropathy gr.1. A scheme of VTD regimen: Velcade 1.3mg/m^2 i.v. day 1.,4.,8.,11., Thalidomide 100-200 mg/per day p.o.-depends on the toleration (100 mg/day for patients older than 65 years), Dexamethasone 40 mg p.o. day 1.-4., 8.-11. (20mg/d for patients older than 65 years). Every cycle of therapy repeated after 21 day, in total 6-8 cycles. *Results*. Overall response rate (ORR) 63.6% (7/11) was observed with: 9.0% (1/11) of complete response, 27.3% (3/11) of very good partial response, 27.3% (3/11) of partial response. In 9.0% (1/11) of patients was reported stable disease and 27.3% (3/11) patients progressed on the treatment. The most common toxicity effects were neurogical-91.0% (neuropathy grade 3/4 was observed in 2 patients-18.2%, all of them previously treated with Thalidomide) and haematological, especially thrombocytopenia-63.6% (grade 3/4 was observed in 3 patients-27.3%). Other toxicity effects: gastrointestinal (grade 1/2 was observed in 7 patients-63.6%). *Conclusions*. VTD regimen is effective in the treatment of R/R multiple myeloma with ORR 63.6% and with manageable toxicity. A serious neuropathy grade 3/4 was more frequently observed in the group of patients previously treated with Thalidomide.

PO-619

BORTEZOMIB SURVIVAL PROJECTION IN RELAPSED MULTIPLE MYELOMA

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Introduction. Multiple myeloma (MM) is a disease with poor survival outcomes. Recent trials have suggested improved overall survival with newer agents like bortezomib. We noted the lack of long-term survival data for new and existing agents used to treat MM. We therefore sought to extrapolate short term overall survival data to 10 years with several MM therapies. The therapies included were bortezomib, high dose dex (HDD), and thalidomide regimens. Thalidomide regimens consisted of a weighted average of thalidomide monotherapy and thalidomide combination therapy with steroids. *Methods.* The APEX trial results were used to obtain the 3-year clinical benefits of bortezomib and HDD for relapsed MM (Richardson, 2005). Three-year survival for bortezomib was available from the APEX study. Due to positive interim results, HDD patients were allowed to switch to bortezomib, thus limiting the HDD data available to one year. One to three year survival with HDD and thalidomide regimens were estimated from published trials. The natural survival history

of relapsed MM patients from Kumar (2004) was used to extrapolate survival to 10 years. Kumar studied the clinical course of 578 relapsed MM patients at the Mayo clinic from 1985 to 1998. From this study, the conditional survival, S(t|t-1) was calculated as the ratio of survival at the end of year (S(t)) to the survival at the end of the year before (S(t-1)). We assumed the rate of death in years 4 to 5, 5 to 6, etc. was the same for all three therapies. *Results*. Based on the above method of survival extrapolation over the 10 years, a typical patient would have an average life of 2.84 years for bortezomib, 1.81 years for HDD and 2.39 years for thalidomide regimens. Bortezomib provides the largest mean overall survival over a 10-year time horizon. *Conclusion*. This is a conservative estimate of the overall survival advantage of bortezomib as it assumes the mortality rate in year 4 onwards is the same for all three therapies. Long-term extrapolated analyses may be used to estimate the full benefit of therapies for which only short-term survival trial data is available.

PO-620

DOXIL® + VELCADE® IN PREVIOUSLY TREATED ≥65Y MYELOMA PTS

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Introduction. DOXIL (D) + VELCADE (V) is an effective combination for previously treated multiple myeloma (PTMM) (Orlowski, Blood 2006). The majority of myeloma patients are elderly with higher comorbidities and compromised ability to tolerate therapy. Our objectives were to determine whether (1) the safety and efficacy of D+V in elderly pts (≥65 years) was comparable to younger pts (<65 years) and (2) the efficacy was improved with the combination compared to V alone in both subgroups. *Methods.* We performed a prespecified analysis of the above-mentioned Phase III trial comparing D+V vs. V alone. PTMM pts were randomized to receive D at 30 mg/m^2 given on day 4 and V at 1.3 mg/m^2 on days 1, 4, 8, 11 vs V alone for up to eight 21-day cycles or at least 2 cycles beyond CR or optimal response unless disease progression or unacceptable toxicities occurred. Subgroups based on elderly (≥65 years) or younger (<65 years) patients were analyzed. *Results*. Baseline disease characteristics were comparable between treatment groups within the elderly and younger subgroups, including Beta-2 microglobulin (elderly: 4.16 [V], 4.79 [D+V]; younger 3.72 [V], 3.92 [D+V]). Both in elderly and younger patients D+V therapy showed significantly longer TTP and increased duration of response as compared to V (Table 1). In both subgroups, OR was higher with D+V vs. V. Further, D+V therapy showed comparable TTP, duration of response, and OR in elderly and younger pts. For D+V, safety profiles were comparable between elderly and younger pts: hand foot syndrome (all grades:13% vs. 18%; Grade 3: 3% vs. 6%; no grade 4), symptomatic cardiac events (9% vs. 6%), grade 3/4 neutropenia (29% vs. 30%), peripheral neuropathy (all grades: 35% vs. 35%; grade 3/4: 5% vs. 4%) and thrombovascular events (2% vs. 1%). *Conclusion*. D+V combination is well tolerated by elderly PTMM patients, is as efficacious as in younger patients, and shows better efficacy than V alone in both younger and older patients.

Table 1.

	Elderly pts (≥ 65 years)		*Younger pts (<65 years)*	
	V	*D+V*	*V*	*D+V*
N* (n=evaluable#)	129 (127)	121 (112)	193 (183)	203 (191)
Median TTP (Days) ,*[a]	205	276 *p*=0.0056 HR= 1.82	190	295 *p*=0.0008 HR= 1.75
CR+nCR (%)#	10	9	11	17
OR (%)#	39	49	46	47
Median Duration of Response (Days)	227	311	204	310

**ITT Population. #Evaluable population. [a]KP estimate-HR >1--- Adv for V+D vs. V*

PO-621

BORTEZOMIB, DOXORUBICIN AND DEXAMETHASONE (PAD) IN ADVANCED MULTIPLE MYELOMA

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Introduction. The association of Bortezomib, Doxorubicin and Dexamethasone (PAD) has shown encouraging results in Multiple Myeloma (MM). In this study, the safety/efficacy profile of this combination was evaluated in patients with advanced disease. *Material and Methods*. Sixty-four relapsed/refractory MM patients (median age 65 years) were treated with PAD: Bortezomib 1.3 mg/m^2 days 1,4,8,11; Doxorubicin 20 mg/m^2 i.v. days 1,4 or Pegilated Lyposomal Doxorubicin 30mg/m^2 i.v. day 1; and Dexamethasone 40 mg p.o, days 1,2,3,4. Each cycle was repeated every month. *Results*. Patients received a median number of 4 cycles (range 1-8). Median time from diagnosis was 31 months (range 2-181 months) and median number of prior therapies was 2 (range 1-7): 58% patients received prior transplantation, 27% prior Bortezomib based-regimens and 75% prior Thalidomide regimens. Forty-three patients (67%) achieved at least partial response (PR) including 16 patients (25%) who showed at least very good partial response (VGPR). In patients who received PAD as 2nd line treatment, the PR and VGPR rates were 80% and 27%. Responses were equal or superior to that induced by previous treatment schedules in 69% of patients. The PR (59% vs 66%) and VGPR (18% vs 28%) rates were similar between patients who received prior Bortezomib regimens or those who did not. Similarly, the PR (67% vs 69%) and VGPR (27% vs 19%) rates were analogous between patients who received or not prior Thalidomide regimens. From the start of PAD therapy, the 1-year progression free survival (PFS) and the 1-year overall survival (OS) were 34% and 67%. In patients treated with PAD as 2nd line, the 1-year PFS was 57%. The 1-year PFS was not different between patients who received prior Bortezomib based-regimens and those who did not (16% vs 39%, p<0.1). Hematological toxicities ≥grade 3 included trombocitopenia (48%), neutropenia (36%), anemia (13%); non-hematological toxicities ≥grade 3 included infections (17%), neuropathy (13%), gastrointestinal (11%), fatigue (6%) and cardiac (3%). Sixteen patients died for infections (2), cardiac heart failure (1) and progressive disease (13). *Conclusions*. The PAD regimen showed a high proportion of responses. Both responses and PFS were not influenced by previous Bortezomib treatments. Toxicities were predictable and manageable.

PO-622

HEMATOLOGIC PROFILES WITH BORTEZOMIB IN RELAPSED MM

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Introduction. Bortezomib (VELCADE®) ± dexamethasone was associated with transient, cyclical thrombocytopenia and neutropenia in the phase 2 SUMMIT[1,2] and CREST[2,3] and phase 3 APEX[4] trials in relapsed/refractory multiple myeloma (MM). Bortezomib-associated thrombocytopenia may result from transient inhibition of platelet budding,[5] which may also influence the rate of thromboembolic (TE) complications, although other mechanisms may be involved. Awareness is increasing regarding the risk of TE events in MM, and its relation to underlying disease- and patient-related factors and choice of therapy, most

notably with high-dose dexamethasone, concomitant erythropoietin (EPO) and lenalidomide or thalidomide.[6] Here we present the hematologic toxicities associated with bortezomib vs high-dose dexamethasone in APEX and the incidences of deep-vein thrombosis (DVT) and pulmonary embolism (PE) in each trial. *Materials and methods*. Hematologic toxicities were collected for patients receiving bortezomib (N=333) or dexamethasone (N=336) in APEX. DVT/PE rates were analyzed in patients receiving bortezomib or dexamethasone ± EPO in APEX and bortezomib ± dexamethasone ± EPO in SUMMIT/CREST (N=256). *Results*. Hematologic toxicities in APEX are shown (Table 1). Bortezomib-associated thrombocytopenia and neutropenia were transient and rapidly recovered to baseline during the rest period of each cycle. More patients receiving bortezomib (15%) vs dexamethasone (1%) had platelet transfusions, but the incidence of significant bleeding events was similar (" 5% in each arm); few patients required growth-factor support (bortezomib 6%, dexamethasone <1%). Anemia rates were similar between arms (bortezomib 26%, dexamethasone 22%). Hemoglobin levels increased in both arms during treatment, with a clearer trend in the bortezomib arm. There was no increase in DVT/PE with concomitant EPO in either arm of APEX; there appeared to be a decrease in DVT/PE with bortezomib vs dexamethasone, controlling for EPO (p=0.0459; odds ratio=0.207). In SUMMIT/CREST, DVT/PE rates were low with bortezomib alone (1%) and did not notably increase with the addition of dexamethasone ± EPO. *Conclusions*. Bortezomib-associated hematologic toxicities are predictable and manageable. Both thrombocytopenia and neutropenia are transient and reversible. There was no evidence of an increased TE risk with bortezomib ± dexamethasone ± EPO. Moreover, preliminary data from this analysis and others suggest using bortezomib with agents of thrombogenic potential may lower TE risk.

Table 1. Hematologic toxicities and thromboembolic events in the phase 3 APEX trial (bortezomib vs dexamethasone in relapsed MM).

Toxicity/event (n=331)	*Bortezomib (n=332)*	*Dexamethasone*
Thrombocytopenia, n (%)		
All grades	115(35)	36(11)
Grade 3/4	97(29)	22(7)
Neutropenia, n (%)		
All grades	62(19)	5(2)
Grade 3/4	48(15)	4(1)
Anemia, n (%)		
All grades	87(26)	74(22)
Grade 3/4	33(10)	35(11)
DVT, n (%)		
All grades	1(0.3)*	6(1.8)*
Grade 3/4	0*	6(1.8)*
PE, n (%)		
All grades	1(0.3)*	5(1.5)*
Grade 3/4	1(0.3)*	4(1.2)*

DVT, deep-vein thrombosis; PE, pulmonary embolism.
**± Erythropoietin.*

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PO-623

PHASE I TRIAL OF BORTEZOMIB (VEL) + SAMARIUM (SAM) IN MM

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Introduction. Recent preclinical studies have demonstrated that the bone-seeking radionuclide Samarium Sm153 lexidronam in combination with the proteasome inhibitor, bortezomib, can synergistically inhibit growth of multiple myeloma (MM) both *in vitro* and *in vivo*. These results provide the basis for a new therapeutic approach combining Vel and Sam to overcome resistance and to minimize non-tumor tissue toxicity among refractory and relapsed MM patients. *Materials and Methods.* Relapsed or refractory MM patients who failed more than 2 prior treatments were enrolled on this phase I dose-escalation trial. Previous treatment with bortezomib was permissible. Enrollment in six cohorts occurred in two parallel arms (Vel 1.0 or 1.3 mg/m^2) with escalating Sam (0.25, 0.50 or 1.0 mCi/kg). Each cycle was 8 weeks with a maximum of 4 cycles. Vel was given on days 1, 4, 8, and 11 followed by a 45 day rest period. Sam was administered on day 3. The cycle was repeated on Day 57 if disease was stable or improved and platelets and neutrophils recovered to at least Grade 1 toxicity (delayable up to four weeks). Dose-limiting toxicity (DLT) was defined as cycle 1 grade 4 hematologic or Grade ≥3 non-hematologic toxicity. *Results*. Twenty-seven patients have been enrolled in 6 cohorts and 26 are currently evaluable. There have been three DLTs, including grade 4 neutropenia (n=1) and grade 4 thrombocytopenia (n=2). Responses (minimal [MR], partial [PR], or complete [CR]) occurred in 5 of 26 patients (19%), including three immunofixation-negative CRs (12%), two MRs (7%). Two of the five patients who were progressing on bortezomib therapy at the time of enrollment responded to this therapy. Five patients (19%) have stable disease. Only 2 patients (4%) demonstrated a worsening of peripheral neuropathy. *Conclusion*. In this phase I trial evaluating the novel combination of Vel and Sam, we have determined the maximum tolerated dose to be Vel 1.0 mg/m^2 and Sam 1.0 mCi/kg. This regimen appears to be a promising and safe new treatment option that deserves further evaluation for MM patients. Thus, we are starting a phase II trial at these doses in March 2007.

PO-624

PAD IN RELAPSED AND REFRACTORY MYELOMA. A PHASE II STUDY

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Introduction. The combination of Bortezomib, Adriamycin and Dexamethasone (PAD) has been shown to be highly active in patients with *de novo* myeloma producing response rates of up to 95% (Oakervee *et al.*, BJH 2005;129(6):755-62) and would therefore seem to offer opportunities for the treatment of relapsed patients. We postulated that the efficacy of PAD could be determined by studying patients who were previously treated with VAD (Vincristine, Adriamycin, Dexamethasone) or VAD-like regimen (VAMP, C-VAMP, Z-Dex etc) by comparing the response to PAD to the response previously obtained with VAD or VAD-like regimen in terms of relative and absolute changes in paraprotein or light chain (serum or urine) levels. *Materials and Methods.* A Phase II study was developed with three cohorts, each planned to recruit 23 patients. Cohort 1; patients treated with VAD or VAD-like regimen who previously had an autologous transplant; Cohort 2 - similar patients but without previous transplant and Cohort 3 - patients refractory to VAD. Patients in groups 1 and 2 were allowed to have received one further line of treatment following VAD but patients in group 3 would proceed directly to PAD. *Results and Conclusion*. To date 30 patients have been enrolled, 18 in group 1, 3 in group 2 and 9 in group 3. Response rates have been high in both groups 1 and 2 with many patients obtaining better responses than with their initial VAD or VAD-like therapy. Response rates in group 3 have been mixed, some patients remaining relatively refractory but other obtaining complete responses. These results will be presented in detail and suggest that in many patients the synergy demonstrated between Adriamycin and Bortezomib *in vitro* may also be present *in vivo*.

PO-625

DOXIL + VELCADE IN PREVIOUSLY TREATED MYELOMA W/PRIOR SCT

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Introduction. A recent report on the Phase III randomized trial with DOXIL + VELCADE (D+V) vs. VELCADE alone (V) in previously treated multiple myeloma (PTMM) patients demonstrated improved time to progression (TTP) with D+V (Orlowski, Blood 2006). This prespecified subgroup analysis was conducted to further assess the effect of prior stem cell transplant (SCT) on TTP. *Methods.* Patients were randomized to receive a combination of D at 30 mg/m^2 given on day 4 and V at 1.3 mg/m^2 on days 1, 4, 8, 11 vs. V alone for up to eight 21-day cycles or at least 2 cycles beyond CR unless disease progression or unacceptable toxicities occurred. In this analysis, safety and efficacy were compared between patients who received prior SCT vs. those that did not. *Results*. The treatment groups were balanced for the key baseline prognostic risk factors. D+V therapy showed significantly longer TTP ($p \leq 0.0011$), increased duration of response, and higher overall response (OR) rates as compared to V alone in both subgroups (Table 1). Further, when D+V combination was compared between the patients with or without prior SCT, these improved therapeutic outcomes were not appreciably compromised by the receipt of SCT. Also, the safety profile was comparable between the two groups of patients with or without prior SCT, including drug-related serious adverse events (25% vs. 18%), symptomatic cardiac events (7% vs. 8%), peripheral neuropathy (all grades: 39% vs. 30%; grade 3/4: 5% vs. 4%), grade 3/4 neutropenia (33% vs. 26%), hand-foot-syndrome (all grades: 15% vs. 17%; grade 3/4: 6% vs 3%) and thromboembolic events (1% vs. 1%), respectively. *Conclusion*. D+V combination is well tolerated, and shows superior efficacy compared with V alone regardless of prior SCT.

Table 1.

	Prior SCT		*No Prior SCT*	
	V	*D+V*	*V*	*D+V*
N* (n=evaluable#)	173 (164)	186 (173)	149 (146)	138 (130)
Median TTP* (Days) a	197	276 *p*=0.0011 HR= 1.76	197	331 *p*=0.0009 HR= 1.94
CR+nCR (%)#	11	14	10	14
OR (%)#	46	50	39	45
Median Duration of OR (Days)	227	309	204	394

** ITT population; #Evaluable population; aKP estimate-HR >1--- Adv for V+D vs. V*

PO-626

BORTEZOMIB/DEX/DOXIL: BREAKS PLATEAU IN MM

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While most MM patients respond to standard induction regimens, the majority do not achieve a complete response (CR). Our group has looked for methods to increase the percentage of patients achieving CRs by incorporating sequential, non cross-resistant agents. This study is designed to treat patients post first line therapy to determine whether the introduction of bortezomib/dexamethasone ± pegylated liposomal doxorubicin for six cycles (DoVeD study) can further cytoreduce and improve quality of the response. Thus far, the majority of subjects have initiated DoVeD in a PR response plateau (<25% change in paraprotein x 3 sequential determinations) on initial induction regimens.All patients enrolled on the study were intended to proceed into autologous stem cell transplantation. Patients were treated for six 21-day cycles receiving bortezomib at 1.3 mg/m^2 on days 1, 4, 8, and 11, with three, four day pulses of high dose (40 mg) dexamethasone. If a PR or better was not achieved after cycle 2, pts received liposomal doxorubicin on day four of every cycle. Patients who did not achieve a CR by the end of cycle four, received day four liposomal doxorubicin for cycles five and six. Seventeen pts have been accrued to the study, eleven of which are evaluable for response, and six of those with the poor prognostic factor, deletion 13q14. Within the 6 DoVeD treatment cycles there have been 3 CRs, 2 nCRs, 3 PRs, 3 cases of stable disease, and 1 case of progression leading to early termination. In post study evaluations, four of the six deletion 13q14 were in CR. Four patients achieved a cytogenetic CR (standard cytogenetics and FISH) as determined by post study bone marrows. The DoVeD regimen is able to break the plateau typical of pts receiving standard induction therapy, inducing further cytoreduction. Our data suggest that patients with del 13q14, in particular, may benefit from bortezomib as part of their up-front induction therapy. Though further study of this approach is warranted, the evidence suggests a possible paradigm shift for induction therapy in general. Non cross-resistant agents given in tandem can increase the percentage of patients achieving CR.

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PO-627

QUALITY OF RESPONSE WITH BORTEZOMIB IN APEX

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Introduction. In the phase 3 APEX trial in patients with relapsed multiple myeloma (MM), bortezomib was superior to high-dose dexamethasone in terms of response rate, time to progression (TTP), and overall survival (OS);[1] updated data showed a complete/partial response (CR/PR) rate of 43%, median TTP of 6.2 months, and median OS of 29.8 months.[2] CR/quality of response is prognostic for improved survival.[3–7] This analysis using updated APEX data assessed whether higher quality of response with bortezomib was associated with greater clinical benefit. *Materials and methods*. Patients (N=333) received bortezomib 1.3 mg/m^2 on days 1, 4, 8, and 11 (eight 3-week cycles), then on days 1, 8, 15, and 22 (three 5-week cycles). Responses (N=315) were assessed per modified EBMT[8] with the addition of very good (VG)PR from the updated uniform response criteria.[9] Treatment-free interval (TFI), time to alternative therapy (TTAT), TTP, and OS were evaluated among responders (CR, VGPR, PR), and patients with minimal response (MR; ≥25% and <50% M-protein reduction) and non-responders (stable/progressive disease). *Results*. Data for responders are shown (Table).

Table. Clinical benefit of bortezomib by response in the APEX trial.

	CR	VGPR	PR
N (%)	27 (9)	31 (10)	77 (24)
Kaplan–Meier median, months (95% CI)			
Treatment-free interval	24.1 (9.2, 24.4)	6.9 (5.5, 11.1)	6.4 (4.4, 10.0)
Time to alternative therapy	27.1 (15.2, 32.0)	13.6 (10.0, 17.3)	14.0 (12.6, 16.1)
Time to progression	10.6 (7.8, NR)	10.3 (8.1, NR)	8.3 (7.0, 9.9)
Overall survival	NR (NR, NR)	NR (23.8, NR)	NR (29.8, NR)

CR, complete response; VGPR, very good partial response; PR, partial response; NR, not yet reached.

Median TFI appeared longer in patients with CR (24.1 months) vs those with VGPR (6.9) or PR (6.4). TTAT appeared longer for CR (27.1 months) than VGPR (13.6) or PR (14.0). TTP was similar with CR (10.6 months) and VGPR (10.3), and appeared longer than with PR (8.3). Median OS has not been reached for all responders (median follow-up 22 months). Patients with MR (n=21, 7%) appeared to have longer TFI, TTP, TTAT, and OS than non-responders (n=159; 50%; TFI 6.1 vs 2.9 months; TTAT 11.3 vs 6.5 months; TTP 4.8 vs 2.3 months; OS 24.9 vs 18.7 months). *Conclusions*. Bortezomib has substantial activity in relapsed MM, including a high CR rate (9%).2 Higher quality of response to bortezomib, especially achievement of CR, is associated with greater clinical benefit in terms of extended TFI, TTAT, and TTP. MR also appears to be associated with clinical benefit relative to non-responders. These data support CR as a surrogate marker for clinical benefit in patients with relapsed MM and suggest that even MR may be beneficial.

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PO-628

EDA-V IN ADVANCED MM PATIENTS PREVIOUSLY TREATED WITH THALIDOMIDE

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Introduction. Bortezomib has shown its efficacy in patients with relapsed/refractory multiple myeloma particularly when combined with other agents. Aim of this phase II study was to determine efficacy and safety of the EDA-V combination (etoposide, dexamethasone, cytosine arabinoside and bortezomib) in advanced MM patients previously treated with thalidomide. *Patients and Methods*. Drug administration was as follows: Etoposide 80 mg/sm iv days 1-4; cytosine arabinoside 1 g/sm day 5; bortezomib 1.3 mg/sm iv days 1, 4, 8, 11 and dexamethasone 20 mg days 1-2, 4-5, 8-9, 11-12. Non progressing patients received 6 cycles of EDA-V every 28 days (induction therapy) followed by 3 cycles of bortezomib 1 mg/sm day 1, 8, 15 and dexamethasone 20 mg days 1-2, 8-9, 15-16 every 2 months (consolidation therapy) and prednisone 50 mg every other day (maintenance therapy). Actually 18 patients have been enrolled and 14 (6 M, 8 F; median age 64 yrs, range 58-78) are assessable for response and toxicity. Six patients (43%) had WHO performance status (PS)> 1, 5 (36%) had refractory disease, 7 (50%) had received more than 2 lines of prior therapy (all patients had received thalidomide) and 10 patients (64%) had had one previous autologous stem cell procedure. *Results*. The overall response rate was 64%, a PR or better was achieved in 57% and a VGPR or better in 29% of patients. In total, 42 cycles have been administered. Non-hematological toxicities included: grade 3 neurotoxicity (10% of cycles), grade 3 diarrhea (5%) and constipation (12.5%), grade 3 cardiotoxicity (2,5%,one patient with acute heart failure). Grade 1-2 fatigue was observed in 45% of cycles, no report of DVTs but one patient developed acute ischemic stroke. Grade 3 hematological toxicities were reported in 10% of cycles while no grade 3-4 infections were observed. In 3 patients bortezomib dosage was reduced and 2 discontinued treatment due to neurotoxicity. Three patients were dropped due to cardiotoxicity (one) and disease progression (two). *Conclusion*. EDA-V has significant activity in advanced MM patients who had received prior thalidomide with a manageable toxicity.

PO-629

DOXIL® + VELCADE® IN PREVIOUSLY TREATED HIGH RISK MYELOMA

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Introduction. Recently, a significant improvement in time to progression (TTP) was reported for DOXIL + VELCADE (D+V) combination therapy vs. VELCADE (V) monotherapy in a Phase III randomized trial in previously treated multiple myeloma (PTMM) patients (Orlowski, Blood 2006). This prespecified analysis assessed the efficacy of D+V in PTMM in patients with poor prognosis factors, including (1) serum beta-2 microglobulin ≥ 5.5 mg/L, or (2) the presence of disease refractory to initial treatment. *Methods.* Patients with ≥ 1 prior therapy were randomized to receive D at 30 mg/m^2 on day 4 and V at 1.3 mg/m^2 on days 1, 4, 8, and 11 vs V alone for up to eight 21-day cycles or at least 2 cycles beyond CR or optimal response unless disease progression or unacceptable toxicities occurred. *Results*. The improved TTP reported previously with D+V over V alone in total study population was also observed in both subgroups studied (Table 1). TTP was significantly longer with D+V vs V for both high risk subgroups, indicating therapeutic benefit favoring the D+V combination. Moreover, TTP with the D+V combination was comparable between the high risk subgroups and their corresponding better prognosis groups (β-2 microglobulin < 5.5 mg/L or not refractory to initial treatment). In the β-2 microglobulin ≥5.5 mg/L subgroup, CR+PR was higher with D+V vs V; however, CR+PR was comparable between the 2 arms for those with refractory disease. Incidence of symptomatic cardiac events was comparable between the two arms in both high risk subgroups (not shown). *Conclusion*. These results demonstrate that D+V combination therapy compared to V alone significantly improves TTP and may overcome poor treatment outcomes associated with β-2 microglobulin levels ≥ 5.5 mg/L and disease refractory to initial treatment.

Table 1.

	β-2 ≥ 5.5 mg/L		*β-2 < 5.5 mg/L*	*Refractory*		*Not Refractory*
	V	D+V	D+V	V	D+V	D+V
N* (n-evaluable#)	99 (95)	98 (95)	226 (208)	27 (23)	29 (28)	295 (275)
Median *β*-2 M (mg/L)	7.94	8.14	3.44	4.17	4.72	4.08
Median TTP (Days)[a]	178	276	282	189	Not Reached	276
	p=0.0007; HR-2.11			p=0.0322; HR- 2.99		
CR+PR (%)#	33	49	47	48	43	48

** ITT population; #Evaluable population; aKP estimate-HR >1--- Adv for V+D vs. V;*

PO-630

NATURAL POLYPHENOLS ANTAGONIZE THE ACTION OF PS-341 BY DIRECT CHEMICAL INTERACTION OF VICINAL DIOL ON POLYPHENOLS WITH BORONIC ACID OF PS-341: TWO CONTRASTING SIDES OF POLYPHENOL ACTION ON MULTIPLE MYELOMA CELLS

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Introduction. Bortezomib (PS-341) is a FDA-approved anticancer agent which shows a remarkable antiproliferative activity on MM cells. Nat-

ural polyphenols, abundant in vegetables and fruit, show antioxidant activity as efficient ROS scavengers, while they have potential antiproliferative activity on certain tumor cells. To investigate the drug-drug interactions between natural polyphenols and PS-341, we treated the multiple myeloma cell lines and primary myeloma cells from patients with polyphenols and PS-341. *Materials and Methods.* We screened the antiproliferative effect of natural-abundant polyphenols (quercetin, rutin, dihydroxycinnamic acid, gallic acid, tannic acid) and PS-341 on 3 kinds of genetically different MM cell lines (U266 with trisomy 1q, Rb deletion & IgH rearrangement, RPMI-226 with K-ras mutation & hyperdiploid, MC/CAR with normal karyotype). Antioxidant potential assay, western blot and flow cytometric analysis were done. We performed all the same experiments with CD138 sorted primary myeloma cells from patients. *Results*. When MM cell lines and myeloma cells were treated with PS-341 (5 nM) in combination with various concentration of polyphenols, the antiproliferative effect of PS-341 was abolished even at low concentrations of polyphenols (30 uM). To validate the structure-activity relationship as well as direct cause of antiagonism, we tested antagonistic effects of polyphenols with various antioxidant potentials and structures. Polyphenols showed potent antiproliferative effect by alone, but they antagonized the effect of PS-341. Antioxidant potentials of polyphenols did not show high correlation with antagonistic effect, while structural variations of polyphenols demonstrated striking correlations with antagonism, which supports our hypothesis of direct drug-drug interaction with vicinal diol on polyphenols. Western blot and flow cytometric study revealed combined treatment of PS-341 and polyphenols inhibited ROS generation by caspase independent pathway. *Conclusion*. We confirmed the antagonistic interaction between PS-341 and polyphenols *in vitro* and vivo. We infer that vicinal diol on polyphenols interact with boronic acid of PS-341, which convert active boronic acid (sp2 character) of PS-341 to inactive boronate (sp3 character). This equilibrium of this conversion is controlled by structures and concentration of polyphenols, and this conversion abolished the anti-myeloma activity of PS-341. We suggest that avoiding the intake of natural polyphenols could be considered during the treatment with PS-341 in patients with MM, just as avoiding vitamin K containing foods in patients during Warfarin medication.

PO-631

BORTEZOMIB, DOXORUBICIN AND DEXAMETHASONE (PAD) COMBINATION THERAPY FOLLOWED BY THALIDOMIDE AND DEXAMETHASONE (TD) AS A SALVAGE TREATMENT FOR RELAPSED MULTIPLE MYELOMA (MM): PRELIMINARY ANALYSIS OF EFFICACY AND SAFETY

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Introduction. PAD was reported to be highly effective regimen as an induction therapy before high dose therapy. TD is an another effective regimen with no cross resistance. We conducted a phase II study with PAD followed by TD in relapsed MM to test effectiveness of this combination. *Methods*. Patients were planned to receive 6 cycles of PAD, (bortezomib 1.3 mg/m^2 days 1, 4, 8 and 11, doxorubicin 4.5 mg/m^2 days 1-4, dexamethasone 40 mg days 1-4, every 21 days). Responders following 6 cylces of PAD received 12 cycles of TD (thalidomide 100 mg days 1-28 and dexamethasone 40 mg days 1-4, every 28 days). In patients with progression during PAD therapy, regimen was changed to 12 cycles of thalidomide 200 mg days 1-28 and dexamethasone 40 mg days 1-4, every 28 days. Response was assessed by EBMT criteria, with additional categories of nCR and VGPR. Adverse events were graded by the NCI-CTC, Version 3.0. *Result*. This study aimed to enroll 35 patients till Oct 2007 and we are reporting preliminary result with 25 patients. Efficacy could be assessed in 20 patients. After two cycles of PAD, 14 patients showed response with 4 CR. Overall response rate to 6 cylces of PAD was 84% with 32% CR. Five of total 11 patients with TD showed further improvement of response status with 2 additional CR. Overall response to PAD followed by TD was 90%: CR 42%, nCR 11%, VGPR 5%, PR 32%, MR 5%, PD 5%. There was no prognostic factor for CR+nCR achieving in the univariate analysis. The median follow-up was 7.7 months with 1 year PFS 64% and 1 year OS 85%. Ninty-five PAD cycles in 24 patients were assessable for safety. The most common hematologic toxicity was thrombocytopenia, with grade 3/4 in 21%. Grade 3/4 neutropenia was observed in 20%. Sensory neuropathy occurred with grade 2 in 38% and grade 3 in 4%. The median dose intensity was 1.52 mg/m^2/week for bortezomib and 5.37 mg/m^2/week for doxorubicin, which correspond 88% and 90% of the planned dose intensities, respectively. A total of 42 TD treatment cycles (median 3, range 1-6 cycles) was administered. One patient developed grade 3 neutropenia and thrombocytopenia. Non-hematology toxicities occurred infrequently and mild. *Conclusion*. PAD followed by TD in patients with relapsed multiple myeloma is very active and tolerable.

PO-632

SINGLE CENTRE VELCADE 03-06: STEROIDS, TIME TO RESPONSE & IGA CORRELATE WITH OUTCOME

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Patients treated with bortezomib for relapsed or refractory myeloma 2003-06 were audited. 40 patients of mean age 61.2 years (45-78) were treated, at median 45.5 months from diagnosis (8-152). 57.5% were IgG, 32.5% IgA, 62.5% BJ+. 1 2nd-line & 39 ≥3rd-line; median 4 prior lines (1-8). 95% had prior thalidomide & 55% prior autologous-SCT (18% 2 autos). 67.5% refractory to most recent treatment. 57.5% commenced monotherapy, with steroid for non-response (as per SUMMIT) in 52%. Post 25/07/05 BCSH guidelines recommended steroids in all (overall 72.5%). Median 4.8 cycles bortezomib were given (range 0.5-12). Overall toxicity ≥GI: neuro. 80%, fatigue 75%, gut 60% & breathlessness 10% (reversible). 25% patients had GIII-IV neurotoxicity, & mean neurotoxicity grade was 1.7. 52.5% required dose-reduction (at median 4 cycles), majority for neuropathy. 39 patients were evaluable (≥1 cycle) for best response, by EBMT criteria. 23 monotherapy: 8.5% IF-CR, 39% PR & 22% MR; 8.5% SD & 78% non-PD. Steroids improved response in 17% (1 MR & 1 PR). 16 with initial steroids: 56% PR & 6% MR; 6% SD & 75% non-PD. Overall 5% IF-negative CR, 49% PR & 15% MR; 8% SD & 77% non-PD. PR/CR correlated with IgA, & not with steroid, refractory status to or lines of prior treatment. Overall time to progression 5.3 months (0-25), & time to next therapy 7.1 months (0-27); TTP correlated with PR/CR (8.1 months), cycles to maximum response & IgA, with trend to initial or additional steroid. Time to next treatment also correlated with PR/CR (10 months), IgA & initial steroid, with trend to cycles to maximum response. Survival post-bortezomib cessation was 8.0 months (0-44), & overall survival median 11.4 months (1-52). Overall survival correlated with PR/CR (16.3 months), & initial/additional steroid, with trend to IgA & cycles to maximum response. This heavily pre-treated cohort has higher responses than comparable SUMMIT group, exceeding APEX which had median 2 prior therapies. Steroids did not significantly affect PR/CR rates, but appear to increase time to progression/next treatment & overall survival. Time to maximum response correlates with progression & survival. IgA also correlates with response & survival.

PO-633

CONTEMPORARY INTRAVENOUS ADMINISTRATION OF BORTEZOMIB, MELPHALAN AND DEXAMETHASONE IN PREVIOUSLY TREATED MYELOMA PATIENTS

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Bortezomib, Melphalan, and Steroids are among the most effective drugs for treatment of multiple myeloma and their combination has already shown impressive results in elderly myeloma patients. In the attempt to optimize the administration of these drugs and to take advantage of their synergism when used in combination, we designed a protocol with 6 monthly courses of contemporary intravenous administration of these drugs in patients with advanced multiple myeloma. Bortezomib was given at dosage of 1.3 mg/m^2 i.v. days 1,4,8,11, Melphalan 5 mg/m^2 i.v. days 1,4,8,11, Dexametasone 40 mg days 1-2, 4-5, 8-9, 11-12. So far, 21 patients have been enrolled. Median age was 64.5 (range 53-82). All patients had been already treated with a median of 2 previous

lines of treatment (range 1-6) including autologous bone marrow transplant in 7 patients and and Bortezomib alone or in combination in 5 patients. All patients included in this study were no longer eligible for a bone marrow transplant procedure. Fourteen patients were resistant to previous therapies while 7 were considered as relapsed. Since the study is still ongoing and very few patients are beyond the fourth course, we have evaluated toxicity and efficacy data for the first 4 cycles only. Hematological toxicity grade 3 occurred in 38% of patients after the first cycle. After the second cycle hematological toxicity was grade 3 in 15% and grade 4 in 21% of patients. The third cycle induced grade 3 haematological toxicity in 20% and grade 4 in 13% of patients. After the fourth cycle, hematological toxicity was grade 3 in 30% and grade 4 in 20% of patients. Three patients developed also grade 3 non-hematological toxicity (Herpes zoster, vomit). So far, 4 patients have stopped treatment for toxicity after 1, 3, 3, and 4 courses. All of these patients were in stable disease. Five patients achieved a very good partial remission (M-protein not detectable at electrophoresis), 5 patients a partial remission (reduction of M-protein > 50%) while in two other patients reduction of the M-protein was associated with increase of bone marrow plasmacells. Two patients were in stable disease, one was in progression and 2 are not yet evaluated. In conclusion, the contemporary intravenous administration of Bortezomib, Melphalan, and Dexametasone, appears to be an highly effective treatment even for heavily pretreated patients. However, in these patients, haematological toxicity was the limiting factor. Therefore, the dosage of the drugs or their schedule has to be modified.

PO-634

EFFICACY OF COMBINATION THERAPY OF BORTEZOMIB AND DEXAMETHASONE FOR SEVEN PATIENTS WITH EXTRAMEDULLARY PLASMACYTOMA

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Despite the use of aggressive local and systemic treatment including autologous stem cell transplantation in multiple myeloma, extramedulary recurrences are common and the prognosis of these patients is poor. Many novel drugs such as thalidomide, lenalidomide and bortezomib improve the response of treatment of multiple myeloma, but some reports failed to describe thalidomide has effect in extramedullary plasmacytoma. Bortezomib is a potent and selective proteasome inhibitor recently introduced in the treatment of myeloma. This drug produces significant responses in about one-third of patients with relapsed/refractory disease. We report 7 cases of relapsed or refractory plasmacytomas associated with or without multiple myeloma with bortezomib and dexamrthason at our institution. Characteristics of patients with extramedullary palsmacyroma Case Sex/age Previous treatment Site of plasmacytoma Bone marrow disease Response Case 1 24/M VAD Orbit, nasal cavity Absent CR (pathogic) Case2 67/F MP Orbit, zygomatic bone present CR Case 3 56/F VAD, autologous PBSCT Orbit, breast, sucutaneouse soft tissue present PR Case4 48/M VAD, autologous PBSCT TD Pleura, scalp Present PR Case 5 69/ F VAD Orbit, present CR Case 6 55/F TD pleura present PR Case 7 72/M MPT Chest wall Present PR M*p*=melphalan, prednisone, VAD=vincristine, doxorubicin, dexamethasone, TD=thalidomide dexamthasone, MPT= melphalan, prednisone, thalidomide We recognized all these patients soft tissue plasmacytomas decreased and showed more than partial response. This report lends support to the efficacy of bortezomib and dexamethason treatment in the treatment of plasmacytoma and describes the safe use of bortezomib.

PO-635

SAFETY AND EFFICACY RESULTS FROM AN INTERNATIONAL PHASE 3B STUDY FOR EXPANDED ACCESS TO BORTEZOMIB IN PATIENTS WITH RELAPSED AND/OR REFRACTORY MULTIPLE MYELOMA

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Introduction. The proteasome inhibitor Bortezomib (VELCADE®) showed significant activity in previous phase 2 and 3 studies in patients (pts) with previously treated MM. This international multicenter open-label phase 3b study was designed to allow expanded access to bortezomib. *Methods.* Pts with MM were eligible if they had ≥ 2 prior lines of therapy and required treatment of relapsed or progressive disease. Pts received 1.3 mg/m^2 IV bolus bortezomib on days 1, 4, 8, and 11 of each 3-week cycle for up to 8 cycles; dexamethasone (20 mg/d PO on the day of and day after bortezomib) could be added after cycle 2 for progressive disease or after cycle 4 for stable disease. Adverse events (AEs) were graded based on NCI-CTC version 2.0. Efficacy assessment was performed based on changes in monoclonal (M)-protein concentration in serum and urine every 2 cycles, response assessed using modified SWOG criteria: complete response (CR) being 100% reduction in M-protein; very good partial response (VGPR), 75-99% reduction; partial response (PR), 50-74% reduction; minimal response (MR), 25-49% reduction; stable disease, <25% reduction, and increasing M-protein levels progressive disease. *Results*. 624 pts enrolled in 93 centers from 21 countries were evaluable for safety (55.3% male; median age 62.7 years). 68% of pts received 3-11 lines of previous therapy. Karnofsky performance status was 70 in 25.6% of pts; 141 pts (22.6%) were ≥ 70 years of age. Pts completed a median of 5 cycles of therapy (range, 0-13). Grade 3/4 (related and unrelated) AEs were reported in 430 pts (68.9%), with thrombocytopenia (29.2%), neutropenia (13.3%), and anemia (11.7%) the most common hematologic AEs. Grade 3/4 PN was reported in 8.2% of patients. Overall, 165 patients (26.4%) discontinued therapy due to treatment-related AEs. Efficacy results of evaluable pts (n=590) were 12.0% CR, 23.2% VGPR, 18.8% PR, and 16.6% MR. Median time to first response 42 days (range, 7-125), median time to best response 63 days (range, 7-235). *Conclusions*. Bortezomib was well tolerated with manageable toxicities in pts with relapsed and/or refractory MM. Safety and efficacy findings confirm results of previous clinical studies.

PO-636

CORRELATING RESPONSE TO BORTEZOMIB IN RELAPSED/REFRACTORY MULTIPLE MYELOMA TO ADVERSE CYTOGENETICS: RESULTS OF A CANADIAN COHORT

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Background. Bortezomib, a reversible proteasome inhibitor active in patients with relapsed and/or refractory multiple myeloma (MM), appears to overcome poor prognosis associated with certain cytogenetic abnormalities. *Methods.* Relapsed and/or refractory MM patients from 13 centres across Canada who had received ≥2 previous lines of therapy were enrolled in this open-label, non-randomized, phase IIIb study. Patients received up to eight 3-week cycles of bortezomib (twice weekly). *Results*. 104 pts were enrolled. Mean age was 60.7 years, and 62.5% were male. Thirty-nine patients (37.5%) had a KPS of ≥70. 81 patients (77.9%) had received ? 3 lines of prior therapy and 69.2% of patients had received prior thalidomide. Mean number of bortezomib cycles received was 5.22 (0-12 cycles); 37.5% of patients completed 8 cycles. Overall, 66.3% of patients experienced Grades 3/4 treatment related adverse events, with only 5.8% experiencing Grade 3/4 peripheral neuropathy. Overall best response for 96/104 evaluable patients was 69.8%. Fifty-two patients were evaluable for cytogenetic abnormalities: 13 out of 24 patients tested (54%) were positive for del (q13), 9 out of 28 patients (32%) were positive for t(4;14) and 4 patients were positive for both. Response rates were as follows: All patients: 34.3% had a complete response (CR) or very good partial response (VGPR), 35.4% had a partial response (PR) or minimal response (MR), 14.6% had stable disease (SD) and 15.6% had progressive disease (PD). Out of 13 patients who were positive for del (q13), 30.8% had a CR or VGPR, 46.2% had a PR or MR and 23.1% had SD or PD. Out of 9 patients who were positive for t(4;14), 44.4% had a CR or VGPR, 44.4% had a PR or MR and 11.2% had SD or PD. Out of 4 patients who were positive for both del (q13) and t(4;14), 50% had a CR or VGPR, and 50% had a PR or MR. Response was, therefore, not significantly different for patients with cytogenetic abnormalities. *Conclusion*. This Canadian cohort confirms that response to bortezomib in a heavily pretreated population was similar in the subset of patients with del (q13) and t(4;14).

PO-637

USE OF BORTEZOMIB IN NORTHERN IRELAND- COMBINATION WITH DEXAMETHASONE ROUTINELY USED TO IMPROVE RESPONSE RATE

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Introduction. Bortezomib has been available for NHS patients with myeloma in Northern Ireland only from 2006 due to financial constraints. It has therefore been particularly important for us to obtain maximal response rates in patients in order to obtain maximal benefit from this expensive agent. It has been part of our policy in Northern Ireland to use Dexamethasone with Bortezomib on a routine basis - a commonly accepted practice but supported by relatively few clinical studies. *Methods*. Bortezomib treatment was prescribed by eight different consultants from 5 different units. 29 patients (21 males, 8 female) median age 65, range 51-80 were treated. The group was heavily pre-treated with a median of 3 previous lines of treatment range 1-7; 16 patients had previous autologous transplant and 26 patients had previous Thalidomide, while 5 patients had previous Bortezomib. Of the 29 patients, 26 received Bortezomib with Dexamethasone, 24 using a regimen of 20mg per day on days 1, 2, 4, 5, 8, 9 and 11, 12. Two patients had anthracycline and dexamethasone as part of a PAD type regimen. Three patients did not receive dexamethasone on medical grounds. *Result*. Of the 29 patients, 4 patients have had unconfirmed complete remissions, 6 patients very good partial responses and 10 patients partial responses giving a total partial response rate of better than 69%. This compares with the response rate using Bortezomib monotherapy in the APEX trial of 42% in a similarly heavily pre-treated group. The median duration of remission has not yet been reached. *Conclusion*. We suggest that to optimise the clinical effect of Bortezomib, it should be used in conjunction with Dexamethasone on a routine basis. The additional of further agents which may further synergise with this combination should also be considered.

PO-638

PROGNOSTIC IMPACT OF UNFAVORABLE CYTOGENETIC ABNORMALITIES IN MULTIPLE MYELOMA PATIENTS TREATED BY BORTEZOMIB (VELCADE)

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Introduction. The aim of this study is to investigate if Bortezomib (Velcade) is able to antagonize the impact of negative cytogenetic prognostic markers. We have focused on four chromosomal aberrations known as negative prognostic factors in multiple myeloma (MM) treated by conventional or myeloablastive treatment: deletion of RB gene (13q14), deletion of p53 (17p13), amplification of CKS1B gene (1q21) and translocation t(4;14). *Material and Methods.* We have identified monotypic plasma cells and studied chromosomal aberrations by cytoplasmic light-chain fluorescence *in situ* hybridization (cIg-FISH) technique. Up-to-date group of 18 patients (pts.) has following characteristic: 67% of men, median age 62,5 years (44,4-77,9). 50% (9 pts.) were in stage IIIA, 28% (5 pts.) in IIA and 22% (4 pts.) in IA. 61% (11 pts.) were in first relapse, 22% (4 pts.) were in second relapse. The others 17% (3 pts.) were in third relapse. Treatment with Velcade based regimens was continued for up to 8 cycles in 66% pts. (12/18). *Preliminary Results*. Cytogenetic findings: Deletion of RB gene was found in 8/16 (50%) pts., t(4;14) in 8/15 (53%) pts., deletion of p53 gene in 7/15 (47%) cases and amplification of CKS1B gene in 10/15 pts. (66%). Overall Response (OR) was 39% (0% CR, 11% VGPR, 28% PR). OR was not higher in pts. with cytogenetic findings for all aberration types except t(4;14): OR was 38% vs. 38% for pts. with vs. without deletion of RB gene; 63% vs. 14% for t(4;14) (p=0,240); 29% vs. 50% for deletion of p53 and 40% vs. 40% for amplification of CKS1B. Further, Velcade based therapy showed comparable TTP, DOR and OS in pts. with or without cytogenetic aberations including t(4;14). iv) *Preliminary conclusions*. We have found no significant difference when compared positivity vs. negativity of each cytogenetic aberration (including t(4;14)) for OR, TTP, DOR, PFS, and OS. This should have been caused by small size of our sample. It is possible that Velcade antagonizes the negative prognostic value of studied cytogenetic findings in MM.

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PO-639

WEEKLY BORTEZOMIB + CYCLOPHOSPHAMIDE + PREDNISONE IN MYELOMA

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Introduction. Oral cyclophosphamide (CY) and prednisone (P) produces partial remission (PR) in approximately 40% of relapsed/refractory myeloma patients(pts). We performed a phase 1-2 trial in which increasing doses of bortezomib (B), given once or twice per week, were added to CY + P. The maximum tolerated dose was B 1.5 mg/m^2 on days 1, 8 and 15 of a 28-day schedule. *Materials and Methods.* Each 28-day cycle consisted of CY 300 mg/m^2 on days 1, 8, 15, and 22, B 1.5 mg/m^2 on days 1, 8 and 15 and P 100 mg every 2 days. A maximum of 8 cycles was given. Thirteen pts were entered between 11/05 and 09/06. Toxicity was graded as per the CTC version 3 scale while the EBMTR criteria was used to assess responses. *Results*. The median age was 59 (48-70), 8 were male and the median pre-treatment beta 2-microglobulin level was 266 nmol/L (155-496). All but 1 had undergone prior ASCT. Six have finished all 8 cycles and 5 continue on study after 7 (3 pts), 5 (1 pt) and 4 (1 pt) cycles. 76 cycles are evaluable for toxicity. Grade 4 neutropenia, grade 4 thrombocytopenia and grade 4 thrombocytopenia occurred in 1.3%, 2.6% and 1.3% of cycles, respectively. Grade 3 nausea, vomiting and/or diarrhea were seen in 8% of the cycles, the majority in 1 pt with long-standing gastrointestinal symptoms. One pt with a MR withdrew from therapy after 4 cycles due to steroid intolerance. Only grade 1 neurotoxicity was seen (7 pts). Shingles occurred in 4. Seven (54%) achieved CR/nCR, 4(31%) PR, 1 (7.5%) minimal response (MR) and 1 (7.5%) stable disease. All 13 are alive, and only 1 has progressed (after cycle 4) at a median of 8 mos (range 5-11) after starting therapy. *Conclusions*. 1) Weekly B 1.5 mg/m^2 along with oral CY and P produced an overall response rate of 85% with a CR/nCR rate of 54%; 2) this regimen is well-tolerated and convenient; 3) shingles prophylaxis is recommended; 4) further evaluation of this regimen as either first-line therapy or as maintenance therapy is warranted.

PO-640

RESPONSE TO BORTEZOMIB IN RELAPSED/REFRACTORY MULTIPLE MYELOMA SERIE

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Background. Bortezomib has been shown to be effective in multiple myeloma (MM), but there is limited experience in response to re-treatment. *Aims.* To evaluate the efficacy and safety of Bortezomib in an every day clinical use in refractory/relapsed MM between December 2003 to November 2006 in a single institution. *Patients and methods.* 43 patients with relapsed/refractory MM receiving Bortezomib alone (1,3 mg/m^2 on days 1,4,8,11 in a 21-day course) as second or more line of therapy. The response was evaluated according EGBMT criteria (Blade J, Samson D, Reece E *et al.*). Patients without response after 4 courses and patients that relapsed after reached CR or PR with Bortezomib alone, a combination of bortezomib + dexamethasone (BD) or bortezomib+melphalan+prednisone (BMP) were administered. Adverse effects were registered. *Results*. 43 patients (males 41.8%), mean age 67.3 years (34-89), over 65 years (51.1%). Bortezomib was administered in second line: 14 (32.5%), in third or more: 29 (67.4%). Overall response: 31 (77.5%); CR+PR: 29 (72.5%); MR: 2 (5.0%); CR: 16 (40.01%); CR-EIF negative: 11 (27.5%); failure: 9 (22,5%), mean courses to reached response: 3.6. No relation to response and presence or not chromosomal aberrations. At 36 months on follow-up, of 40 valuable patients, 18 (45%) are in stable response without therapy. Seven patients (16.3%) do not reached response after 4 courses and received a Bortezomib combination therapy, 10 patients (23.2%) were relapsed after a mean of 18 months follow-up in stable response. In 12 patients (70.5%) a combination of BD was applied and 5 patients(29.5%) received BMP by relapse or progression. Responses: group BD 1 CR EIF+, 7 PR, 4 F; group BMP 1 CR-EIF-, 2 PR,

1 F, 1 NV. 14 patients died by progression or infectious complications (35%), Adverse events: thrombocytopenia 38.4 (grade III: 17.9), fatigue 38.5%, peripheral neuropathy 33.3%, constipation 35.8%, diarrhoea 20.5%, ZHV 12.8%, non documented infection 33.3%, fever 10.2%, hypotension 5.1%, leucopenia grade 3 12.8%. Only 3 patients (7.6%) need disrupted therapy by toxicity. No more adverse events were observed in patients treated with Bortezomib in combination. Comments: In our experience a high response to Bortezomib in an every day clinical use in relapsed/refractory MM was observed. In addition retreatment with Bortezomib in combination induces response (64.7%). The safety is good with tolerable adverse effects. It is necessary prolonged follow-up time and to perform more studies in order to establish the best schedule for relapsed/refractory MM.

PO-641

EFFICACY OF BORTEZOMIB IN ELDERLY MM PATIENTS

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Aims. The aim of this non-interventional, observational-study (AWB, 26866138MMY0003) was to document the efficacy and safety of Bortezomib (VelcadeR, Vel) monotherapy in Multiple Myeloma (MM) patients (pts) as 3rd-line therapy under routine conditions. *Methods.* Pts were treated up to 24 weeks. At baseline, demographical and medical history data were collected, including type and number of preceeding therapies and concomitant diseases. Adverse events (AEs) were continuously documented. *Results*. Between July 2004 and December 2005, 202 pts were treated (93 (46%) female, 109 (54%) male). Median age was 67 years (y) [37-87y], whereby 135 (67%) pts were ≤ 70 y and 67 (33%) > 70 y. The median number of prior therapies was 2 [0-4] (melphalan ± prednisone 26,3% (≤ 70y) and 37,7% (> 70y), VAD or similar 24,8% (≤ 70y) and 20,0% (> 70y), steroid mono 5,8% (≤ 70y) and 5,2% (> 70y), bendamustine 14,3% (≤ 70y) and 29,7% (> 70y), cyclophosphamide 3,8% (≤ 70y) and 0,7% (> 70y), others 25,1% (≤ 70y) and 6,7% (> 70y)). Response was evaluated in 176/202 pts (87%). From these pts objective responses (CR+PR+MR+SD) occurred in 164 (93,2%) pts, including 9,7% CR, 55,1% PR, 7,4% MR and 21,0% SD; 12 (6,8%) pts showed progressive disease (PD). The overall response (OAR) (CR+PR+MR) as best response occurred in 127 (72,2%) pts. OAR was observed in 88 (72,7%) pts ≤ 70 y and 39 (70,9%) > 70 y. Median time to response (TTR) was 2.5 cycles (1-8) in both groups. 77,3% (≤ 70 y) versus 79,4% (> 70y) of responding pts achieved an OAR (CR+PR+MR) as best response after 4 cycles. The most common AEs were: Thrombocytopenia grade 3 in 12,4% and grade 4 in 3,0%; PNP grade 3 in 4,0%; anemia grade 3 in 2,5% and grade 4 in 0,5%. 5 (≤ 70y) and 2 pts (> 70y) died from PD. *Conclusion*. Bortezomib in 3rd-line therapy of MM is efficient with manageable side effects. No differences in RR or TTR between pts below and above 70 years of age were seen. Findings confirm the results of large randomized clinical trials.

PO-642

BORTEZOMIB (VELCADE) FOR REFRACTORY/RELAPSED MULTIPLE MYELOMA-RESULTS OF CZECH MYELOMA GROUP (CMG) AND SLOVAK MYELOMA SOCIETY (SMS)

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Summary. The efficacy of bortezomib in the treatment of newly diagnosed and/or relapsed multiple myeloma (MM) has been shown repeatedly. This retrospective case analysis evaluated the feasibility and activity of bortezomib-based therapy in MM patiens in Czech and Slovak republic. One hundred fifty consecutive patients, 88 men and 62 women, aged 33-78 years, with refractory/relapsed myeloma were treated with bortezomib-based therapy. This was a heavily pretreated population (1-11 lines of prior therapies), including high-dose therapy with stem cells support (86 patients, 57.3%) and/or thalidomide (57 pts, 38%). The overall response rate (ORR), assigned by IMWG criteria, was 35.3%, including 15.3% of complete remission (CR). The response was higher in patients without prior thalidomide in comparison with those with previous thalidomide therapy - CR 18.3% vs. 10.5%, ORR 40.9% vs. 26.3% (p<0.03). The most common adverse events were thrombocytopenia (56.8%) and neuropathy (56.1%), grade 3/4 thrombocytopenia developed in 30.3% and neuropathy in 20.1% of patients, respectively. The risk of both complications was substantially higher in patients with baseline involvement (gr.1 neuropathy, thrombocytopenia). *Conclusion*. Our experience confirmed that bortezomib provides clinical benefit with manageable toxicities in this heavily pretreated and high-risk population. Higher effect and lower risk of the complications in our study supports the statement that it would be advantageous to start treatment with bortezomib earlier in the course of disease.

PO-643

PROLONGED SURVIVAL WITH ACTIMID (CC-4047)

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Introduction. The introduction of thalidomide for myeloma has been associated with improvements in overall response rates, progression free and overall survival. However thalidomide toxicity frequently leads to therapy discontinuation. Analogues of thalidomide have been developed with the aim of enhancing anti-tumour activity and reducing therapy associated toxicity. Two of these analogues (CC-4047 [Actimid] and CC-5013 [Lenalidomide]) are currently in clinical development. CC-5013 has demonstrated significant anti-myeloma efficacy in the Phase 3 setting. We have previously reported a Phase 1 study of CC-4047 as a daily or alternate day drug with MTD of 2 mg od or 5 mg alt day and overall response rates (>50% reduction) of 50 and 54% respectively. We now report on the long term follow-up and outcomes of this group of patients. *Results*. 44 patients have received CC-4047 at doses ranging from 1mg alternate days to 10 mg daily. Patients had received a median of 3 prior lines of treatment (range 1-8). 18/44 had received previous high dose therapy, 24/44 had received thalidomide and 2/44 had previously received bortezomib. The median duration of therapy with CC-4047 was 37 weeks (2-213 weeks) and median follow-up is 110 weeks (5-291). Therapy has been withdrawn in 36/44 patients. This was due to PD in 18/36, DVT in 4/36, recurrent neutropaenia in 7/36, relapsing/remitting fever in 1/36, non-therapy related death in 2/36 and unrelated to therapy or myeloma in 4/36. Maximum response to single agent CC-4047 was: CR 6/43, VGPR 9/43, PR 8/43, MR 4/43, SD 13/43 and PD 3/43. 1 patient withdrew from study after 2 weeks and is not eligible for response assessment. Overall response rate to single agent CC-4047 was 53%. 11 patients received additional dexamethasone for PD (10/11) and non-response (1/11). Overall best response was: CR 6/43, VGPR 11/43, PR 7/43, MR 5/43 and SD 11/43. The median time to maximum response was 16 weeks (2-109 weeks). The overall median progression free survival was 55 weeks and median overall survival was 109 weeks. *Conclusion*. Actimid is well tolerated and addition of dexamethasone is associated with improved clinical responses. Further studies are necessary.

PO-644

DEVELOPMENT OF EXTRAMEDULLARY MYELOMA MANIFESTATIONS FOLLOWING THALIDOMIDE THERAPY

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Thalidomide targets marrow stromal cells, alters IL-6 and TNF-α production and decreases adhesion of malignant plasma cells. Due to modified cell-to-cell contacts and interactions within bone marrow microenvironment, malignant plasma cells may become resistant to therapy and may develop tendency to disseminate and infiltrate other tissues in the periphery. Of 7 patients on thalidomide 3 developed extramedlullary symptoms of plasma cell proliferation: 1st. A 54 year -old man with IgG kappa myeloma from 2004. VAD and EDAP resulted in partial remission. Due to disease progression, he received thalidomide 100-400 mg/d +high-dose dexamethasone from April 2006 to September 2006, when submandibular lymph node enlargement appeared, caused by diffuse

infiltration of atypical ($CD38^{++}$) plasma cells. Lymph nodes were irradiated. Marrow plasmacytosis then amounted 0,5% and serum M-protein 2,63g%. In November 2006 M-protein increased to 4,9 g% and he died in January 2007 due to disease progression. 2nd. A 71-year -old man with lambda -free light chain myeloma from April 2005. Initial treatment with VAD, melphalan and VMBCP resulted only in disease stabilization. From March to August 2006 he received thalidomide 100 -300 mg/d and in 6th therapy month he developed plasma cell leukemia (26% of $CD38^{++}$, $CD138^{+}$, CD56- plasma cells in blood) with hypercalcemia, kidney failure and 3,25 g% BJ lambda concentration in urine. Trephine biopsy revealed mixed aplastic /hypercellular marrow heavily (80%) infiltrated by $CD138^{+}$ plasma cells. 3rd .A 50 year -old man with IgG kappa myeloma infiltrating Th3 vertebra from June 2004, treated with laminectomy, spine irradiation, VAD and EDAP without remission. From May to November 2006 thalidomide 200-400 mg/d + high-dose dexamethasone were given. After 2 months, marrow plasmacytosis decreased from 93% to 4% and serum M protein from 4,7 g% to 2,0 g%. In November 2006 plasma cell leukemia developed (in blood 40% of $CD138^{+}$, $CD56^{+}$ plasma cells) while bone marrow plasmacytosis was 83%. Our findings suggest that thalidomide is effective in initially reducing more mature plasma cell compartment confined to the marrow and allows a relatively immature myeloma cell compartment to escape marrow microenvironment (Saba 2005).

PO-645

THALIDOMIDE INDUCED-LEUKOPENIA IN JAPANESE PATIENTS WITH REFRACTORY MULTIPLE MYELOMA

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Introduction. Thalidomide is effective for refractory multiple myeloma, however induce many adverse events including peripheral neuropathy, deep vein thrombosis, constipation, and neuropsychological symptoms. Hematological adverse events have rarely been reported in U.S.A and Europe. We report here severe leucopenia, which has been observed in JMSG phase II study *low-dose thalidomide plus low-dose dexamethasone therapy for refractory myeloma*, and analyze the factors related to leucopenia. *Patients and methods.* Patients with refractory myeloma were eligible. Thalidomide 100 mg/day was administered on day 1-7, and increased to 200 mg/day without severe adverse events. Dexamethasone 4 mg/day was administered on day 1-28, and decreased 1mg/week, and maintained with 1 mg/day. *Results*. Sixty-six patients (male/female ratio; 0.7, median age 64.5, range40-74) have been accrued to the study. The myeloma subtypes include 48 IgG, 6 IgA, 1 IgD, and 11 light chain only. Forty-nine patients were refractory for conventional chemotherapy, and 17 patients had relapsed disease after autologous stem cell transplantation. Responses were observed in 63.6% (4 nCR, 13PR, and 25MR). Median progression-free and overall survivals were 6.2 and 25.4 months. The incidence rates of grade 2 or higher peripheral neuropathy, hyperglycemia, skin rash, constipation, and DVT were 7.5, 1.5, 4.5, 4.5, and 4.5%, respectively. Grade 1-2 leukopenia and thrombocytopenia were observed in 30.3 and 24.2%, and Grade 3 or higher leucopenia was observed in 10.6%. One patient was died with sepsis caused by severe leucopenia. Thalidomide-induced leucopenia was closely related to pretreatment white blood cell count ($p<0.02$) and platelet count ($p<0.001$). Thrombocytopenia was related to pre-treatment platelet count ($p<0.01$). In addition, the incidence of leucopenia was significantly higher in patient with pretreatment platelet count $<100\times10^{9}$/L. There was no relationship between hematological adverse events and disease period. *Conclusion*. Low-dose thalidomide plus low-dose dexamethasone therapy was as effective as low-dose thalidomide plus high-dose dexamethasone therapy in patients with refractory multiple myeloma. The incidence of adverse events including DVT and hyperglycemia is lower than the data in the United States and Europe. However, leukopenia is one of the most serious adverse events in Japanese patients, especially in patients with low pretreatment white blood cell and platelet count.

PO-646

MONOTHERAPY WITH LOW-DOSE THALIDOMIDE FOR RELAPSED OR REFRACTORY MULTIPLE MYELOMA - IS 100 MG DAILY ENOUGH?

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Introduction. Thalidomide is an immunomodulatory drug used in the treatment of relapsed or refractory multiple myeloma (MM). The optimal dosing regimen of thalidomide in not known and is currently being studied in a number of clinical trials. *Patients and methods.*We retrospectively analysed the overall response rate and response duration of 53 patients with MM who received thalidomide in a median dose of 100 mg daily in monotherapy for relapse or progression of MM between 2000 and 2005. The aim of the study was to compare response rates of thalidomide given as the 2nd line treatment to those of thalidomide given as the 3rd line therapy and to evaluate the occurrence of toxicities with this treatment regimen. *Results*. Of 33 patients receiving thalidomide as 2nd line, 13 (39%) had overall response, including two patients (6%) with complete response (CR) and 11 (33%) patients with partial response (PR). Of 20 patients treated with thalidomide monotherapy as 3rd line treatment, there were three responses (15%), all CRs. The difference between the overall response rates in the two groups of patients was statistically significant (39% vs. 16%; $p=0.039$). There was no significant difference between the duration of responses between the two groups. Only 6% (3 of 53 patients) had to stop the treatment because of grade 3 or 4 toxicities (one patient each neuropathy, allergic reaction, and prolonged leukopenia) *Conclusions*. Low-dose thalidomide in monotherapy achieves treatment responses in approximately 30% of patients with advanced MM. The response rate appears to be higher if thalidomide treatment is started after the 1st relapse or progression in comparison with the 2nd relapse or progression. The occurrence of toxicities is acceptable even with prolonged exposure to the drug.

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PO-647

PROGNOSTIC FACTORS IN PROGRESSIVE MM TREATED WITH THAL/DEXA

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Introduction. The outcome of patients with relapsed/refractory multiple myeloma (MM) treated with the combination of thalidomide and dexamethasone (Thal/Dexa) is quite variable. The aim of the present study was to evaluate the prognostic impact of simple clinical and laboratory disease parameters on progression-free survival (PFS) of these patients. *Materials and methods*. Between 2000 and 2007, 72 patients with relapsed/refractory MM were treated in our department with thalidomide 200 mg p.o. daily at bedtime and dexamethasone 40mg p.o.x 4 days/2 weeks. Gender, age, ECOG status, disease status (primary refractory disease, relapse, refractory relapse), MM type, presence of extramedullary disease (ED), number of previous therapeutic regimens administered, levels of hemoglobin, platelets, albumin, creatinine, LDH, beta-2-microglobulin (β2m), CRP and percentage of bone marrow plasma cells were included in a univariate analysis in order to assess their impact on PFS. Those variables found to bear statistical significance, entered subsequently a multivariate Cox regression analysis. *Results*. Median follow up was 25 (range:1-72) months. Overall response rate was 62.5% and median RD was 21 (95%CI:15-27) months. Median PFS and OS were 10 (95%CI:6-14) months and 24 (95%CI:17-31) months respectively. Parameters bearing a statistically significant ($p<0.05$) impact on PFS were the following: 1)ECOG status ≤1 vs. >1 with PFS 23 (95%CI:12-34) vs. 8 (95%CI:5-11) months 2) disease status with PFS 22 (95%CI:12-32), 14 (95%CI:4-24) and 7 (95%CI:4-10) months for primary refractory disease, relapse and refractory relapse respectively 3) absence vs. presence of ED with PFS 12 (95%CI:2-22) vs. 5 (95%CI:1-9) months 4) β2m <3.5 mg/L vs. ≥3.5 mg/L with PFS 22 (95%CI:12-32) vs. 7 (95%CI:4-10) months 5) LDH ≤480 U/L vs. >480 U/L with respective PFS 22 (95%CI: 18-26) and 9 (95%CI: 5-13) months 6) number of previous therapeutic regimens 1 vs. >1 with PFS 19 (95%CI:10-28) vs. 8 (95%CI:6-10) months. During subsequent Cox regression analysis, B2m proved to be the only variable with independent prognostic significance for PFS. *Conclusion*. ECOG status >1, refractory relapse, presence of ED, β2m ≥3.5 mg/L, LDH >480 U/L and previous therapeutic regimens >1, were associated with a significantly worse outcome in patients with progressive MM treated with Thal/Dexa, with β2m being the only independent predictive variable.

PO-648

PROGNOSTIC VALUE OF ISS IN PROGRESSIVE MM TREATED WITH THAL/DEXA

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Introduction. The International Staging System (ISS) is based on the simple combination of beta-2-microglobulin (β2m) and albumin and has already demonstrated high prognostic power in previously untreated multiple myeloma (MM) patients. Our aim was to apply ISS in the setting of relapsed/refractory MM treated with thalidomide-dexamethasone (Thal/Dexa) and evaluate its prognostic significance. *Materials and Methods*. Between 2000 and 2007, 72 patients with relapsed/refractory MM were treated in our department with thalidomide 200 mg p.o. daily at bedtime and dexamethasone 40mg p.o.x 4 days/2 weeks. Patients were stratified into 3 groups according to the pretreatment levels of B2m and albumin using the ISS (I: β2m <3.5 mg/L, albumin ≥3.5 g/dL, II: neither I nor III, III: β2m ≥5.5mg/L). Response rates were compared between groups using chi-square tests. Response duration (RD), progression-free survival (PFS) and overall survival (OS) were estimated according to Kaplan-Meier method and differences between groups were assessed using the log-rank test. *Results*. Twenty-three (31.9%) patients were classified in ISS I, 27 (37.5%) in ISS II and 22 (30.6%) in ISS III. Objective responses were observed in 17 (73.9%) of ISS I patients, in 18 (66.7%) of ISS II patients and in 10 (45.5%) of ISS III patients (p>0.05). Median RD in ISS I, II and III was 27 (95%CI:20-34), 18 (95%CI:12-24) and 3 (95%CI:1-5) months respectively (pI,II=0.026, pI,III <0.0001, pII,III=0.0003). Median PFS was 24 (95%CI:20-28) months in ISS I, 10 (95%CI:5-15) months in ISS II and 4 (95%CI: 2-6) months in ISS III (pI,II=0.006, pI,III <0.0001, pII,III=0.002). Median OS in ISS I, II, III was 49 (95%CI:34-64), 28 (95%CI: 22-34) and 9 (95%CI: 5-13) months respectively (pI,II=0.04, pI,III <0.0001, pII,III=0.0001). *Conclusion*. ISS status did not affect response rates but proved highly predictive of RD, PFS and OS in patients with progressive MM treated with Thal/Dexa, thus providing an easy and simple alternative to assess prognosis in these patients.

PO-649

THAL/DEXA IN PROGRESSIVE MM: 7-YEAR EXPERIENCE OF A SINGLE CENTRE

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Introduction. The combination of thalidomide and dexamethasone (Thal/Dexa) has proved effective as salvage treatment in multiple myeloma (MM) yielding response rates above 50%. Our aim was to assess the long term outcome of such patients, since so far relevant data is limited due to the short follow up in most studies. *Materials and methods*. Between 2000 and 2007, 72 patients of median age 64 years (range:44-80) with progressive MM, 16 (22.2%) with primary refractory disease, 39 (54.2%) with relapse and 17 (23.6%) with refractory relapse, were treated in our department with thalidomide 200 mg p.o. daily at bedtime and dexamethasone 40 mg p.o.x 4 days/2 weeks. Anticoagulant prophylaxis with low dose aspirin or low molecular weight heparin was administered in 49 (68.1%) patients. A significant proportion of patients had features of advanced disease: 43.1% ECOG status >1, 20.8% extramedullary lesions, 47.2% albumin <3.5g/dL, 34.7% LDH >480 U/l, 58.3% β2m >3.5 mg/L and 55.6% CRp>6 mg/L. The median time from initial diagnosis was 25 months (range:2-75) and the median number of previous regimens administered was 2 (range:1-5). Response duration (RD), progression-free survival (PFS) and overall survival (OS) were estimated with the Kaplan-Meier method and differences between responders and non-responders were assessed using the long-rank test. *Results*. Median follow up was 25 months (range:1-72). Overall response rate was 62.5% (complete response: 5.6%, partial response: 56.9%). Minor response was observed in 11.1%, stable disease in 13.9% and disease progression in 12.5%. Median time to response was 42 days (range:14-182) and median RD was 21 (95%CI:15-27) months. Median PFS was 10 (95%CI:6-14) months (22 months for responders vs. 3 months for non-responders, p<0.0001) and median OS was 24 (95%CI:17-31) months (29 months for responders vs. 11 months for non-responders, p=0.002). The most common adverse effects were constipation, peripheral neuropathy, somnolence and skin rash observed in 59.7%, 52.8%, 38.9% and 18.1% respectively, but grade 3-4 toxicities were limited. Thrombotic events occurred in 11.1% with significantly lower incidence in patients receiving anticoagulant prophylaxis (6.1% vs. 21.7%, p=0.048). *Conclusion*. Thal/Dexa combination induces high response rates and maintains long-lasting remissions in MM patients with progressive disease, while toxicity remains acceptable.

PO-650

THALIDOMIDE SALVAGE THERAPY FOLLOWING IMMUNOMODULATORY TREATMENT

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Introduction. Despite the introduction of new agents and targeted therapy myeloma remains an incurable disorder. The introduction of thalidomide marked a new treatment paradigm with response rates of 32%. The thalidomide analogues CC-5013 and CC-4047 were developed to improve the immunomodulatory and toxicity profile associated with thalidomide. Laboratory studies confirmed that cytotoxicity and anti-TNF activity were improved. However there was also the suggestion that although there was substantial overlap in spectrum of activity that there were also differences in drug activity. Initial clinical studies of both lenalidomide (CC-5013) and actimid (CC-4047) confirmed that responses occurred following development of thalidomide resistance. However the response following development of progressive disease whilst receiving CC-4047 or CC-5013 is unknown. We present our experience of thalidomide post CC-4047 therapy. *Results*. Six patients received CC-4047 as part of a phase 1 dose escalation study. A median of three previous regimens had been given prior to CC-4047. One patient had previously received thalidomide. Responses to CC-4047 were: VGPR, PRx3, MR and SD. Four patients were withdrawn from CC-4047 due to PD, 1 patient stopped following a DVT having achieved MR and 1 patient withdrew due to recurrent neutropaenia. 5/6 patients received thalidomide as initial salvage therapy following CC4047 treatment failure. 1/6 patients received thalidomide as 3[rd] line salvage therapy. All patients had progressive disease at the time of thalidomide commencement. 3/6 patients received single agent thalidomide, 1/6 received thalidomide + dexamethasone and 2/6 received cyclophosphamide / thalidomide / dexamethasone. Thalidomide was received for a median of 58 weeks (range 5-196). 1/6 patients withdrew due to neuropathy. The median dose of thalidomide received was 100 mg/day. 1/6 patients achieved a VGPR, 2/6 achieved a PR and 1/6 achieved MR. The remaining 2/6 patients had no response to thalidomide. The overall response was therefore 66%. Duration of response to thalidomide was a median 91 weeks (range 26-196). *Conclusion*. Patients progressing on CC-4047 may respond to the introduction of thalidomide with durable responses.

PO-651

THE PRESENCE OF T CELL CLONES IS INCREASED AFTER THALIDOMIDE MAINTENANCE THERAPY (ALLG-MM6) AND IS ASSOCIATED WITH AN IMPROVED SURVIVAL

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We have previously reported that clones of $CD8^+$ $CD57^+$ $CD28^-$ perforin+ T cells are present in 59% of patients with multiple myeloma (MM) and are associated with a prolonged survival. The aim of this study was to confirm this observation with a different patient cohort and to investigate a possible relationship between the generation of T cell clones and the immunomodulatory agent, thalidomide. ALLG -MM6 is a randomised trial of maintenance therapy ± thalidomide (max 200 mg/day) post autologous stem cell transplant in 243 patients. Patients were >18y, non-progressive, <12 months prior therapy, ECOG <3 and serum creatinine <2 mg/dL. At 2y median follow-up both progression free survival (PFS) and overall survival (OS) were significantly prolonged in the thalidomide arm (p=0.0005 and p=0.021 respectively) (Blood 2006; 108:22a). A total of 221 blood samples from 120 of these patients (104 pre-transplant and 117 after maintenance) were available for analysis of TCR Vb expression by a 4-colour flow cytometry assay that covered approximately 70% of the TCR Vb repertoire (Beta Mark). Controls were 42 age-matched normals. Clonality of TCR Vb expansions was verified in 6 samples by CDR3 length analysis and direct sequencing. 93% of all clones were $CD8^+$ and all 24 TCR Vb families studied were represented. T cell clones were detected in 48% of patients pre-transplant, 68% after 8 months maintenance (76% in thalidomide and 60% in control arm) and 57% after 12 months maintenance. The incidence of patients with multiple clones was significantly greater after thalidomide (49%) compared to the control arm (23%) (χ=6.8; p=0.01). There was a trend for patients to develop new T cell clones after thalidomide (52%) compared with the control arm (40%). The presence of T cell clones

regardless of therapy was associated with a longer PFS (median = 32.1 vs 24.1 months; χ= 4.2; p=0.04) and a trend to a longer OS. Median PFS in the thalidomide arm was 40.1 months for patients with clones and 28.3 months for patients without clones while in the no thalidomide arm, median PFS was 15.9 months without and 21.3 months with T cell clones (χ=9.5; p=0.002). These observations confirm the prognostic significance of circulating T cell clones and demonstrate their enhanced impact after thalidomide therapy.

PO-652

MELPHALAN,PREDNISONE,THALIDOMIDE AND DEFIBROTIDE IN ADVANCED MYELOMA

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Introduction. Defibrotide(DF)has demonstrated increased responsiveness of Multiple Myeloma(MM)cells to cytotoxic agents both *in vitro* and *in vivo*. A multicenter, phase I/II trial in relapsed and relapsed/refractory advanced MM has been conducted to define the Maximun-Tolerated-Dose (MTD), the overall safety and efficacy of the combination melphalan-prednisone-thalidomide-DF(MPTD). *Material and Methods*. Between March and November 2006, 24 patients (median age 69 years) were enrolled. MPTD regimen included 6 cycles of oral melphalan 0,25 mg/Kg) and prednisone (1,5 mg/kg) D 1-4, thalidomide(50-100 mg/day)continuously and DF at 3 dose levels (17 mg/Kg i.v. or 2.4g p.o. D 1-4, 1.6 g p.o. D 5-35; 34 mg/Kg i.v. or 4.8 g p.o. D 1-4, 3.2g p.o. D 5-35; 51 mg/Kg i.v. or 7.2g p.o. D 1-4, 4.8g p.o. D 5-35). *Results*. Nineteen patients who received at least one cycle of MPTD were evaluable for toxicity and response. We observed 1 DLT (grade 3 ileus, later deemed unrelated to DF) in the 1st level, 1 acute myocardial infarction (AMI) in the 2nd and none in the 3rd. MTD was therefore not reached in any cohort. Hematological toxicities ≥grade 3 included neutropenia(47%), thrombocytopenia(10%), anemia(21%); non-hematological toxicities ≥grade 3 were observed in <5%. No DVTs were reported. Three patients stopped experimental drugs because of adverse events: AMI (because of other anticoagulation treatment), ileus (because of the finding of amyloidosis AL and progression), and persistent G4 neutropenia. No significant bleeding has been reported. After a median of 3 cycles, partial response rate was 41% including 16% very good partial response. No significant difference in response rate was noted between the 3 DF levels but follow up remains short. Pharmacokinetic studies and analysis of surrogates are ongoing. *Conclusions*. MPTD is feasible and effective salvage regimen with a high proportion of responses in relapsed and relapsed/refractory MM. A protective role of DF on thrombosis is suggested by this study, with no significant attributable toxicity seen. The convenience of an oral regimen without the need for therapeutic monitoring of anticoagulation is compelling and further studies are warranted. An update of these data will be presented.

PO-653

THAL MONOTHERAPY INDIVIDUAL PATIENT ANALYSIS: RESPONSE

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Introduction. Thalidomide has proven efficacy as monotherapy in relapsed/refractory Multiple Myeloma (MM). However, to date several questions regarding response and survival after thalidomide treatment remain unanswered. The objective of this analysis was to address the question of response and survival utilizing individual patient data from published studies. *Methods.* Authors of published studies (Glasmacher *et al.*, Br J Haematol. 2006; 132: 584-93) evaluating thalidomide monotherapy in patients with relapsed/refractory MM were asked to send individual patient data including parameters on dose of thalidomide, response based on reduction of paraprotein and toxicity. *Results*. So far, data from 827 patients have been collected with 509 of these are evaluable for this analysis. Of these patients, 52% had relapsed disease, 48% were refractory. The median number of previous treatment lines was 2 (interquartile range, IQR, 2-3). The best response to thalidomide was complete remission in 4% (95% CI 2.6-6) and partial remission in 37% (95% CI 33-41) of patients (CR+PR 41%). This was achieved after a median of 3 months (IQR 2-5). In the first 3 months patients received a median cumulative dose of 18000 mg (IQR 9000-32775 mg) and took a median daily dose of 200 mg/d (IQR 100-400 mg/d). Interestingly, no difference regarding the cumulative dose after 3 months (responders 19500 mg, IQR 9000-33750, non-responders 18000 mg, IQR 9000-32900; p=0.58) or the daily dose at 3 months (responders 200 mg/d, IQR 100-400, non-responders 300 mg/d, IQR 100-400; p=0.15) could be detected. The Kaplan-Meier estimated median event-free survival (EFS) was 11 months (95% CI 10-12), and overall survival (OS) 24 months (95% CI 20-29). There was a clear benefit of response to thalidomide: Patients with response had a median EFS of 17 months (95% CI 14-19) versus 7 months (95% CI 6-9) without response (p<0.001). Similarly, the OS was 29 months (95% CI 26-31) for patients with response but only 15 months (95% CI 12-19) without response (p<0.001). *Conclusions*. This first analysis of our large data base of patients with thalidomide monotherapy confirms good efficacy, shows no dose-response relationship and demonstrates a clear survival benefit for patients who achieved a response to treatment.

PO-654

CTD TREATMENT OF MYELOMA- A SINGLE INSTITUTION'S EXPERIENCE

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Introduction. Many studies have demonstrated the effectiveness of thalidomide in the treatment of patients with relapsed/refractory myeloma as a single agent and in combination with other treatments, such as dexamethasone. Further studies have shown better results for its use as first line therapy. There are a few published studies on the use of combinations of CTD (cyclophosphamide, thalidomide, and dexamethasone) and these have shown a higher response rate compared to thalidomide/dexamthasone combinations in both relapsed/refractory myeloma and as first line therapy. *Methods.* A retrospective analysis of patients receiving CTD between 2004 and 2006 was performed. The regime used consisted of monthly cycles of weekly oral cyclophosphamide 500 mg, daily thalidomide 100-200 mg nocte according to response and pulsed dexamethasone 40 mg daily days 1-4 repeated on days 15-18 in first cycle only. Thrombophrophylaxis was used after the first three cases were treated. *Results*. 23 patients were treated with CTD, 7 as first line therapy and 16 with relapsed or resistant disease. 4 had undergone prior autologous stem cell transplant. The mean age was 66.09 years (range 49-80). 17/23 (73.9%) had detectable paraprotein (IgG or IgA), 5/23 (21.74%) had light chain myeloma and 1/23 (4%) was non-secretory. The overall response rate was 87%. Complete response was observed in 9% (2/23), near complete response in 22% (5/23), partial response in 39% (9/23) and minimal response in 17% (4/23). The median number of courses given was 4 (range 2-10). At the time of analysis 11 patients showed no disease progression, 1-17 months after completing treatment. 12 had progressed with a mean progression free survival of 8 months (range 1-18 months). Two out of the first three patients treated developed DVT and because of this thromboprophylaxis was used in the following 20 patients. No other grade 3-4 adverse events were observed. *Conclusion*. CTD is effective and generally well tolerated treatment for myeloma with a very good response rate and compares favourably with other conventional treatment.

PO-655

THE *DTZ* PALLIATIVE REGIMEN FOR RELAPSED/REFRACTORY MM

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Introduction. We previously reported that treatment of 26 patients with relapsed/refractory multiple myeloma (MM) with a regimen combining low dose Dex and Thal plus higher frequency zoledronic acid (Zol) - *dtZ* - was associated with a reasonably good initial response rate (RR) of 61.6% (Xth International MM Workshop 2005, Sydney). *Materials and Methods*. The same cohort of patients was treated with intensive *dtZ* cycles until maximum response. Thereafter, responses were maintained using monthly pulses of Dex 20 mg OM on D1 to D4; Thal 50 mg ON; and monthly to 4-monthly Zol 4 mg. Event free survival (EFS) was the primary end point. We now report the long term (8 to 58 months; median 25 months) follow-up of these patients. *Results*. The overall RR had increased to 73.1% (19 patients) and there were 2 (7.7%) patients who achieved immunofixation (IF)-negative complete remission (CR), and 3 (11.5%) who achieved near CR (nCR). Grade 1 or 2 toxicities included chest infections [9], peripheral neuropathy [4], mild hypocalcemia [3], mild hepatitis [2], and osteonecrosis of the jaw (ONJ) [1]. One patient developed a grade 3 bronchopneumonia. There were no grade 4 toxicities, deep venous thromboses or pulmonary embolism. Patients also demonstrated improvements in quality of life, pain scores, renal function, β2-microglobulin, hypercalcemia, pancytopenia, hypoalbuminemia, and normal immunoglobulins. Of the responders, 13 (50.0%) patients who achieved partial response (PR) progressed and were further treated with a bortezomib-based salvage regimen. Importantly, all patients in CR or nCR did not progress. The median EFS was 14.0 months. All 7 (26.9%) patients who failed to respond to *dtZ* died. In addition, 8 (30.8%) patients who achieved PR and then progressed also died despite further salvage therapy; thus giving an overall survival rate of 42.3%. The median overall survival rate, which is an aggregate of *dtZ* and bortezomib-based salvage therapies, was 48.0 months. All deaths were attributable to advanced MM. *Conclusions*. We conclude that the *dtZ* regimen was effective and well tolerated in patients with relapsed/refractory MM. Since *dtZ* was associated with a better RR, CR/nCR rates, and EFS than Dex alone or melphalan/prednisolone, *dtZ* could be considered for use as a palliative regimen for patients with relapsed/refractory MM.

PO-656

PROGNOSTIC VALUE OF SELECTED CHROMOSOMAL ABNORMALITIES IN MULTIPLE MYELOMA PATIENTS TREATED BY THALIDOMIDE

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Introduction. The aim of this prospective study is to investigate if Thalidomide is able to antagonize the impact of cytogenetic negative prognostic markers. We have focused on four chromosomal aberrations known as negative prognostic factors in multiple myeloma (MM) treated by conventional or myeloablastive treatment: deletion of 13q14 (RB), deletion of 17p13 (p53), amplification of CKS1B gene (1q21) and translocation t(4;14). *Material and Methods.* For identification of malignant plasma cells in bone marrow samples, we use cytoplasmic immunoglobulin (cIg) labelling methodology. This method allows us to identify simultaneously monotypic plasma cells by monoclonal antibody fluorescence (anti-k or anti-l) and to detect chromosomal abnormalities by fluorescence *in situ* hybridization (cIg-FISH). Overall characteristics of up-to-date set of 24 patients (pts.): median age 65.0 years (range 48.3-83.3). 66% (16/24 pts.) in stage IIIA, 29% (7/24 pts.) in stage IIA and 1 patient in IIIB. 75% (18/24 pts.) were in first relapse; the others were in second relapse. *Preliminary Results*. Cytogenetic findings: Deletion of 13q14 was detected in 62% (13/21) pts., t(4;14) in 66% (14/21) pts., deletion of 17p13 in 41% (7/17) pts. and amplification of CKS1B gene in 63% (12/19) pts. Overall response (OR) was 83% (0% CR, 29% VGPR, 54% PR). OR was not significantly higher in pts. with or without cytogenetic findings for all aberration types. There were no difference when compared OS, TTP, PFS and DOR for groups with or without each aberration except deletion of RB gene or amplification of CKS1B: Average OS was 10,2±3,4 months vs. 6,6±2,2 months in patients with vs. without the RB deletion and 7,1±3,1 vs. 10,0±3,8 months for amplification of CKS1B. *Conclusions*. Despite of the observed trends in patients with CKS1B amplification or deletion of RB gene, we have found no significant difference when compared groups with or without of each aberration for TTP, DOR, PFS, OS and response rate. This should have been caused by small size of our sample. It is still possible that Thalidomide antagonizes the negative prognostic value of some cytogenetic findings in MM.

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PO-657

THAL MONOTHERAPY INDIVIDUAL PATIENT ANALYSIS: DURATION

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Introduction. Although there is proven efficacy of thalidomide monotherapy in relapsed/refractory Multiple Myeloma (MM), the question of optimal duration of treatment remains unanswered. To address this we analysed individual patient data from published studies with a focus on survival outcome. *Methods.* Authors of published studies evaluating thalidomide monotherapy in patients with relapsed/refractory MM were asked to send individual patient data of their study. To assess the influence of duration of treatment, this analysis included patients who continued on thalidomide as long as they tolerated it and those who remained on drug until the study they were enrolled in was complete. Patients who discontinued treatment because of progression or early death were excluded. *Results*. Of 509 patients evaluable for preliminary analysis, 30 patients had planned completion of a trial and 124 patients discontinued because of adverse events or reasons other than progression. Thus, 154 patients were included in this analysis. Forty-seven percent of patients had refractory disease, the median number of previous lines of therapy was 3 (interquartile range, IQR, 2-4). The response rate in this population was 53% after a median of 3 months (IQR 3-6). Although there was no difference in cumulative dose or daily dose at 3 months, responders had a significantly longer duration of treatment (median 9 months, IQR 4-15) than non-responders (median 4 months, IQR 2-8; $p<0.001$). There was a clear effect on survival: if patients managed to take thalidomide 10 months or longer, event-free survival (EFS) was 31 months (95% CI 18-43) and overall survival (OS) not reached ($p<0.001$). In contrast, patients with a shorter duration of treatment (< 10 months) had an EFS of 12 months (95% CI 9-14) and OS 28 months (95% CI 22-33; $p<0.001$; median follow-up 26 months 95% CI 20-33). *Conclusions*. A clear survival benefit of longer duration of thalidomide treatment in the setting of relapsed/refractory MM could be demonstrated in this analysis of individual patient data.

PO-658

RAD (REVLIMID, ADRIAMYCIN, DEX) IS A NEW TREATMENT REGIMEN FOR RELAPSED MULTIPLE MYELOMA

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Introduction. Lenalidomide (Revlimid) has repetitively been associated with reliable anti-myeloma effect in clinical trials. It induced significant objective response rates both alone or in combination with dexamethasone in relapsed/refractory disease. We hypothesized that addition of lenalidomide (R) to adriamycin (A) and dexamethasone (D; RAD) might further enhance efficacy. *Materials and methods*. A Fibonacci-like scheme was chosen for dose escalation. Patients after no more than three preceding therapeutic regimens were eligible when having measurable disease parameters and normal organ function and blood counts. Exclusion criteria were previous administration of R and any thromboembolic complications in the patient's history. Aspirin 100 mg/day was mandatory for prevention of thrombotic events. Objectives were to determine the maximum tolerated dose (MTD) of R in the combination protocol and its tolerability and efficacy when given for six cycles. *Results*. On the fourth dose level two cases of non-febrile neutropenia occurred, thus the protocol was amended for G-CSF support (Pegfilgrastim [Neulasta] 6 mg, day 6). Lenalidomide 25 mg/day (d 1-21) in combination with A 9 mg/m^2 (d 1-4 continuous infusion); and D 40 mg, days 1-4, and 17-20 was found to be the MTD. By this update of the still ongoing trial, 61 subjects with a median age of 64 years (range, 44-77) have been enrolled, 47 of whom had received autologous and and an additional six autologous followed by allogeneic stem cell transplants. Forty-eight pts are currently evaluable for toxicity and 40 for response. Incidence of grades 3 and 4 toxicity was very acceptable with six episodes of asymptomatic neutropenia/thrombocytopenia, four infectious episodes and one case of deep venous thrombosis. No treatment-related mortality was observed. Overall response rate (paraprotein reduction of at least 50%) is 83% including three patients with immunofixation-negative CR. Neither somnolence, constipation, nor neuropathy occurred. *Conclusions*. RAD chemotherapy significantly contributes to the therapeutic armamentarium in intensively pretreated myeloma pts combining promising response with a favourable toxicity profile.

PO-659

ELIGIBILITY FOR LEN/DEX TREATMENT IN MM

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Introduction. In January 2007, an international group of multiple myeloma (MM) specialists reached a consensus on practical recommendations regarding the management of lenalidomide treatment (in combination with dexamethasone) in patients with relapsed/refractory MM, who have received at least one prior therapy. This communication deals with patient-eligibility for treatment with lenalidomide and dexamethasone. *Methods.* A moderated round table discussion. *Results*. Response rates of combination therapy with lenalidomide/dexamethasone (Len/Dex) in the setting of relapsed/refractory MM are approximately 60% and lead to a survival advantage over treatment with dexamethasone alone. This effect was seen in all groups of patients, including patients of all age groups, independent of disease stage, duration of disease prior to Len/Dex treatment, ECOG performance status, cytogenetics including t(4;14) and del(13q), level of β2-microglobulin and renal or hepatic impairment. Of note, patients with a creatinine >2.5 ng/dL had not been included in clinical trials. Response to Len/Dex was superior to Dex alone, independently of the type of prior therapy (bortezomib, thalidomide, or prior transplant). It is important to note that patients refractory to thalidomide still respond to Len/Dex, although the overall response might be less than in patients not refractory to thalidomide (not significant). Patients with only 1 previous line of therapy had a greater survival advantage than patients with more than 1 previous line of therapy. There is some evidence that a lower dose of Dex may result in less toxicity (Rajkumar *et al.*, Blood 2006; 108:799), but efficacy data are not yet available. However, the dexamethasone dose may be adjusted in elderly, fragile patients >75 years. Also, as lenalidomide is mainly renally excreted, dose reduction of lenalidomide depending on the severity of renal impairment will be provided. Adjustments for mild to moderate hepatic dysfunction or potential drug-interactions are not required. *Conclusion*. Len/Dex is recommended for patients with relapsed/refractory MM regardless of baseline factors.

PO-660

PHASE 2 STUDY OF REV/VEL/DEX IN RELAPSED/REFRACTORY MM

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Introduction. Bortezomib (VELCADE®, Vel) and lenalidomide (Revlimid(r), Rev) are highly effective in multiple myeloma (MM). Preclinical studies show Rev sensitizes MM cells to Vel and dexamethasone (Dex). In a phase 1 study, Rev/Vel (±Dex 20-40 mg) was well tolerated (MTD: 1.0 mg/m^2/15 mg) and resulted in a response rate (CR+PR+MR) of 58% in relapsed and/or refractory MM patients. The aim of this multi-center phase 2 study is to evaluate the efficacy and safety of Rev/Vel/Dex at the phase-1 MTD in up to 64 relapsed and/or refractory MM patients. *Materials and methods*. Patients with 1-3 prior lines of therapy received up to eight 21-day cycles of Vel 1.0 mg/m^2, days 1, 4, 8, 11, Rev 15 mg, days 1-14, and Dex 40 mg (cycles 1-4)/20 mg (cycles 5-8), days of/after Vel dosing. Patients received required concomitant antithrombotic and antiviral prophylaxis. Toxicities were assessed using NCI CTCAE v3.0. *Results*. Twelve patients (9 men, 3 women) have been enrolled to date, median age of 67 years (range: 51-83), including 8 with relapsed/4 with relapsed and refractory MM. Median number of prior lines of therapy is 2 (range: 1-3); prior therapies include stem cell transplant (SCT) in 3 patients, Vel in 9, Rev in 1, and thalidomide in 11. Dose reductions have been required for Rev in 3 patients, and for Dex in 7; no dose reductions have been required for Vel. No significant (Grade ≥3) fatigue, DVT, or peripheral neuropathy has been seen. There have been two episodes of Grade 3 atrial fibrillation, reversible with cardiac medication, that were possibly attributed to the combination of Rev and Dex, and that prompted Dex dose reduction. In 10 evaluable patients (completed ≥1 treatment cycle), overall response rate (CR+PR+MR, modified EBMT criteria) is currently 50%, including 4 PR/1 MR. *Conclusions*. Rev/Vel/Dex is active in patients with relapsed and/or refractory MM, including patients who have received prior Rev, Vel, thalidomide, and SCT. Dex dose reduction has been required in the majority of patients, but the combination has been otherwise well tolerated. Accrual is continuing. Rev/Vel/Dex is also being investigated in front-line patients in a phase 1/2 study.

PO-661

A RANDOMIZED PHASE 3 TRIAL OF ORAL LENALIDOMIDE AND DEXAMETHASONE VERSUS PLACEBO AND DEXAMETHASONE IN PATIENTS WITH RELAPSED OR REFRACTORY MULTIPLE MYELOMA. FINAL ANALYSIS OF MM010 STUDY

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Introduction. Thalidomide in combination with dexamethasone has demonstrated enhanced activity in patients with refractory or relapsed multiple myeloma. However, treatment with thalidomide is associated with a number of toxicities, including sedation, fatigue, constipation, rash, deep vein thrombosis and peripheral neuropathy, that often require dose reduction and even discontinuation. Lenalidomide is an oral derivative that lacks many of the toxicities of thalidomide. Previous studies have shown that lenalidomide can overcome disease resistance not only to conventional treatment but also to thalidomide. Purpose of the study was prospective randomized comparison of the combination of lenalidomide plus dexamethasone versus placebo plus dexamethasone in patients with refractory or relapsed multiple myeloma. *Materials and Methods.* Patients with relapsed or refractory disease where randomized to 25 mg per day oral lenalidomide on days 1-21 or placebo plus 40mg oral dexamethasone on days 1-4, 9-12 and 17-20 of each 28-day cycle (for

the first four cycles). After four cycles, dexamethasone was administered only on days 1-4. Patients continued on treatment until disease progression or unacceptable toxicity. Patients were stratified by baseline β2-microglobulin levels (<2.5 mg/L vs ≥2.5 mg/L), prior transplantation (none vs ≥1) and number of prior antimyeloma regimens (1 or ≥2). Essential inclusion criteria were the presence of measurable disease (serum monoclonal protein ≥0.5 g/dL or urine monoclonal protein ≥0.2g/day), an ECOG performance status of at least 2 and the absence of disease progression during high-dose dexamethasone containing therapy (total monthly dose of dexamethasone >200 mg). Primary study endpoint was time to progression and secondary endpoints were overall survival, response rate and safety. Toxicity was graded according to the NCI CTC (version 2); response and progression were evaluated according to the EBMT criteria. *Results*. Of 351 patients, 175 were randomized to lenalidomide/dexamethasone (len/dexa) and 176 to placebo dexamethasone (placebo/dexa). Patients had received a median of 2 prior regimens and average time form initial diagnosis was more than 4 years; about one-third of the patients had previously received thalidomide. Treatment arms were well balanced for all baseline characteristics. With a median follow-up of 16 months, time to progression was significantly improved in the len/dexa arm compared with placebo/dexa (median of 11.3 versus 4.7 months; $p<0.001$) and this superiority was maintained for all stratified groups. Moreover, the combination of len/dexa resulted to superior time to progression among thalidomide pretreated patients (median 8.4 vs 4.6 months for the placebo/dexamethasone arm, $p<0.001$). Previous resistance to thalidomide did not affect time to progression in patients treated with len/dexa. Similarly among patients with no prior exposure to thalidomide, median time to progression was 13.7 months for the len/dex arm vs 5.3 for the placebo/dexa arm ($p<0.001$). Len/dexa induced at least partial response in 59.1% of patients (CR rate was 15.3%) compared with 24% of patients in the placebo/dexa arm (CR rate was 3.4%) ($p<0.001$). Median time to first response was 9 and 7 weeks respectively in the len/dexa and placebo/dexa group. Moreover, overall survival was significantly improved with len/dexa (HR 1.524, $p=0.031$). Neutropenia was the most frequent grade 3 or higher adverse event in the len/dexa arm (29%), whereas a higher incidence of deep vein thrombosis and pulmonary embolism was associated with len/dexa. *Conclusions*. Len/dexa was well-tolerated and significantly more effective than high-dose dexamethasone alone in patients with relapsed or refractory multiple myeloma, resulting in a two-fold increase of both time to progression and response rate and an overall survival advantage. This benefit is still obvious among patients who were previously treated with thalidomide. The primary toxicities were hematologic and therefore predictable and manageable with dose adjustment. Lenalidomide did not induce peripheral neuropathy. Although there was a higher incidence of thromboembolic events in the len/dexa arm the overall incidence of these events were low and prophylactic anticoagulation may prevent this incidence. Studies are ongoing in patients with newly diagnosed multiple myeloma and in the minimal residual disease setting as consolidation after high dose therapy and autologous stem cell transplantation.

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EFFECT OF LEN/DEX IN MM DESPITE THAL RESISTANCE

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Introduction. Lenalidomide (Len), a thalidomide (Thal) analog is effective against multiple myeloma (MM). In 2 Phase III trials, Len/dexamethasone (Dex) induced a significantly higher overall response rate (OR) and complete remission rate (CR), as well as longer time-to-progression (TTP) compared with Dex alone. This analysis assessed whether Len/Dex remains effective in the presence of Thal resistance. *Methods.* We evaluated 704 patients (pts) from 2 Phase III trials who had relapsed/refractory MM, who received either Len/Dex or Dex. In the Thal-naïve group, pts were never exposed to Thal. In the Thal-exposed group, pts received Thal but could have had any response (CR, PR, MR, SD or PD). Pts in the Thal-exposed group were divided into 3 subgroups according to their degree of Thal resistance. In the R1 (Progressed-on-Thal) group, pts had progressive MM while on Thal but could have had prior CR or PR or SD. In the R2 (Never-Responded) group, pts never responded to Thal but could have had SD. In the R3 (Thal-Worst) group, pts never responded to Thal, not even SD. *Results*. In all subgroups, Len/Dex was more effective than Dex in OR and TTP ($p<0.01$). The longest TTP was observed for the Thal-naïve group. The OR and TTP were similar among the resistant (R1-R3) groups. Even the group with strongest resistance to Thal (R3) benefited from Len/Dex with prolonged TTP and OR ($p<0.01$). *Conclusion*. Len/Dex was more effective than Dex in patients with heavily-treated, relapsed/refractory MM despite prior Thal resistance. Future prospective trials are needed to explore potential cross-resistance between Len and Thal.

Table 1.

	Thal Naïve		*Thal Exposed*		*P Value*	*R1 Progressed on Thal*		*R2 Never Responded*		*R3 Thal - Worst*	
	n=430		n = 274		LD vs. LD	n=116		n = 70		n=44	
	LD	D	LD	D		LD	D	LD	D	LD	D
n	227	203	127	147		54	62	31	39	20	24
OR	64	28	60	18	ns	43	11	45	15	50	21
TTP	61	20	36	20	<0.01	30	16	30	16	31	16

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EFFECT OF LEN/DEX IN MM IN DIFFERENT AGE GROUPS

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Introduction. Elderly patients with relapsed or refractory multiple myeloma (MM) have limited therapeutic options due to co-morbidities and poor tolerability to chemotherapy. Age can also be an independent prognostic factor. Lenalidomide (Len), an analog of thalidomide (Thal) is a novel, oral, immunomodulatory agent that is effective against multiple myeloma (MM). In 2 prospective, randomized, double-blind, placebo-controlled Phase III trials, Len with dexamethasone (Dex) induced a significantly higher overall response rate (OR) and complete remission rate (CR), as well as longer time-to-progression (TTP) in comparison with Dex alone. Here, we investigate the efficacy and safety of Len/Dex in different age groups. *Methods.* We evaluated the pooled results of 704 patients from both randomized trials (MM009, MM010) who had relapsed or refractory MM, without prior resistance to Dex, who received either Len (25 mg daily for 3 weeks every 4 weeks) plus Dex (40 mg on days 1-4, 9-12, 17-20 every 4 weeks for 4 cycles) or placebo plus Dex. For this analysis, patients were divided into 3 age groups <65 years (n=390), 65-75 years (n=246), and >75 years (n=68). *Results*. OR was significantly higher for patients treated with Len/Dex compared with Dex for all 3 age groups (Table).

Table.

	Age <65 years		Age 65-75 years		Age >75 years	
	Len/Dex	Dex	Len/Dex	Dex	Len/Dex	Dex
Response,%	n=192	n=198	n=125	n=121	n=36	n=32
CR	16.7	2.5	9.6	0.8	25.0	3.1
RR	26.6	9.1	31.2	9.9	22.2	6.3
PR	18.2	10.6	17.6	10.7	16.7	12.5
OR	61.5	22.2	58.4	21.4	63.9	29.9
SD	28.6	57.1	33.6	59.5	27.8	43.8
PD	2.6	16.2	1.6	9.9	2.8	18.8
PFS, median weeks	46.0	20.1	47.9	20.1	57.0	18.1
Grade 3/4 adverse events,%						
Neutropenia	35.4	4.5	39.2	1.7	13.9	16.1
Thrombocytopenia	9.4	6.6	19.2	5.8	22.2	3.2
Anemia	8.9	2.5	12.8	9.1	11.1	6.5
Febrile neutropenia	1.6	0.0	3.2	0.0	2.8	0.0

Len/Dex response was similar for the 3 age groups with an OR of 61.5%, 58.4%, and 63.9% for patients aged <65, 65-75, and >75 years, respectively. Progression-free survival (PFS) was higher for patients treated with Len/Dex compared with Dex for all 3 age groups (Table). PFS in the Len/Dex arm was similar for the 3 age groups with a median PFS of 46, 47.9, and 57 weeks for patients aged <65, 65-75, and >75 years, respectively. Grade 3/4 cytopenia was more common with Len/Dex compared with Dex alone for all 3 age groups (Table). Age did not affect adverse events. *Conclusion*. Len/Dex was more effective than Dex alone for the treatment of relapsed or refractory MM, irrespective of age. Age did not affect the incidence of adverse events in these patients. An update of overall survival based upon age cohorts will be presented.

PO-664

NECESSITY OF ADAPTATION OF LENALIDOMIDE IN CASE OF ACUTE RENAL FAILURE: A CASE REPORT

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Introduction. lenalidomide is a new molecule used for the treatment of multiple myeloma. Even if the necessity of dose adaptation in case of renal failure is probably relevant, at the present time it is not completely established. Patient: a 50-year-old male patient presented with an IgA kappa stage III myeloma (M component level at 70 g/L) with multiple bone lesions and 2 pejorative prognostic factors as del 13 positivity and beta2-microglobulinia > 3 mg/L. He received VAD induction treatment (vincristin: 0,4 mg/D D1 to D4 ; adriamycin: 9 mg/m^2/D D1 to D4 ; dexamethasone: 40 mg/D D1 to D4 ; D9 to D12; D17 to D21 [5 cycles]) followed by high dose therapy with melphalan (200 mg/m^2) supported by autologous stem cell transplantation in October 2004. This patient considered as CR, relapsed 18 months after graft with increased M component. A salvage therapy with thalidomide (100 mg/D) / dexamethasone (40 mg/D) D1 to D4 every month was ineffective. A partial response (>50%) was reached after 6 cycles of bortezomib (1.3 mg/m^2/D) D1, 4, 8, 11. An impairment of renal function (serum creatinine level: 600 micromol/L) following the intake of NSAI for lower limbs pain required hospitalization. No other hematological abnormality was found at this time. While the renal failure was worsened (and serum creatinine level still at 600 micromol/L), the patient relapsed and lenalidomide (25 mg/D) D1 to D21 every month associated with dexamethasone (40 mg/D) D1 to D4 every month was initiated with dialysis (metabolic trouble). *Results*. eight days later, the patient presented a febrile grade 4 pancytopenia complicated by pneumonia with hypoxemia. He died 48 hours later from a septic shock. Taking into account the clinical trials results of lenalidomide treatment, grade 3-4 thrombocytopenia is noticed in patients with renal failure as well as WBC decreased (with no grade 3-4 neutropenia). For chronological reason, a causal role of lenalidomide cannot be excluded in the occurrence of hematological abnormalities in this patient despite dialysis. *Conclusion*. in case of renal failure lenalidomide dose reduction in accordance with the creatinine clearance should be considered.

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SURVIVAL FROM RELAPSE AND THE INFLUENCE OF THERAPY

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Aims. Analysis of survival from relapse *Methods.* 1372 of 2528 patients (54%) receiving melphalan (845 patients), ABCM (1622 patients) or cyclophosphamide (61 patients) based conventional dose chemotherapy in the MRC trials between 1980 and 1997 achieved a stable plateau phase. With a minimum follow-up of 7.5 years analysis of trial files was made for course of disease and its relationship to therapy and prognostic factors. *Results*. 1372 patients achieving plateau phase had an overall median survival of 3.9 years (95% confidence interval 3.7-4.0). Duration of plateau phase (<1 yrs, 1-3 yrs and >3 yrs) was associated with median survivals from relapse of 11, 17 and 21 months, respectively (χ^2=44.73, p<0.0001). Response to initial therapy did not influence survival from relapse (p=0.29). Skeletal disease, renal disease, high percentage bone marrow plasma cells and beta2microglobulin at diagnosis were associated with poor survival from relapse (p<0.0001). 225 patients (16%) survived >7.5 years; of these 126 (56%) patients achieved a second plateau phase and, having relapsed a second time, 47 (37%) achieved a third plateau. The mean durations of first, second and third plateaus were 4.8, 2 and 1 years respectively. Median survival from relapse, for the 1151 patients who had a recorded date of relapse, was 1.2 years (95% confidence interval 1.1-1.4 years). In 639 of these patients the nature of second line therapy (non-randomised) was known and was associated with only minor differences in survival from relapse: ABCM (182 patients), melphalan (224 patients), VAD (103 patients) and cyclophosphamide (130 patients) based therapies. In 247 patients the treatment used as first line therapy was reintroduced at relapse and survival from relapse was better for these patients when compared to the 392 patients who received second line therapy different to that used for first line (χ^2=8.03, p=0.005); this was still the case when the patients were stratified by β2-microglobulin line (χ^2=7.85, p=0.005). However, when the two second line treatment groups were stratified by duration of plateau there was only a borderline difference in survival from relapse (χ^2=4.45, p=0.04). *Conclusions*. Choice of second line conventional dose chemotherapy has little impact on survival post relapse.

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MODIFIED SURVIVAL DATA REDUCES THE DIVERSITY OF MYELOMA RELAPSE

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Many studies on refractory or relapsing multiple myeloma are uncontrolled phase 2 studies. Even when limiting the study populations to patients at first relapse the prognosis of the patients are variable. 1;2. The prognosis is worse in patients relapsing within the first year after high-dose melphalan with autologous stem cell support (ASCT). We believe that time to first relapse reflects the biology of the individual myeloma patient. From our population of 255 patient who have past one or two ASCT as part of initial treatment we have compared the overall survival (OS) and event free survival (EFS(II)) after the first relapse in 134 of the patients with the EFS(I) after their initial ASCT. OS is significantly shorter in patients relapsing within the first year compared to later relapsing patients (OS: 11,8 vs. 30.0 months, p<0.0015). However, when using Kaplan Meyer analysis of patient survival divided by the individual patients time-to-first relapse the difference between patients early or later relapse is no longer significant (p>0.15) in this patient population mainly treated with conventional chemotherapy at relapse. EFS(II) was 47% of EFS(I) with less than 7% of patients experiencing longer EFS(II) than EFS(I). *Conclusion.* We suggest that phase 2 studies in relapse patients that are compared to historical controls uses this modified survival analysis to compensate for variations of the biology in the different populations.

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PO-667

LOW-DOSED THALIDOMIDE REGIMENS IN THERAPY OF RELAPSED OR REFRACTORY MULTIPLE MYELOMA

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Introduction. Thalidomide is a useful drug in therapy of refractory or relapsed multiple myeloma. Its effectivity can be higher when combined with other drugs. Recently, several studies have shown a combination of thalidomide, cyclophosphamide and dexamethasone to be effective, but high doses of thalidomide used lead to a serious toxicity. *Methods.* In our study, we performed a therapy with low doses of thalidomide, continual oral or pulsed intravenous cyclophosphamide and pulsed dexamethasone in a group of 97 patients with relapsed or refractory multiple myeloma. For younger patients up to 65 years we used a *CTD-junior* regimen, consisting of oral thalidomide 100-200 mg daily (according to tolerability), pulsed intravenous cyclophosphamide 800mg on day 1 and pulsed oral dexamethasone 40 mg on days 1-4 and 12-15, for every three weeks. For patients over the age of 65, the *CTD-senior* regimen was used: oral thalidomide 50-100 mg daily (according to tolerability), oral cyclophosphamide 50mg daily and pulsed dexamethasone 20 mg on days 1-4 and 15-18, for every four weeks. *Results*. From the group of 97 patients with progressive or resistant myeloma, 85 patients were evaluated: From 29 patients treated by the *CTD-junior* regimen we observed 52% of partial response (PR), 7% of near complete remission (nCR), 30% of minimal response (MR), 4% of stable disease (SD) and 7% of progression, the total response rate (CR+PR) was 59% (17 patients). From 56 patients with the *CTD-senior* regimen we observed 48% of PR, 7% of nCR, 2% of CR, 18% of MR, 16% of SD and 9% of progression, the total response rate was 57% (32 patients). Toxicity of both regimens was mild and well manageable, when weakness (27%), obstipation (30%), mild neuropathy of lower extremities (33%), respiratory infections (13%), leucopenia Gr. 2-3 (18%) and glycoregulation worsening (6%) occured most often. With LMWH prophylaxis, DVT occured only in 2% patients. *Conclusion*. These results show that low doses of thalidomide are still effective when combined with other drugs, such as cyclophosphamide and dexamethasone. This combination therapy is effective and safe also for patients with advanced and heavily pretreated multiple myeloma and the oral regimen is suitable also for elderly people.

GROUP 7: Treatment of newly diagnosed patients

PO-701

CYCLOPHOSPHAMIDE/DEXAMETHASONE AS INITIAL TREATMENT IN NEWLY DIAGNOSED SYMPTOMATIC MULTIPLE MYELOMA- A PILOT STUDY

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Aim. Historically VAD has been the most common induction regime used in the treatment myeloma but the contribution of the Vincristine and Doxorubicin in tumour burden reduction has not been clear. VAD is a relatively complicated therapy to administer and requires an indwelling catheter for the continuous infusion or daily visits for four days to administer the drugs with the shorter IV infusion regime. We wanted to have a simple, effective regimen that reduced toxicity, had less catheter-related infection but resulted in adequate harvests for high dose therapy and stem cell transplantation. A previous randomised trial from the Nordic Myeloma Study Group[1] had shown similar response rates when comparing Cy/Dec and VAD methods. Methods We analysed a group of 16 patients (9 males, 7 females) under the age of 69 with symptomatic myeloma. Patients were required to have bone marrow plasma cells greater than 10% and measurable disease defined as a serum monoclonal M protein (greater than 20 g/L) and/or elevation of serum free light chains. The patients had a median age of 55 years and no significant co-morbidities. There were 10 IgG, 2 IgA, 1 IgM and two light chain myeloma. Treatment Schedule All received three cycles of Cy/Dex (Cyclophosphamide 1000 mg/m^2 day 1 and Dexamethasone 40mg days 1-4 and 9-12; new cycle day 22 as initial treatment. The patients then received Cyclophosphamide 1000 mg/m^2 IV plus Filgrastim SC as mobilisation therapy followed by stem cell harvest. Patients then went forward for subsequent high dose Melphalan 200 mg/m^2 followed by autologous stem cell infusion (ASCT). All patients had bone marrow aspirates submitted for cytogenetic testing by FISH using an extensive cocktail of probes including T(4:14), T(14:16) and P53 mutation. *Results*. All 16 patients were judged to have responsive disease with post therapy plasma cell samples reduced to under 10% in all cases. Two of the patients (both over the age of 65, and one with prior DXT) failed to mobilise. The rest mobilised very well with an average CD34 dose of 8.2×10^6/kg so that enough stem cells were collected for a potential second transplant. Cytogenetic abnormalities were detected in 60% of the patients including two patients with T(4:14) translocation that is associated with a poor outlook post ASCT. 14 out of 16 (80%) of patients received an ASCT and major responses (CR plus PR three months after ASCT) were 84% in this group which is the same as was achieved in the Nordic Study. Surprisingly the two 4:14 patients are currently doing reasonably well 12 months and 13 months post ASCT. Toxicity was found to be acceptable apart from the man of 70 who had several episodes of neutropenia and one of herpetic oesophagitis. All transplants were trouble free with mortality of 0%. Three have subsequently gone on to have tandem transplants. One patient relapsed and died 14 months post transplant. *Conclusions*. In our unit historically around 87% of VAD treated patients have received ASCT compared to 80% in this Cy/Dex group but the numbers are small in the latter and did include a 70 year old man. The response rate in terms of plasma cell reduction was excellent. We conclude that Cy/Dex is an effective alternative to VAD and does not require any need for hospitalisation or central venous access for continuous infusion. It also has the potential of being able to be combined with such agents as Thalidomide and Bortezomib to produce higher CR rates in induction therapy.[2]

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PO-702

A PROSPECTIVE STUDY OF T(4;14)MULTIPLE MYELOMA

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The t(4;14)(p16.3;q32) leads to the ectopic expression of two potential oncogenes, the Multiple Myeloma Set Gene (MMSET) and the Fibroblast Growth Factor 3 (FGFR3), which has proven transforming activity. This translocation is found in 15% of patients with multiple myeloma (MM) and indicates a poor prognosis (median survival:30 months). To identify the clinico-biological features associated with this adverse prognosis, we prospectively studied a series of 63 patients with t(4;14) MM. Between March 2002 and July 2006, a t(4;14) was detected, using real time quantitative PCR of the IGH/MMSET and FGFR3 transcripts, in 63 patients. The clinico-biological data were analysed at diagnosis and at relapse. Response rate and time to progression (TTP) after successive lines of chemotherapy, and overall survival (OS) were evaluated. Among the 63 patients (54% male; median age: 55 years), 9 (14.2%) had a MGUS or smouldering myeloma and 54 (85.8%) had a symptomatic MM. At diagnosis, no clinical or radiological feature was significantly associated with t(4;14). IgA isotype was found in 28 (44%) and del(13) in 38 (76.2%) patients. However, only 46% (23/50) had elevated beta2m(>3 mg/L). Twelve patients (19%) had a t(4;14) without expression of FGFR3. Thirty-three patients (63.4%) received autologous stem cell transplantation (ASCT) with a 85% response rate. TTP after ASCT was 10.4 months. At relapse, aggressive features (plasmocytoma, cytopenias, acute renal failure, circulating plasma cells) were observed in 23% of cases. After second line chemotherapy, response rate was only 34.6% and TTP was 6.4 months. Median OS after ASCT and conventional chemotherapy was respectively 44 and 30 months, independently of FGFR3 expression. In this study, t(4;14) MM are characterized by a high response rate after ASCT contrasting with a short TTP and aggressive, chemoresistant relapses. Here, the OS, better than previously published, may be due to the use of novel drugs (Thalidomide, Bortezomib). This suggests the potential interest of a consolidation treatment after ASCT. Moreover, t(4;14) and FGFR3 expression are significantly detected in early stage of disease. Given that FGFR3 expression has no impact on OS, these results plaid for an early transforming role of FGFR3 subsequently leading to molecular additional events.

PO-703

MP VS MD AS FIRST LINE THERAPY IN MM PATIENTS NOT CANDIDATES FOR ASCT

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Aims. Initial therapy of MM usually consists of VAD or thalidomide and dexamethasone (TD) followed by ASCT. Many patients are not candidates for ASCT for various reasons *e.g.* high cost, lack of facilities, old age or co morbid conditions. Treatment options in these patients are MP or VAD, or TD. MP is standard therapy for such patients. One obvious disadvantage of VAD is continuous infusion and its complications. Daily use of thalidomide and its side effects are of concern. We feel for patients who are not candidates for ASCT combination of melphalan and dexamethasone (MD) may be a good oral alternative to MP or VAD if it results in comparable responses and time to responses. To assess efficacy of MP Vs MD we have undertaken a small randomized trial at our center from September 2003 to March 2005. *Material and Methods.* 45 newly diagnosed patients who were not willing to undergo ABMT, were enrolled onto this study and randomized to one of the treatment arm: arm (A) melphalan 8 mg/m^2 PO day 1-4, prednisone 60 mg/m^2 PO day 1-4 and arm (B) melphalan 8 mg/m^2 PO day 1-4, dexamethasone 40 mg PO day 1-4 and 9-12 every 4 weeks with PCP prophylaxis using Septran. Both the arms were balanced in terms of age, performance status, myeloma isotype and clinical stage. *Results*. Patients characteristics are shown in Table 1. Therapy was discontinued if; patient became intolerant, disease progressed, or 6 cycles were completed.. Results are shown in table 2. Toxicity- no significant difference in toxicities was noted in 2 arms. Three patient died during study period 1 in MP arm because of PD and 2 in MD arm. *Conclusion*. Present study has shown superior response rates with MD as compared to MP. In addition safety of MD in all the patients including those with renal failure has been demonstrated. This study justifies use of MD in selected population and warrants further randomized trials.

Table 1. Patients characteristics.

	MP (N=22)	MD (n=23)
Age	55 (38-83)	58 (40-72)
Sex M:F	13:9	17:6
Performance Status		
0-2	13(60%)	15 (65%)
3-4	09 (40%)	08 (35%)
Stage		
IIA	02 (9.1%)	01 (4.5%)
IIIA	12 (54.3%)	15 (65.5%)
IIIB	08 (36.6%)	07 (30%)

Table 2. Response summary.

Variable	*MP*	*MD*	*p value*
After 4 cycle			
CR	0	2(8.7%)	0.05
PR	4(18%)	13(56.3%)	0.012
Overall response	4(18%)	15(65%)	0.008
After 6 cycle			
CR	2(%)	8(34.8%)	0.02
PR	10(45.5%)	7(30.4%)	0.26
Overall response	12(54.5%)	15(65%)	0.05

PO-704

REACTION AFTER FIRST OF CHEMOTHERAPY VAD AS PROGNOSTIC FACTOR IN MYELOMA PATIENTS

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Background. High-dose melphalan with peripheral blood stem cell rescue represents today the standard therapy for young multiple myeloma (MM) patients. The the most frequent therapy inducing remission is chemotherapy according to VAD protocol (vincristine, adriamycin, dexamethason). *Aims.* The aim of this study was to determine prognostic factors and overal survival (OS) according to results of first VAD chemotherapy. The aim of this study was to find a criterion , which could determine the decision about the further treatment after first course of VAD. *Materials and metods.* The study group consisted of 86 MM patients (43M, 43F), median age 62.5 yr, range 35-71 yr; 12 at stage I, 20-II, 54-III acc. to D.S.; monoclonal protein IgG was in 46 patients, IgA-17, IgD-1, Bence Jones-21 and nonsecretory MM-1. Patients achieved following types of chemotherapy: - 69 pts received chemotherapy according to VAD with following auto-PBSCT; - 17 pts receives VAD as first line treatment We divided pts in 2 groups: First group: Pts, who are living; OS >1000 days (median OS-1000 days) Second group: Pts, wha are died; OS <1000 days All of the results have been statistically tested by using T-student test for the independent groups. For statistically significant results were $p<0.05$. *Results*. In both groups we detected the decrease in serum concentration monoclonal protein after first VAD chemotherapy. The decrease was significant larger in first group according to second group ($p=0.039$). After first course VAD chemotherapy we achieved following results: - a decrease in serum concentration of monoclonal protein >80% in 14 pts; - a decrease in serum concentration of monoclonal protein >50% in 46 pts; - the serum concentration of monoclonal protein didn't changed in 10 pts; - a increase in serum concentration monoclonal protein in 16 pts. We detected statistically significant differences in baseline concentration of monoclonal protein between investigated groups ($p=0.034$). We confirmed statistically significant difference in reduction of β2-microglobulin after first VAD course ($p=0.023$). There weren't statistically significant differences between: others parmeters of MM, OS and answer to first VAD chemotherapy.

PO-705

A PILOT STUDY TO EXPLORE THE TOLERABILITY AND EFFICACY OF THALIDOMIDE CONTAINING REGIMENS TO REDUCE TUMOUR CELL LOAD PRIOR TO AUTOLOGOUS TRANSPLANTATION IN MULTIPLE MYELOMA

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Aim. To explore the role of thalidomide in pre-transplant induction treatment in multiple myeloma. *Patients and methods.* Between Sept 2002 and Dec 2006, 48 patients with advanced, de-novo multiple myeloma (median age 58 years, median S. albumin 34g/L, and β-2-m 3.6 mg/L respectively) were entered into a multicentre, phase 2 study of pre-transplant induction treatment. The regimen included DTx3 (pulse dexamethasone 32mg TDS x 5d every 3 weeks PO and thalidomide 400 mg/d), followed by DT-PACEx2 (thalidomide 400 mg/d, dexamethasone 40 mg/d x 4 PO and cisplatin 10 mg/m^2/d, doxorubicin 10 mg/m^2/d, cyclophosphamide 400 mg/m^2/day, etoposide 4 0 mg/m^2/d as 4 day infusion administered 4 weeks apart, supported with G-CSF 10 microg/Kg/d). Thromboprophylaxis was warfarin (target INR 1.5-2.0) during DT and enoxaparin 40 mg/day (adjusted according to platelet count) during DT-PACE. Stem cells were harvested at recovery from the second cycle of DT-PACE in the first 27 patients, the remaining patients being harvested after first cycle of DT-PACE with an option to re-harvest after the second if the initial harvest was insufficient. Paraprotein and BJP responses were compared to a historical cohort of 58 patients treated with VAD (same dexamethasone dosing) and mobilised with cyclophosphamide 5G/m^2 and G-CSF 5microg/Kg. *Results*. 29/48 patients completed study treatment (3 yet to complete), 16 patients were withdrawn from study treatment at various stages. 26 had successful stem cell harvests. There were 2 deaths (1 sepsis, 1 haemorrhage). Tables 1 and 2 show the comparison of response to trial therapy and VAD + HD cyclophosphamide. Table 1. Median percent of baseline paraprotein or BJP levels remaining following therapy Therapy Para/BJP p DT X 3 11% VAD X 3 32% 0.009 DT PACE x 2 3% Cyclo 5G/m^2 25% 0.000 Table 2. Quality of response following therapy Therapy CR+VGPR p CR p DTX3 47% 3% VADX3 26% 0.259 12% 0.136 DTPACEx2 68% 32% HDCyclo 27% 0.000 9% 0.011 69 serious adverse events included 25 episodes of infection, 3 haemorrhage, 1 pulmonary embolus and 2 deaths and 1 late myelodysplasia. *Conclusion*. 1. Thalidomide dexamethasone combination achieves a greater depth of response than VAD. 2. The addition of DT-PACE improves the pre-transplant CR rate. 3. Thalidomide dexamethasone followed by DT-PACE is associated with tolerable but not insignificant toxicity.

PO-706

DOXORUBICIN, INTERMEDIATE DOSE OF DEXAMETHASONE, AND THALIDOMIDE (DDT) IN NEWLY DIAGNOSED AND ADVANCED MULTIPLE MYELOMA

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Introduction. We evaluated the combination of thalidomide, dexamethasone and doxorubicin administrated on an outpatient basis to untreated and refractory/progressive myeloma patients. *Material and Methods.* From January 2005 to January 2007 we treated 29 patients (17 untreated and 12 advanced patients) with DdT: standard doxorubicin 40 mg/m^2 intravenous on day 1, dexamethasone 40 mg orally on days 1-4, 9-12 and 17-20 on the first cycle (40 mg/d, days 1-4 every other cycle) and thalidomide was given at 50 mg/d orally increasing if tolerated by 50mg every week, not to exceed 400 mg/d. The DdT regimen was repeated every 4 weeks for a maximum of 6 cycles. Patients"65 years are proposed to high dose chemotherapy (HDC) and autologous transplantation (AT). *Results*. Eleven patients were men. The median age was 59 years (43-90). The median disease history is 7 months (2-62) and the median of previous treatments is 2 (1-6). Four patients had IgA M-protein, 18 IgG and 7 had light-chain. Hb<10,0 g/dL in 12 patients. Serum creatinine>2 mg/dL in 4 patients, only one in dialysis. Serum calcium>11,0 mg/dL in 2 patients. Urine M-component >200 mg/24h in 8 patients. Salmon-Durie stage II/III in 23 patients (79%). International Staging System: stage II/III in 14 patients (48%). Cytogenetic assessable in 26 patients and unfavourable in 11. All the patients were available for response and toxicity. The overall response rate (OR) was 58%: complete response=17%; very good partial response (VGPR)=7% and partial response (PR)=34%. One out of 12 patients with advanced disease achieved VGPR and 5 patients PR, with an OR of 50%. Four patients died: 3 of disease progression and 1 of lung cancer. Ten patients harvest PBSC with a median CD34$^+$ cells of 2,13×10^6/kg (0,017-5,98) and nine underwent AT. Neutropenia grade 3/4 and grade 3 infectious complications occurred in 20% and 10% of the patients. Peripheral neuropathy grade I/II occurred in 24% but one patient with grade III, and venous thromboembolic events in 7% of the patients. No treatment-related deaths were reported. *Conclusions*. DdT is effective in MM patients with advanced or untreated disease, with low toxicity and it is feasible in an outpatient basis. DdT could be an alternative to VAD as induction regimen in multiple myeloma.

PO-707

MELPHALAN, PREDNISONE AND THALIDOMIDE IN NEWLY DIAGNOSED MYELOMA

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Introduction. For patients older than 65 years of age oral melphalan and prednisone (MP) has remained the treatment of choice since 1960. In this multicentre randomised trial we compared oral MP plus thalidomide (MPT) with MP alone in 60 to 85 years old patients. *Material and Methods.* Patients with newly diagnosed multiple myeloma, age > 65 years, Durie and Salmon stage II or III, measurable disease were randomly assigned to receive oral MP for six four-week cycles plus thalidomide 100 mg per day continuously until relapse or progression (N=129) or MP alone (N=126). On December 2003, anti-thrombotic prophylaxis with enoxaparin at 40 mg per day for the first 4 MPT cycles was introduced. The primary objective was response rates and event-free survival (EFS). Secondary end points included overall survival (OS), prognostic factors and incidence of any grade 3-4 adverse event. *Results*. Patients treated in MPT arm experienced higher response rates and a longer event-free survival than patients who were not. The partial response rates were 76.0% for MPT and 47.6% for MP alone, including near complete response of 27.9% and 7.2%, respectively. The 2-year EFS rate was 54% in patients receiving MPT and 27% in patients receiving MP (p=0.0006). The 3-year OS rate was 80% in patients taking MPT and 64% in patients taking MP (p=0.19). In MPT arm, no significant differences in term of OS were observed between patients with high or low β2-microglobulin: at 18 months OS rate was 80% and 84%, respectively (HR 1.21, 95% CI 0.50-2.93, p=0.67). By contrast, in MP group, β2-microglobulin remained a prognostic factor: at 18 months OS rate was 71% for patients with high B2-microglobulin and 86% for patients with low B2-microglobulin (HR 2.8, 95% CI 1.26-6.18, p=0.01). Severe adverse events were 48% in MPT patients and 25% in MP patients (p=0.0002). In the MPT group, the most frequent grade 3-4 adverse events were haematological, thromboembolism, infections and peripheral neuropathy. The introduction of enoxaparin prophylaxis significantly reduced the incidence of thromboembolism from 20% to 3% (p=0.005). *Conclusion*. Oral MPT is superior to MP as first-line treatment for elderly patients with multiple myeloma. Longer follow-up is needed to assess the effect on overall survival. Oral MPT seems to cancel the adverse prognostic effect of high β2-microglobulin.

PO-708

CKS1B OVER-EXPRESSION IS A NEGATIVE PROGNOSTIC FACTOR FOR NEWLY DIAGNOSED MULTIPLE MYELOMA PATIENTS TREATED WITH THALIDOMIDE-DEXAMETHASONE AS UP-FRONT THERAPY

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Introduction. Up-front therapy of Multiple Myeloma (MM) patients with thalidomide and dexamethasone (thal-dex) combination improved both the rate of response after induction therapy and the survival after double autologous transplantation (Tx2). Nevertheless, possibly as a consequence of their specific molecular and kariotypic context, a small group of patients failed to respond to this therapy. With the aim to look for factors impacting the survival of MM patients treated with thal-dex, we investigated the prognostic role of CKS1B over-expression, t(4;14) and del(13). *Patients and Methods*. A total of 131 patients were analyzed. CKS1B expression was evaluated by Real-time RT-PCR. CKS1B over-expression was defined by an expression level higher than 2. The presence of t(4;14) was evaluated by IgH/MMSET fusion gene RT-PCR. Del(13) was assessed by FISH. Response to therapy was evaluated according to the Bladè criteria. The statistic analysis included Mann-Whitney test, logistic regression, Kaplan-Meyer and Cox analysis. *Results*. The overall probability to respond (≥ partial response) to up-front thal-dex therapy was 72%, as opposed to a 28% probability either to not respond or to progress. CKS1B over-expression did not correlate with the presence of t(4;14) or del(13). Median CKS1B expression value was significantly higher in non responders in comparison with patients who attained at least a partial response: 1.42 (range 0.15-52.35) vs. 0.89 (range 0-11.88), respectively (p=0.01). In a logistic regression analysis, both CKS1B over-expression and del(13) resulted the most adverse variables independently influencing the response to thal-dex. The univariate analysis of factors impacting survival after Tx2 showed that patients over-expressing CKS1B had a shorter overall survival (p=0.02). Moreover, a multivariate model including CKS1B over-expression, t(4;14) and del(13) showed that CKS1B over-expression was the unique independent variable affecting patients survival. *Conclusions*. In patients with newly diagnosed MM, CKS1B over expression at baseline predicts for a significantly lower probability of response to primary remission induction therapy with thal-dex. Moreover, the poor survival after Tx2 conferred by CKS1B over-expression is independent from the presence of t(4;14) and/or del(13).

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PO-709

PREDICTION OF RESPONSE TO PRIMARY THERAPY WITH THALIDOMIDE -DEXAMETHASONE(THAL-DEX) FOR NEWLY DIAGNOSED MULTIPLE MYELOMA BY GENE EXPRESSION PROFILING (GEP)

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Introduction. The *Bologna 2002* clinical trial has recently demonstrated the superiority of thalidomide and dexamethasone combination (thal-dex) as front-line therapy in preparation for autologous transplantation as opposed to VAD in terms of increased rate of response and magnitude of tumour reduction. In particular, the probability to attain at least a very good partial response was 19%, including 13% of patients in complete response or near complete response (nCR). *Aim*. To identify a set of genes able to predict ≥nCR after thal-dex induction therapy. *Patients and Methods*. Plasmacells obtained at diagnosis from 88 patients enrolled in the *Bologna 2002* clinical trial were used throughout the study. GEP was performed in 32 patients, using the Affymetrix microarray platform. Real-time PCR using micro-fluidic cards was performed in 55 patients. Criteria of response were those established by Bladè. *Results*. Overall, 19 of the 88 patients (22%) obtained at least a nCR to thal-dex. In the first study-phase, a training set of 32 patients was used in order to identify a gene signature of 162 genes able to significantly distinguish patients with ≥nCR from the others (p<0.05); among the genes included in the signature, a list of 31, able to predict the ≥nCR was then selected, by adopting a Nearest-Neighbours (NN) classifier. In the second study-phase, the predictive value of the 31 genes was validated on a test set of 55 patients by Real-time PCR. Moreover, looking for the most significant predictive genes, a final list of seven genes was generated, able to predict the ≥nCR with about 80% sensitivity. Of interest, this list encompasses CCND2, one of the most important cell cycle regulator known to be involved in tumour progression in MM patients and the anti-apoptotic gene CFLAR. *Conclusion*. These results could be the first step to create a custom array or to adopt microfluidic cards using a small number of genes, in an attempt to select at diagnosis patients who will respond very favourably to a particular treatment strategy.

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PO-710

CLINICAL EFFICACY OF THALIDOMIDE CONTAINING REGIMENS AS A FIRST-LINE THERAPY IN PATIENTS WITH MULTIPLE MYELOMA

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Introduction. The aim of this study was to assess the efficacy and toxicity of thalidomide-containing regimens as the first-line therapy in patients with multiple myeloma. *Material and Methods*. A total of 58 patients with multiple myeloma were initially treated with thalidomide-containing regimens between June 2003 and October 2006. Thalidomide was given with the two different regimens repeated every 28 days: (A) TD regimen: thalidomide (200 mg/day, daily) and dexamethasone (20 mg/m^2, intravenously, on D1-4, D9-12, D17-20); (B) TCD regimen: thalidomide (50 mg/day, daily), cyclophosphamide (150 mg/m^2 P.O. on D1-4), and dexamethasone (20 mg/m^2 I.V. on D1-5, D15-19). Autologous peripheral blood stem cells (PBSC) were collected after mobilizing with G-CSF with or without cyclophosphamide. *Results*. Of the patients, 58 patients (TD regimen: 18 patients, TCD regimen: 40 patients) who received at least 4 cycles or more were evaluated for response and toxicity. There were 28 males (48.3%) and 30 females (51.7%). The median age of patients was 68 years (range, 39-80 years). The overall response rate for thalidomide-containing regimens was 84.3%. There were 6 (33.3%) complete responses and 8 (44.4%) partial responses for TD regimen and 11 (27.5%) complete responses and 18 (45.0%) partial responses for TCD regimen, respectively. There was no significant difference in overall response rate between two treatment groups (TD: 77.7% vs. TCD: 72.5%, p=0.43). However, the progression-free survival was significantly shorter in patients treated with TD regimen than with TCD regimen (13.8±6.2 ms. vs. 29.8±8.6 ms. p<0.01). There was no significant difference in overall survival between two treatment groups (p=0.23). Toxicity by NCI-CTC (grade 3/4) included neutropenia in 8 patients (13.8%), thrombocytopenia in 4 patient (6.9%), infection in 9 patients (15.5%) and neuropathy in 10 patients (17.2%). In addition, there were 2 patients (3.4%) with thrombosis. Sixteen patients who achieved more than partial response to the thalidomide-containing regimens proceeded to PBSC collection and the median number of CD34$^+$ cells collected was 3.4×10^6/kg (range, 1.0-18.5×10^6/kg). *Conclusion*. Thalidomide-based combination chemotherapy is safe, well tolerated and effective regimen in patients with newly diagnosed multiple myeloma showing high response rates, relatively low toxicities, and sufficient collection of PBSC.

PO-711

LONG TERM FOLLOW UP OF T-VAD FOR UNTREATED MULTIPLE MYELOMA

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Introduction. Thalidomide is an effective agent for patients with MM both alone and in combination. This study incorporated thalidomide into an induction regimen for patients prior to high dose melphalan (HDM) and stem cell (PBSC) support with maintenance phase thereafter. *Methods*. In this multi-centre non-randomised Phase II study, patients received one cycle of VAD (vincristine 0.4 mg/day and doxorubicin 9 mg/m^2 by iv infusion D1-4; dexamethasone 40 mg D1-4, 8-11,

15-18), followed by a cyclophosphamide primed PBSC harvest (incase thalidomide affected PBSC mobilisation). Up to 6 further cycles of VAD (dexamethasone 40 mg D1-4 only) were given with thalidomide 200mg/day increasing to 400 mg if tolerated. A second cyclophosphamide PBSC harvest was performed (to investigate the effect of thalidomide on PBSC mobilisation) and subsequently patients received HDM with PBSC. 3 months later, thalidomide maintenance was commenced at 50mg/day increasing to 200 mg if tolerated. Routine thromboprophylaxis was not planned for this study. *Results*. 27 patients were enrolled (median age 57 [range: 40-65]) of which 26 were evaluable for response. The response rate following T-VAD alone was 96% (CR 11%, PR 85%) and the best overall response by intention-to-treat analysis was 96% (CR 46%, PR 50%). 3 patients failed to mobilise PBSCs, 20 received HDM and engraftment times were unaffected. 15 received thalidomide maintenance of which 10 remain on therapy. The median dose of thalidomide was 200 mg. The median progression free survival was 47 months and the median overall survival had not been reached (median follow-up 50 months [range 22-75]). Treatment was generally well tolerated with 2 withdrawing from study due to adverse events (1 infective, 1 VTE). During induction therapy, 26% developed VTE; 19% neuropathy and 19% infections. During maintenance, 30% had neuropathy 15% had infections. *Conclusions*. The combination of thalidomide into the VAD induction regimen is safe, did not effect PBSC mobilisation and thalidomide maintenance was well tolerated. Response rates were high and have lead to durable PFS of 47 months and longer overall survival with follow-up of up to 6 years. The most common adverse event was VTE (predominantly related to central venous lines) which would be lower if thromboprophylaxis was used.

PO-712

SURVIVAL AND OUTCOME OF BLASTOID VARIANT MYELOMA FOLLOWING TREATMENT WITH DT-PACE

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Introduction. Blastoid morphology in myeloma is a rare presenting feature associated with poor outcome, and hence effective treatment strategies need to be developed. Extramedullary myeloma also often has this morphology and is known to be associated with a poor outcome. Cells with these features often respond well to cell cycle active classic chemotherapy regimens and combination of these agents with thalidomide offers an interesting new approach, which may be useful in this setting. *Materials and Methods.* We have collected and analysed all such cases presenting in a 5 year period and evaluated their characteristics and outcome after treatment with DT-PACE±Autograft. We analysed the results of 34 patients (median age 51.6 years, range 37-67) of whom 22 (60%) had extramedullary and/or blastoid type and 12 had relapsed/refractory disease. DT-PACE consists of dexamethasone 40 mg day1-4, thalidomide 100-200mg daily, and cisplatin 10 mg/m^2/day, doxorubicin 10 mg/m^2/day, cyclophosphamide 400 mg/m^2/day, and etoposide 40 mg/m^2/day all given on days1-4, on a 28 day cycle aiming to deliver 2 cycles. Results 20 patients received DT-PACE alone, and 14 DT-PACE followed by a melphalan autograft. The overall response rates (CR/PR) were 59% for the blastoid group and 27% for the relapsed/refractory group. Despite these initial good response patients with the blastoid variant had early disease progression (PFS 5 vs 13months p=0.015) and (OS 10 vs 13months p=0.138). Patients who tolerated the DT-PACE and were able to go on to the autograft had better PFS and OS when compared to the group who received the chemotherapy alone (PFS= 12 vs 4 months p=0.023 and OS not reached vs 4 months p=0.006). *Conclusion.* In conclusion we confirm the poor clinical outcome for this group of cases even with the intensive regimen DT-PACE, however, in contrast to previous treatment excellent response rates are obtained and some long term survivors are found. This forms the basis against which future strategies aimed at improving this regimen utilising novel therapies can be compared.

PO-713

EFFICACY OF THALIDOMIDE BASED THERAPY IN THE TREATMENT OF PRIMARY PLASMA CELL LEUKAEMIA

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Primary plasma cell leukaemia is a rare disease with a median survival of only 7 months.[1] Currently there is no consensus as to the most appropriate treatment for this aggressive disease. Here we report the data on 7 patients with primary plasma cell leukaemia treated with thalidomide-based therapy in three European centres. Their characteristics are as follow: mean age 52.8 years, female (71%), light chain disease (57%), anaemia (Hb <10 g/dL) (100%), renal impairment (creatinine >120 umol/L) (71%), hypercalcaemia (42.8%), splenomegaly (42.8%), lytic bone disease (85.7%). Five of the 7 patients have died but the mean overall survival (OS) is 20.3 months from diagnosis, a figure nearly 3 times that reported in the literature.[1] The remaining 2 patients have, to date, survived beyond 19 and 24 months respectively since diagnosis. Of the 2 patients whose serum free light chains were measured, thalidomide- based therapy is also associated with a reduction in free light chains. We conclude that thalidomide-based therapy is associated with increased overall survival and shows very promising results in the effective management of primary plasma cell leukaemia.

Reference

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PO-714

BIRD (BIAXIN®/REVLIMID®/DEXAMETHASONE) IN MYELOMA (MM)

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Introduction. Herewith, we report BiRD (Biaxin(r)(Bi)/Revlimid (R)/Dexamethasone(D)) as initial therapy for Myeloma (MM). Methods and Patients (pts): Symptomatic, treatment naive pts were eligible. BiRD consists of R given po at 25 mg qd days 1-21. D given po at 40 mg q weekly and Bi po at 500 mg bid. Intent-to-treat analysis. Correlative studies: PK analysis of D and the impact of Bi on D levels by HPLC. 72 pts [mean follow-up 10 months (range 1-27) accrued. Median age 63 years (range 36-83), β2m 3 mg/L (range 1.1-17.6), CRP 0.4 mg/dL (range 0.02-14.2), creatinine 1.1 mg/dL (range 0.6-3.1), albumin 3.3 g/dL (range 2.3-4.9), and calcium 9.2 mg/dL (range 7 -11.2). Cytogenetics/FISH: trisomy/tetrasomy 11 (28 pts), del13q14(22 pts), t(4,14)(4pts), t(11,14) (11pts). 50% of the pts stage IIIa, 4.2% stage IIIb, 44.4% stage IIa & 1.4% stage IIb. ISS: 54% have > stage II. *Results*. Of the 72 enrolled pts, 69 (87.5%) have achieved objective response (>PR). Mean time to first response 43.6 days (range 28-255); mean time to max response 141 days (28-692). Response (NURC): sCR: 14/72 (19.4%), CR: 6/72 (8.3%) total >CR of 27.7%. One third of pts had a VGPR, therefore, 60% pts had >VGPR.19/72 patients went on PBSCT while 50 have continued therapy. 60% of those (30/50) achieved >VGPR (10sCR, 3CR, 17VGPR) [mean fu 10 months (range 1-27)]. Only 2 CR patients had POD (at 98 and 191 days) with reappearance of IFX pos but no clinical relapse. PK studies: median AUC for D only was 286827.3 while BD was 451863 and BRD was 348969.6, (p=0.07). Grade 3/4 hematological toxicities: anemia(11%), neutropenia(9%), thrombocytopenia(9%), and leukopenia(11%). Non-hematological toxicities: thrombosis in 7 patients (15%) with 8 (17%) total events: PE (4%), DVT (9%), AMI (2%), and sudden death (2%). 6/7 patients were off ASA. Two of the 7 patients died due to thromboembolic events. Other non-hematological toxicities include myopathy (6%), pain (4%), abdominal cramps (4%), and mood (4%). *Conclusions*. 1) BIRD is safe and very effective yielding high CR+VGPRs. 2) Long term BIRD is feasible in pts who are not candidates for PBSCT. 3) Bi increases AUC of dexamethasone.

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PO-715

PHASE 1/2 STUDY OF UPFRONT REV/VEL/DEX IN MM: EARLY RESULTS

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Introduction. Single-agent bortezomib (VELCADE®, Vel) and lenalidomide (Revlimid®, Rev) plus dexamethasone (Dex) are approved treatments for relapsed multiple myeloma (MM) patients (pts) who have received ≥1 prior therapy. In a phase 1 study, Rev/Vel±Dex was well tolerated and resulted in 58% response rate (CR+PR+MR) in relapsed/refractory MM pts.[1] Primary objectives of this study are to determine the MTD of Rev/Vel/Dex and response rate (CR+nCR+PR) in newly diagnosed MM pts. *Materials and methods*. Pts received Vel 1.0-1.3 mg/m^2, days 1, 4, 8, 11, Rev 15-25 mg, days 1-14, and Dex 40 mg, days of/after Vel, for ≤8 21-day cycles at 4 planned dose levels (Vel/Rev: 1.0/15; 1.3/15; 1.3/20; 1.3/25). Dose escalation proceeded (3-pt cohorts) depending on occurrence of DLTs (G≥3 non-hematologic toxicity; G4 thrombocytopenia with platelets <10,000/mm^3 on >1 occasion despite transfusion support; G4 neutropenia for >5 days and/or resulting in neutropenic fever; inability to receive cycle 2/day 1 dose due to drug-related toxicity). Response was assessed by modified EBMT criteria. Toxicities were graded by NCI CTCAE v3.0. *Results*. To date, 10 pts (5M/5F; median age 58y) have been enrolled and 9 treated in dose levels 1-3, the majority with advanced (Durie-Salmon stage 3/ISS stage 2-3) disease. No DLTs have been seen; MTD has not been reached. Dose reductions beyond cycle 2 have occurred for Vel in 1 pt, Rev in 2 pts, and Dex in 8 pts. Edema attributed to Dex was seen in all 3 pts in dose level 1, prompting dose reduction after cycle 2, but has yet to be seen at other dose levels. No unexpected toxicities have otherwise been encountered. Other common toxicities include anxiety, constipation, fatigue, and diarrhea, all of which have been manageable. No DVT or significant peripheral neuropathy has been reported. Responses among 5 evaluable pts include 1 nCR/4 PR, and have been durable. *Conclusions*. Rev/Vel/Dex is active and well tolerated in newly diagnosed MM pts, with no DLTs seen to date, although Dex dose reduction beyond cycle 2 has been required in most pts. Phase 1 enrollment is ongoing, with 10 additional pts planned at the MTD.

Reference

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PO-716

INITIAL THERAPY WITH THALIDOMIDE/DEXAMETHASONE VERSUS LENALIDIMIDE/DEXAMETHASONE IN NEWLY DIAGNOSED MULTIPLE MYELOMA: A RETROSPECTIVE ANALYSIS OF TWO PHASE II TRIALS

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Introduction. The combination of thalidomide and dexamethasone is effective induction therapy in newly diagnosed multiple myeloma. Recently, the lenalidomide plus dexamethasone has been shown to be a highly active induction regimen with possibly, a better response rate and toxicity profile compared to thalidomide/dexamethasone. However, there is little data comparing the clinical efficacy of these two regimens. *Materials and Methods*. We analyzed the clinical outcomes of patients with newly diagnosed multiple myeloma treated in prospective phase 2 studies with thalidomide and dexamethasone (thalidomide 200 mg daily, dexamethasone 40 mg days 1-4, 9-12, and 17-20, odd cycles and 40 mg days 1-4 even cycles; Rajkumar *et al.*, J Clin Oncol 2002) and lenalidomide and dexamethasone (lenalidomide 25 mg daily on days 1-21 of a 28-day cycle, dexamethasone 40 mg days 1-4, 9-12, and 17_20 of each cycle; Rajkumar *et al.*, Blood 2005). The data regarding disease course, treatment, progression free survival (PFS) and overall survival were updated as of February 2007 by review of clinical records. *Results*. There were no major differences in eligibility criteria between the two trials. 50 patients were treated with a combination of thalidomide and dexamethasone, of which 29 (58%) underwent high dose chemotherapy (HDC) with autologous stem cell transplantation (ASCT) following initial therapy. 34 patients underwent induction therapy with lenalidomide and dexamethasone, of which 13 (38%) proceeded to HDC and ASCT. Despite small sample size, there was a trend towards a higher two year overall survival rate in patients receiving lenalidomide and dexamethasone compared to thalidomide and dexamethasone, 90% and 70%, respectively (p=0.078). The median PFS among patients who did not receive HDC and ASCT (i.e., patients who received these regimens as primary therapy) was significantly higher with lenalidomide and dexamethasone (n=21) compared to thalidomide and dexamethasone (n=21), 29 months versus 12 months (p=0.019), respectively. *Conclusions*. Our study suggests that initial therapy with the combination of lenalidomide and dexamethasone may be superior to that of thalidomide and dexamethasone in patients not undergoing HDC with ASCT. However, a prospective randomized trial of these two regiments is necessary to confirm these findings.

PO-717

MELPHALAN, PREDNISONE AND LENALIDOMIDE FOR NEWLY DIAGNOSED MYELOMA

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Introduction. Lenalidomide plus dexamethasone is an effective treatment in advanced multiple myeloma (MM). No data are available on the combination of Melphalan, Prednisone and Lenalidomide. In a multicenter phase I/II trial, we evaluated the efficacy and safety of the combination melphalan, prednisone and lenalidomide (MPR) in elderly newly diagnosed MM patients. *Material and Methods.* Fifty-four patients (median age 71 years) were enrolled to receive 9 cycles of lenalidomide (5-10 mg/day for 21 days) plus melphalan (0.18-0.25 mg/kg for 4 days) and prednisone (2 mg/kg for 4 days) every 4 weeks followed by maintenance therapy with lenalidomide alone (10 mg/day for 21 days every month). Four different dose-levels were tested to define the maximum tolerated dose (MTD). Aspirin (100 mg/day) was administered as antithrombotic prophylaxis. *Results*. Lenalidomide 10 mg/day for 21 days and melphalan 0.18 mg/kg for 4 days monthly was defined as the MTD. At this level 81% of patients showed at least a partial response (PR), including 47.6% of patients with at least a very good partial remission (VGPR) and 23.8% who showed immunofixation negative complete response (CR). The 1-year event free survival (EFS) and overall survival (OS) rates were 92% and 100%, respectively. No significant differences in terms of response rate and EFS were observed between patients with or without deletion of chromosome 13 or chromosomal translocation (4;14). By contrast, high b2-microglobulin predicted a shorter EFS. Thirty-eight patients started maintenance treatment (median follow-up on maintenance 3,8 months). Major grade 3-4 adverse events were hematological toxicities (69.8%), febrile neutropenia (9.4%) cutaneous rash (7.5%), and thromboembolism (5.7%); two of three thromboembolic events occurred after aspirin discontinuation. *Conclusions*. Oral MPR is a promising first-line treatment for elderly patients with multiple myeloma. The non-hematological adverse events showed a low incidence, the hematological adverse events were manageable. Un update of these data will be presented at the meeting.

PO-718

FRONTLINE VMP IN ELDERLY MM PATIENTS: EXTENDED FOLLOW-UP

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Introduction. Melphalan plus prednisone (MP) has been the frontline standard of care for elderly multiple myeloma (MM) patients not eligible for stem cell transplantation. However, complete responses (CR) are rare and median overall survival (OS) is only 2-3 years. Preclinical studies demonstrated *in vitro* synergy between bortezomib (VELCADE(r)) and melphalan, and the combination was active and well tolerated in relapsed/refractory MM patients. This phase 1/2 study of bortezomib plus MP (VMP) evaluated efficacy and safety in 60 untreated MM patients aged more or equal to 65 years. Here we report updated time-to-events data. *Materials and methods*. Patients received bortezomib 1.0 (N=6) or 1.3 (N=54) mg/m^2 on days 1, 4, 8, 11, 22, 25, 29, and 32 for four 6-week cycles, then on days 1, 8, 15, and 22 for five 5-week cycles, plus melphalan 9 mg/m^2 and prednisone 60 mg/m^2 on days 1-4 of each cycle. Response was assessed using EBMT criteria. Response rate, time to progression (TTP), event-free survival (EFS), and OS with VMP were compared with MP historical control data of 96 patients included in a randomized trial of MP versus melphalan plus dexamethasone reported by our group. *Results*. In 53 evaluable patients, response rate with VMP was 88%, including 32% CR/11% nCR. Half the CR patients tested demonstrated immunophenotypic remission. Response rate was higher vs MP historical controls (42%, including 3% nCR). Responses were rapid; median time to response was 2.7 months. After median follow-up of 26 months (range 15-38 months), median TTP was 27,2 months (95% IC: 21,9-32,4) and median OS time had not been reached. Time-to-events data compare favorably vs MP historical controls. At 26 months, median PFS time was 27,2 months (vs 20 monts, p=0,001), median EFS time was 23 months (vs 16 months, p=0,05), and median OS time has not been reached (vs 26,9 months, p=0,000). Projected VMP 3-year OS rate is 85%. PFS and OS were independent of age and cytogenetics abnormalities. Treatment was well tolerated; median number of cycles administered was 8 (range: 2-9), with a median duration of treatment of 9 months (range: 1-12). Principal toxicities were hematologic, gastrointestinal, and peripheral neuropathy. *Conclusions*. After extended follow-up, VMP continues to demonstrate significant superiority vs MP historical controls in elderly MM patients ineligible for transplant, offering a very high CR rate and prolonged disease control. VMP is being compared with MP in the ongoing phase 3 VISTA trial.

PO-719

BORTEZOMIB + ASCORBIC ACID + MELPHALAN UPFRONT THERAPY

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Introduction. The high response rate observed in our phase I/II trial combining oral melphalan with bortezomib for multiple myeloma (MM) patients with relapsed or refractory disease along with the encouraging results from our preclinical *in vitro* as well as *in vivo* studies involving melphalan and ascorbic acid provided the rationale for this clinical trial. *Materials and Methods.* This is a single-arm phase II study that evaluated the combination of bortezomib, oral ascorbic acid and low-dose oral melphalan (BAM) for newly diagnosed patients with symptomatic myeloma. Treatment consisted of a 28 day cycle for a maximum of 8 cycles. Bortezomib was administered in the morning at a dose of 1.0 mg/m^2 on days 1, 4, 8, and 11 followed by a 17 day rest period. Oral ascorbic acid at a dose of 1g and oral melphalan at 0.1 mg/kg were both administered in the evening on days 1-4 of each cycle. Patients who were treated to maximum response plus two additional cycles or after completion of eight cycles of therapy and without disease progression were subsequently treated with bortezomib at a dose of 1.3 mg/m^2 every other week until progressive disease occurred. *Results*. To date, 23 newly diagnosed patients have been enrolled and 20 patients are currently evaluable for response. Responses (minimal [MR], partial [PR], or complete [CR]) occurred in 14 of 20 patients (70%), including two immunofixation (IF) - negative CRs (10%) with some residual bone marrow plasma cells, one IF-positive CR (5%), four PRs (20%), and seven MRs (35%). Five patients (25%) have stable disease. Five patients experienced reversible grade 3 myelosuppression and two patients experienced grade 3 neuropathy. Only one patient has shown progressive disease with initial BAM treatment. *Conclusion*. Bortezomib, low-dose oral melphalan and oral ascorbic acid administered on a 28 day schedule as first line therapy for MM patients shows very encouraging activity with minimal toxicity. This non-thalidomide, non-steroid containing frontline trial represents a promising new, safe, well-tolerated and active therapeutic option for newly diagnosed patients. Enrollment is ongoing for this trial and we plan to enroll a total of 35 patients.

PO-720

EFFICACY AND SAFETY OF LIPOSOMAL DOXORUBICIN [DOXIL/CAELYXTM], BORTEZOMIB [VELCADETM] AND DEXAMETHASONE IN THE TREATMENT OF PREVIOUSLY UNTREATED MULTIPLE MYELOMA PATIENS: IMPACT OF CYTOGENETIC PROFILE

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Introduction. Bortezomib in combination with liposomal doxorubicin has shown convincing activity as induction therapy in frontline multiple myeloma (MM) patients with CR rates exceeding those seen with conventional induction therapy. Pulsed dexamethasone was added to this induction regimen to improve response rates. *Methods.* Newly diagnosed ASCT eligible MM patients were recruited from nine centres across Canada to this single-arm, open-label, Phase II study. Patients received three to four 21-day cycles of DBd induction therapy (bortezomib 1.3 mg/m^2 days 1, 4, 8, 11 + liposomal doxorubicin 30mg/m^2 day 4 + pulsed dexamethasone 40mg days 1-4, 8-11, 15-18 Cycle 1 and days 1-4 cycles 2-4), depending on rapidity of CR achieved, and were then followed post engraftment to determine response rates and eventually TTP. The primary endpoint was based on percentage of patients with CR + nCR post-induction. *Results*. Fifty patients were enrolled to fulfill the sample size requirement. Mean age was 55.7 years, and 66.7% of patients were male. Sixty-nine percent of the patients had Stage III disease and all but two patients had secretory disease. Mean baseline β2-microglobulin level was 195.5 nmol/L. Overall best response post-induction for 44/50 evaluable patients was 95% (> MR), with a corresponding 20.4% combined CR + nCR rate. Time to best response post-induction was 55 days. Of the patients for whom cytogenetic data [del (q13), t(11;14), t(4;14), p53, CKS] is currently available (50% patients), an ORR of 100% was observed (> MR). In the 29 patients transplanted thus far, stem cell collection was not compromised with this induction therapy. 35.6% of patients experienced Grades 3 adverse events, including only 4% Grade 4 events. Grade 3 HFS or peripheral neuropathy was experienced in only 9% and 2% of patients, respectively, with no corresponding Grade 4 events. Only three patients withdrew from the trial due to intolerable HFS. Response rates post-transplant and TTP will be available at the time of conference. *Conclusion*. These results demonstrate that the response achieved with the DBd regimen in the frontline setting is independent of cytogenetic profile, and toxicity is manageable. A subsequent trial utilizing a bortezomib-containing induction regimen is currently under consideration.

PO-721

COMBINATION THERAPY WITH BORTEZOMIB(VELCADE®), DOXIL®, AND DEXAMETHASONE(VDD) IN NEWLY DIAGNOSED MYELOMA: UPDATED RESULTS OF PHASE II CLINICAL TRIAL

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Introduction. We have completed accrual to phase II trial of the combination of bortezomib, Doxil, and dexamethasone (VDD) as initial therapy for newly diagnosed multiple myeloma and present updated response data as well as follow-up data. In addition, we show prelimi-

nary results of investigation of the mechanism of high efficacy of the regimen. *Methods.* 40 patients (30 male and 10 female) with measurable disease requiring therapy were enrolled. The regimen was given for six 3-week cycles as follows: Velcade at 1.3 mg/m^2 on days 1, 4, 8, and 11, Doxil at 30 mg/m^2 on day 4, and dexamethasone at 40 mg on days 1-4 for the first 10 patients and for the remaining patients at 20 mg per dose on days of Velcade and day after. After completion of VDD, most patients (23 to date) proceeded to stem cell transplant (SCT). Patients who declined transplant or were not eligible, continued treatment with Velcade-based maintenance off protocol. *Results*. 37 patients are evaluable for response: median age 61 (range 39-83), Durie-Salmon stage III disease in 34, stage II in 3, chromosome 13 deletion and/or t(4;14) in 14, median beta2-microglobulin 6.2. Median follow-up is 13 months (range 2-19). At the end of VDD therapy > partial response (PR) was in 89% patients, with > very good partial response (VGPR) in 50% and CR/nCR in 37% of patients. There was no grade 4 hematological toxicity and grade 3 or higher non-hematologic toxicity was limited and not significantly different to previously reported (ASH 2006 Abstract # 3093). Among patients staying on maintenance therapy > VGPR was in 53% of patients (46% CR/nCR), whereas in patients who received SCT > VGPR was 78% (65% CR/nCR). All patients are alive. By Kaplan-Meier method, estimated 1-year progression-free survival is 91%. Evaluation of the effects of VDD on the apoptotic pathway revealed that NOXA, a tumor-restricted modulator of proteasomal control, is up-regulated more by VDD than by Velcade or Velcade and dexamethasone. *Conclusion*. VDD is highly active as initial therapy of myeloma and in combination with subsequent SCT produces high rate of > VGPR associated with improved survival.

PO-722

BORTEZOMIB, THALIDOMIDE AND DEXAMETHASONE AS INDUCTION

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The optimal induction regimen for patients with symptomatic myeloma who are eligible for transplant is currently not known. While thalidomide and dexamethasone is an effective regimen, it only has a 60 to 65% response rate and few complete responses (CR). Bortezomib based inductions have demonstrated a high response rate and an improved CR as well. Recently the IFM reported the initial results of the randomized bortezomib plus dexamethasone versus VAD induction followed by transplant, which demonstrated that fewer patients treated with bortezomib required tandem transplants. Wang *et al.* reported a high induction response rate with the combination of BTD for only 2 cycles given over a 28 day cycle. Here we report our experience with the combination of BTD as induction therapy. 28 patients with symptomatic myeloma were treated with BTD as induction therapy. Patients received standard bortezomib at 1.3 mg/m^2 on days 1, 4, 8, and 11 with thalidomide at 100 mg/day, and 8 days of 40mg dexamethasone every 21 days. The median age was 59 years (38-70) with 15 males. This was first line therapy for 18 patients, second line for 8 patients and 3rd line for 2. 11 patients had ISS stage 2 and 8 had ISS stage 3. The median β2m was 3.77 (1.66-41.89). Median creatinine was 1.1 (0.6-21). Fourteen patients had an IgG paraprotein, 4 an IgA, and 10 patients had light chain only disease. The median number of cycles was 4 (2-8). Thirteen patients developed neuropathy of any grade and 4 patients had to discontinue BTD because of neuropathy. The overall response rate was 89%, with 57% of patients achieving a VGPR or better, and 18% of patients achieving a CR. 1 patient had stable disease and 2 patients had progressive disease (both presented with plasma cell leukemia). The ORR for first line patients was 94% with 61% achieving a VGPR or better. In conclusion, we report a very high response rate with a short course of BTD with an acceptable toxicity profile. Further studies of this active regimen are warranted. Follow up of for patients in CR not going to transplant is in progress.

PO-723

INDUCTION WITH SEQUENTIAL BORTEZOMIB/THALIDOMIDE/DEXAMETHASONE AFTER VAD RESULTS IN SIGNIFICANT CYTOREDUCTION PRIOR TO BMT IN MULTIPLE MYELOMA (MM)

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Bortezomib is effective for MM but is expensive. Tandem autologous bone marrow transplantation (ABMT) improves survival in MM. We tested if sequential bortezomib after VAD may increase response rate, and hence reduce tumor load prior to stem cell mobilization for ABMT. Newly diagnosed myeloma patients received VAD for three cycles. In patients in whom M-protein response was less than 75% after three cycles of VAD, they received four three-weekly cycles of VTD [Bortezomib (1.3 mg/m^2 days 1-4 and 8-11) with thalidomide (200 mg/d) and dexamethasone (20 mg/d from D1-4 and D8-11)]. Patients achieving >75% reduction in M-protein and those completing VTD will proceed to cyclophosphamide-primed peripheral stem cell mobilization and ABMT. There were 10 patients (Durie-Salmon stage II in 1 and II in 9) with a median age of 54.5 years (33-64 years). After VAD, there were one, three, four and two patients with no response (<25% reduction in M-protein), minor (MR) (25%-50%), partial (PR)(50%-90%) and very good partial responses (VGPR) (>90%). Five patients receive VTD, where further response was achieved (two MR to VGPR, one PR to CR, and one each of NR and MR to PR). ABMT has been completed in 8 patients, resulting in 4 CR (negative immunofixation), one near-CR (immunofixation positive), two VGPR and one PR. One patient developed grade 4 neuropathy with CR. Other side-effects from VTD were grade II or less. In conclusion, sequential VTD resulted in significant tumor reduction with manageable side-effects, and is affordable by more patients.

PO-724

BORTEZOMIB WITH DEXAMETHASONE AS INDUCTION THERAPY IN NEWLY DIAGNOSED MULTIPLE MYELOMA: A PRELIMINARY STUDY IN THAI PATIENTS

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Introduction. The efficacies of induction treatment in myeloma patients, who are candidates for autologous stem cell transplant, are still unsatisfactory since they can usually get patients into complete remission in only about 5-10% of cases before transplant. Incorporation of new antimyeloma agents into the induction regimen should improve the outcomes of these patients. We, therefore, assess the response rate and factors that may predict the response in newly diagnosed patients who were treated with bortezomib with dexamethasone (VelDEX) and compare them with the historical cohort of patients who were treated with VAD (vincristine, adriamycin and dexamethsone) regimen at the same institution. *Designs and Methods*. Bortezomib 1.3 mg/m^2 was administered on days 1, 4, 8 and 11, along with dexamethasone 40 mg orally on day 1-4 and 8-11 of a 21-day cycle for 4 cycles. The complete evaluation was done after the fourth cycle of VelDEX, before peripheral stem cell collection. Responses were defined according to the European Group for Blood and Marrow Transplantation criteria. *Results*. 19 out of 20 patients enrolled were eligible for evaluation. All but one completed four cycles of VelDEX. The objective responses were achieved in 15 patients (79%), including complete remission in 7 patients. On an intent-to-treat basis, response rate for complete remission, partial response, minimal response, stable disease, progressive disease were 37%, 42%, 11%, 5%, and 5% respectively. Comparing with the cohort of 30 patients treated with VAD regimen, VelDex is superior in inducing complete remission (37% vs. 10%, $p<0.001$). No significant factors were found to be associated with responses in these patients. The most common side effects were fatigue, diarrhea and peripheral neuropathy. Two patients developed colonic pseudoobstruction that could be related to bortezomib. No deep vein thrombosis or serious hematologic toxicity were observed. Five patients has undergone autologous stem cell transplantation successfully and remains in complete remission. *Conclusion*. VelDEX is a highly effective induction regimen for newly diagnosed multiple myeloma who are candidates for autologous stem cell transplant.

PO-725

LONG TERM FOLLOW-UP OF PAD FOR UNTREATED MULTIPLE MYELOMA

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Introduction. Bortezomib has demonstrated efficacy for patients with relapsed myeloma and has an increasing role in the untreated setting. PAD was developed on the basis of *in vitro* synergistic cytotoxicity of bortezomib with doxorubicin and the additivity with dexamethasone. *Methods.* In this non-randomised open-label Phase I/II trial patients with untreated MM received 4 cycles of PAD (bortezomib D1,4,8,11; doxoru-

bicin 9 mg/m^2 D1-4, dexamethasone 40 mg D1-4, 8-11, 15-18 (cycles 2-4 D1-4 only) followed by high dose melphalan with stem cell support (HDM). Two sequential cohorts of patients were enrolled, at bortezomib 1.3 mg/m^2 (PAD 1) and 1.0 mg/m^2 (PAD 2) to investigate if a lower dose of bortezomib would reduce toxicity. Responses were classified by EBMT criteria. *Results*. 41 patients were enrolled of which 40 were evaluable. Responses following PAD alone were high: PAD 1 (n=21) 95% (CR 24%, nCR 5%, VGPR 33%, PR 33%); PAD 2 (n=19) 89% (CR 11%, nCR 5%, VGPR 26%, PR 47%), and following HDM these overall responses were maintained, with an improvement in CR rate (PAD 1: CR 43%, nCR 14%; PAD 2: CR 37%, 5% nCR). Stem cell mobilisation and engraftment were not prejudiced. Toxicities were manageable; however the PAD 2 regimen had lower all-grade neuropathy (48% vs 20%) and grade 3-4 infections (48% vs 20%). The median progression free survival was 29 months (PAD 1) vs 24 months (PAD 2),median time-to-retreatment was 36 months (PAD 1) vs 29 months (PAD 2) and the median overall survival had not been reached over a median follow-up of 28 months (range 17-39). There was no statistical difference in PFS or TTRT between cohorts. *Conclusions*. PAD is a highly effective and well tolerated induction regimen for patients with untreated myeloma. The overall response rate was 95% (PAD 1) and 89% (PAD 2) with high CR rates even prior to HDM. This was reflected in the TTRT of up to 36 months (which may be a clinically relevant indicator of efficacy due to the high CR rate and the EBMT definition of progressive disease). The PAD 2 regimen was well tolerated with no reduction in efficacy.

PO-726

RAPID REMISSION INDUCTION BY VTD (VELCADE, THALIDOMIDE, DEXAMETHASON) IN PATIENTS WITH MULTIPLE MYELOMA AND HIGH TUMOR BURDEN AT DIAGNOSIS

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Background. VTD was reported to be an active salvage regimen in patients with relapsed/refractory multiple myeloma (MM). Since initial results with VTD as frontline therapy were also promising, we used VTD as induction treatment prior to autologous transplantation in MM patients with particularly high tumor burden. *Methods.* Velcade was administered at 1.3 mg/m^2 on days 1, 4, 8, and 11; thalidomide was given as a daily dose of 100 mg; dexamethasone (20 mg per os) was given on days 1, 2, 4, 5, 8, 9, 11, and 12. Four cycles were scheduled every 3 weeks. *Results*. We here report on three patients (age 36, 38, and 50 years) with newly diagnosed MM and high tumor mass at presentation (patient 1: Bence-Jones kappa MM with 95% plasma cell infiltration, serum free-kappa light-chains 4000 mg/L; patient 2: IgG-κ MM with serum IgG > 9000 mg/dL, bulky plasmacytomas in the iliac bones; patient 3: IgA-lambda MM with 70% plasma cells in the bone marrow, IgA 4500 mg/dL, plasmacytoma in the os sacrum > 10 cm in diameter). Genetically, all 3 patients corresponded to the hyperdiploid variant (multiple trisomies, absence of del(13q14) and del(17p13)); patient 1 also had a t(11;14) and gain of chromosome 1q21. VTD resulted in a paraprotein reduction of at least 75% already after cycle 1. Rapid tumor mass reduction was not associated with signs of tumor lysis, and patient 1 had a significant improvement in renal function (serum creatinine from 2.4 to 1.3 mg/dL). After 4 cycles of VTD, patients 1 and 2 achieved a CR including a normal kappa/lambda ratio in the free-light-chain assay (patient 3 still ongoing). Patient 1 has already completed G-CSF primed peripheral stem cell collection and autologous transplantation. Toxicity of VTD was mild (transient nausea and diarrhea in patient 2), in particular no peripheral neuropathy (grade 2 or higher). *Conclusion*. Induction treatment with VTD results in an effective tumor mass reduction already within one cycle and should be further evaluated in MM patients in need of rapid disease control.

PO-727

SEQUENTIAL VAD (VINCRIESTINE, ADRIAMYCIN, DEXAMETHASONE) AND VTD (VELCADE, THALIDOMIDE, DEXAMETHASONE) INDUCTION FOLLOWED BY HIGH-DOSE THERAPY WITH AUTOLOGOUS STEM CELL TRANSPLANTATION AND MAINTENANCE TREATMENT WITH VELCADE FOR NEWLY DIAGNOSED MULTIPLE MYELOMA : PRELIMINARY ANALYSIS OF EFFICACY AND SAFETY

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Introduction. Thalidomide and velcade are very effective in relapsed and refractory multiple myeloma, and VAD (vincristine, doxorubicin, dexamethasone) is widely used as an induction therapy. We conducted a phase II study with sequential VAD and VTD (bortezomib : Velcade®, thalidomide, dexamethasone) induction followed by high-dose therapy with autologous stem cell transplantation and maintenance treatment with velcade as a first line treatment for the patients with multiple myeloma. *Methods*. Patients are planned to receive 2 cycles of VAD (vincristine 0.4 mg D1-4, adriamycin 9 mg/m^2 D1-4, dexamethasone 40 mg p.o. or i.v. D1-4, 9-12 every 3 weeks), and VTD (bortezomib 1.3 mg/m^2 i.v. D1, 4, 8, 11, thalidomide daily, dexamethasone 40mg p.o. or i.v. D1-4, 9-12 every 3 weeks). High dose melphalan (200 mg/m^2) is used as a conditioning regimen for autologous stem cell transplantation. Velcade (1.3 mg/m^2) as a maintenance treatment is administered weekly x 4 times q 6weeks for 4 cycles after autologous stem cell transplantation. Response was assessed by EBMT criteria, with additional categories of nCR. Adverse events were graded by the NCI-CTCAE, Version 3.0. *Result*. This study aimed to enroll the patients till Feb 2007 and we are reporting a preliminary report of 35 patients. Efficacy could be assessed in 27 patients. After 2 cycles of VAD, response rate was 67% with 4% CR. After VTD, 3 patients showed further improvement with additional CR. Overall response to VAD followed by VTD was 95% (n=20: CR 20%, nCR 15%, PR 60%, PD 5%). So far, autologous stem cells were successfully collected in all 18 patients with a median CD34$^+$ count of 6.38×10^6/kg (range, 2.58-44.7×10^6/kg). In 10 patients who underwent autologous stem cell transplantation, 3 patients gained additional CR. In 4 patients with Velcade maintenance, there is no progression. The median follow-up duration was 5.8 months. Total 144 cycles (sequential VAD and VTD) in 35 patients were assessable for safety. Grade 3, 4 adverse events included neutropenia (18%), anemia (11%), thrombocytopenia (11%) and pneumonia (3%). Peripheral neuropathy with grade 2, 3 was observed in only 4 cases (5%). *Conclusion*. Sequential VAD and VTD induction followed by high dose therapy with autologous stem cell transplantation and maintenance treatment with Velcade is highly effective in newly diagnosed multiple myeloma with tolerable toxicity profiles and did not prejudice stem cell harvest. Keywords: bortezomib, thalidomide, dexamethasone, VAD, multiple myeloma, transplantation

PO-728

EFFICACY OF BORTEZOMIB IN PLASMA CELL LEUKEMIA

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Introduction. Plasma Cell Leukemia (PCL) is characterized by a high number of plasma cells in peripheral blood (>2×10^9/L) and more than 20% of circulating plasmocytes on differential count. Primary PCL or secondary form, seen as the end stage of a previous multiple myeloma (MM) represents the most aggressive form of clonal gammapathy with very poor outcome from both conventional chemotherapy and autologous or allogeneic transplant. For this reason new effective treatment approaches are required. The proteasome inhibitor Bortezomib has been shown to be effective in advanced MM with a rapid response but few reports have been published regarding its potential role on PCL. We report here the preliminary clinical results of a group of PCL patients that received proteasome inhibitor, Bortezomib (Velcade®), as induction therapy. *Patients and Clinical Results*. We describe 10 patients with PCL treated with Bortezomib (Velcade). Median age was 58 y (30-70). 4 cases were primary, *de novo*, PCL and 6 cases were secondary forms of relapsed or progressive MM. Patients with secondary PCL had received a median of 3 previous lines of therapy including autologous transplantation (5 cases). Bortezomib at standard dose iv: 1.3 mg/m^2 days 1,4, 8,11 every 21 days were used alone in 2 patients and in combination with high dose of Dexamethasone ± Adryamicin in the rest. 8 patients were evaluable for drug efficacy with 7 cases showing some grade of favourable response with the first cycle, including 2 cases of CR. 4 patients have died: 1 refractory during first month and 3 for rapid progression 2-3 months after initial response. 2 patients received maintenance therapy that included low dose thalidomide. The median follow up of 6 alive patients is 11 months (1-36 months). Grade 2 thrombopenia, neutropenia and gastrointestinal symptoms were the main secondary toxicity. No complicated lysis syndrome were observed. *Conclusions and Comments*. Bortezomib has been shown to be effective by several mechanisms in patients with MM resistant to multiple previous treatments and as induction therapy alone or in combination with other drugs. The favourable response observed in these cases of PCL, including *de novo* patients, and the biological *ex vivo* results in PCL cell lines, suggest the potential efficacy of this agent combined with dexamethasone and doxorubicin. Although more experience is necessary these data support future trials with Bortezomib combined with other drugs in the induction treatment of PCL.For maintenance of response, the role of this drug or other new drugs as thalidomide or lenalidomide has to be investigated.

GROUP 8: Stem cell transplantation

PO-801

IMMUNE RECONSTITUTION OF THE T CELL COMPARTMENT IN MULTIPLE MYELOMA PATIENTS FOLLOWING ALLOGENEIC NON MYELOABLATIVE HEMATOPOIETIC CELL TRANSPLANTATION

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Introduction. Allografting is the only potentially curative treatment for multiple myeloma (MM). Reduced-intensity/non-myeloablative conditionings were designed to initially establish hematopoietic mixed-chimerism and then to serve as a platform for additional cell immunotherapy aimed at eradicating tumor cells in elderly patients (up to 70 years). However, the risk of post-transplant infections and graft-versus-tumor effects also rely on the residual thymic function, gradually reduced with age. *Materials and methods.* The immune recovery of the T cell compartment was evaluated by flow cytometry in 39 MM patients, median age 54 years (range 34-64), conditioned with low dose TBI (200 cGy), with/without fludarabine (90 mg/m^2 total), followed by G-CSF mobilised peripheral blood stem cell infusion from HLA identical siblings. The analyses were performed at different timepoints: baseline, at day +28, at 3, 6 months, and at 1, 2, 3, 4, 5, 6 years post-transplant. Briefly, fresh peripheral whole blood samples were stained with direct four-colour combinations with the following MoAbs: CD3, CD4, CD8, CD16, CD45RA, CD45R0, CD62L. At least 80000 events for each combination were acquired on a FacsCalibur (Becton Dickinson), and analysed with CellQuest Pro software. T cell Receptor Excision Circles (TRECs) were evaluated by real-time quantitative PCR with an ABI PRISM 7900HT Sequence Detection System at the same timepoints. *Results*. CD4$^+$ T cell >200/uL promptly recovered by day +28 with median values of 272/uL, gradually increasing to 524/uL, 681/uL, and 953/uL, at 1, 3 and 6 years, respectively. Naive CD4$^+$CD45RA$^+$bright T cells increased to 49/uL, 65/uL, 100/uL, 125/uL, at day +28, and at 1, 3, 4 years, respectively. Memory CD4$^+$CD45R0$^+$bright remained stable with median values of 153/uL and 158/uL by day +28 and 3 months, respectively; then increased to 224/uL, 333/uL, 433/uL, 515/uL, at 1, 3, 6 years. Moreover, the evaluation of the coexpression of the CD45 isoforms showed that the number of CD4$^+$CD45RA$^+$CD45R0$^+$ T cells reached median values of 63/ uL by day +28 and 77/ uL at 6 months; then increased to 138/uL, 185/uL, 286/ uL at 2, 3, 5 years, respectively. CD8$^+$ T cells reached median values of 147/ uL by day +28, increasing to 729/uL, 1021/microliters, at 6 months, and at 4 years, respectively. CD4/CD8 ratio was 1.8 by day +28, decreased to 0.7 at 3 months, and remained low at 0.72 and 0.79 at 2 and 5 years, respectively. In a subset of 14 patients the presence of naive CD4$^+$CD62L$^+$CD45RA$^+$bright T cells and of memory CD4$^+$CD62L$^-$ CD45R0$^+$bright T cells was evaluated. Preliminary data showed an increase of these cell populations at 3 years with a median value of 882/uL, and 532/uL, respectively, while they remained stable at 5 and 6 years. TREC copies/100 ng DNA of PBMC were measured in 29 patients at the same timepoints: median baseline value was 0.7, then they gradually increased to 5 at 6 months, and reached 21.5 at 3 years, and remained stable at 5 and 6 years. A significant correlation was demonstrated between TREC values and CD4$^+$CD62L$^+$CD45RA$^+$bright T cells (p<0.0001). *Conclusions*. Our findings suggest a slow T immune reconstitution during the first two years post-transplant that differs from normal T lymphocyte ontogenesis. Prelminary results show a significant correlation between the quantitative analysis of TRECs and the analysis of very naive T cells by CD62L expression and will allow to quantify the residual thymic function in this group of elderly patients.

PO-802

VACCINATION WITH HOST DENDRITIC CELLS IN MULTIPLE MYELOMA AFTER T CELL-DEPLETED RIC-SCT

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Introduction. The beneficial effect of allogeneic SCT in MM is based on graft-versus-myeloma reactivity. The proof of principle is the observa-

tion of clinical responses after donor lymphocyte infusions (DLI). In this study feasibility of T cell-depleted RIC-allogeneic SCT after autologous SCT is studied, and the application of host dendritic cells (DC) as adjuvant immunotherapy. *Materials and Methods.* 13 MM patients received a T cell-depleted allogeneic SCT after conditioning with fludarabine 30 mg/m^2/d and cyclophosphamide 1200 mg/m^2/d on days -6 to -3. After discontinuation of cyclosporine A and 2 low-dose DLI, patients without GVHD and minimal residual disease (MRD) will be vaccinated with *in vitro* -cultured, monocyte-derived autologous DC. Currently, the median follow-up is 301 (range, 81-360) days. *Results*. Prior to RIC-SCT, PBMC from all these patients were collected by leukapheresis and cryopreserved (2-4 bags of 6-12×10^9 cells/patient). Validation experiments showed that thawed monocytes can be used to generate mature monocyte-derived DC with a good yield. Functional assays demonstrated that the generated products have a mature phenotype (>80% CD80$^+$CD83$^+$), a good migration capacity towards lymph-node chemokines and the ability to stimulate T cells in a MLR reaction. All 13 patients engrafted. Three months after RIC-SCT, 7 patients were complete donor chimeras, 4 patients were mixed chimeric (5-26% recipient cells) and 2 patients were not evaluated (1 patient died of cardiac failure, 1 patient was transplanted recently). Six patients developed GVHD, 5 patients grade II and 1 patient grade I. Six patients with residual disease were treated with low-dose DLI (1.0×10^6 T cells/kg), and 4 patients received a second DLI (5×10^{6}T cells/kg). None of these patients developed GVHD. Currently, 3 patients with MRD are receiving vaccinations with autologous DC. *Conclusions*. The combination of T cell-depletion and RIC is feasible in MM-patients treated with autologous SCT before. All patients engrafted and aGVHD grade I and II occurred in 46% of the patients. Thawing- and culture procedures were optimized to use monocytes collected before RIC-SCT for culturing mature DC. Currently three patients are receiving vaccinations with autologous DC.

PO-803

HOST DENDRITIC CELL VACCINATIONS TO IMPROVE THE GRAFT-VS-MYELOMA EFFECT OF DONOR LYMPOCYTE INFUSIONS

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Introduction. Allogeneic stem cell transplantation (allo-SCT) followed by donor lymphocyte infusions (DLI) can induce powerful Graft-vs-Myeloma (GvM) effects in 30-50% of Multiple myeloma (MM) patients. Sustained remissions occur predominantly in association with graft-versus host disease (GvHD) and are most likely dependent on the presence of host dendritic cells (DC) at the time of DLI. Thus, improving the GvM effect of DLI may be possible by combination of DLI with infusion of host DCs. To test the safety and efficacy of this novel approach, we are conducting a phase I/II trial where DLI-nonresponder MM patients are vaccinated with autologous DCs in combination with DLI. *Materials and Methods.* Monocytes derived from G-CSF mobilized, cryopreserved PBMC of the recipients are differentiated into mature DCs in a 5-day protocol. Patients receive three DC vaccinations with two week intervals. The first vaccination is combined with DLI. DCs are administered both iv and id. A fraction of DCs is loaded with KLH for immunomonitoring purposes. During and periodically after vaccination, blood samples are collected to monitor the immune status and the development of anti-host and KLH-specific T cell responses. *Results*. So far one patient has been treated. While he had no response to previous DLI without DCs, two weeks after the first DC vaccination+ DLI his very mild pre-existing GvHD of mouth and eyes was exacerbated, requiring systemic prednisone treatment. A skin test at week six revealed positive induration against host-DCs. *In vitro* assays demonstrated the development of T cell responses against KLH and host DCs starting from the last vaccination date. The KLH- and anti-host responses diminished one month after vaccination, probably due to systemic prednisone treatment. No GvM responses were observed during this period. *Conclusions*. These preliminary results indicate the feasibility of boosting the anti-host responses in MM patients by combination of DLI with host DC vaccinations. These anti-host responses increased the severity of pre-existing GvHD. However, data from more patients are required to determine the safety and efficacy of this novel immunotherapy strategy.

PO-804

GENETIC LINKAGE ANALYSES TOWARDS IDENTIFICATION OF HLA CLASS-II RESTRICTED MINOR H ANTIGENS

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Introduction. Donor T cells directed at minor Histocompatibility antigens (mHags) are involved in the Graft-vs-Host Disease (GvHD) and the Graft-vs-Myeloma (GvM) effect after HLA-matched allogeneic stem cell transplantation (allo-SCT). Identification of these mHags can provide important insights for the development of novel strategies for the separation of GvM from GvHD. We previously isolated several HLA-class II restricted mHag-specific T cells from a multiple myeloma (MM) patient after allo-SCT from his HLA-identical brother. Here we used genetic linkage analyses (GLA) to localize the gene locus encoding the mHag recognized by the HLA-DQ2 restricted T cell clone #21. *Materials and Methods.* The GLA made use of large pedigrees from the CEPH family collection, who are typed for thousands of genetic markers. The mHag phenotype of 107 CEPH members was determined by testing their (HLA-DQ2 transduced) EBV-transformed B cell lines for recognition by clone #21. Subsequently, pairwise linkage analyses (PLA) were executed to determine the segregation of mHag phenotypes with genetic markers. The genetic locus of the searched mHag was further narrowed by haplotype association analyses in which mHag phenotypes of CEPH members were compared with the genotypes of all known single nucleotide polymorphisms (SNPs) in the determined region. *Results*. The PLA revealed that the gene for the searched mHag was strongly linked to a large cluster of markers on chromosome 16 p11.2-16q22. This 16,8 cM region contained 289 genes and 4146 *tagging* SNPs. Comparison of the mHag phenotypes with the genotype of these 4146 SNPs in 25 CEPH members revealed 100% match between the mHag phenotypes and genotype of 2 *tagging* SNPs located in close proximity to the RABEP2 and CD19 genes. Since clone 21 recognizes B cells but not monocytes, the CEPH members, recipient and the SC donor were analysed for CD19 polymorphisms. All mHag$^+$ subjects carried the same CD19 allele, which was absent in all mHag$^-$ subjects. *Conclusion*. The mHag recognized by HLA class II-restricted clone 21 is located at chromosome 16. Our data points to CD19 gene as a strong candidate. Studies are underway to explore this intriguing possibility.

PO-805

REBUILDING HLA CLASS-II RESTRICTED, MYELOMA ASSOCIATED MINOR H ANTIGEN SPECIFICITY IN RECALL ANTIGEN-SPECIFIC T CELLS BY T CELL RECEPTOR TRANSFER

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Introduction. Purpose: Donor T cells directed to hematopoietic minor histocompatibility antigens (mHags) are appealing tools for adoptive immunotherapy of haematological malignancies after allogeneic stem cell transplantation (allo-SCT). Towards development of a convenient strategy for *ex vivo* generation of HLA class II-restricted mHag specific T cells, we evaluated the feasibility of rebuilding mHag-specific T cell functions in donor-derived recall antigen-specific T cells via T cell receptor (TCR) transfer. *Material and Methods.* TCR α- and β-chains of an HLA-DPB1*0401-restricted T cell clone recognizing a multiple myeloma-associated mHag were retrovirally transferred into a Tetanus Toxoid (TT)-specific clone derived from the original SC donor. TCR double transduced cells were compared with the parent mHag- and TT-specific clones for antigen-specificity, cytokine secretion, cytotoxic activity and were analyzed for their *in vitro* expansion capacity in a TT- or mHag-specific fashion. *Results*. mHag-TCR transduced TT-specific cells displayed both TT- and mHag-specificity. Similar to the parent cells, they secreted Th-1 cytokines and exerted significant cytotoxic activity against TT-pulsed or mHag+ target cells, including multiple myeloma cells. 4-Week expansion of TCR transduced cells via the TT specific TCR had no negative influence on the mHag specific cytotoxic activity and resulted in 10 100 fold better cell yields as compared to mHag-specific expansion. *Conclusion*. HLA class II-restricted, mHag-specific effector functions can be successfully reconstructed in donor-derived TT-specific T cells via TCR transfer. Effective expansion of these T cells via TT-specific TCRs illustrate the suitability of this strategy for *ex vivo* expansion, and maybe also for *in vivo* TT-specific reboosting of HLA class II-restricted immunotherapeutical T cells.

PO-806

CD4+CD25+FOXP3+ T REGULATORY CELLS RECONSTITUTE AND ACCUMULATE IN THE BONE MARROW OF PATIENTS WITH MULTIPLE MYELOMA FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION

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Introduction. Peripheral tolerance is largely maintained by immunosuppressive regulatory T cells (Treg), such as $CD4^+CD25^+$ T cells co-expressing transcription factor forkhead box P3 (FOXP3) and it has been suggested that Treg contribute to the prevention of graft-versus-host disease (GVHD) following allogeneic stem cell transplantation (alloSCT). Unfortunately, Treg also represent a main obstacle of an effective anti-tumor T cell response and depletion of $CD4^+CD25^+FOXP3^+$ Treg seems to enhance anti-tumor immunity. It is unclear, however, whether a reduced number or function of Treg might play a role in the induction of graft-versus-myeloma (GVM) effects in multiple myeloma (MM) patients post alloSCT and very little is known about Treg residing in the bone marrow (BM) of these patients. *Methods*. We performed the first systematic analysis of Treg numbers and function in the BM and in the peripheral blood (PB) of MM patients treated with alloSCT (N=40), newly diagnosed MM patients (N=17), and healthy BM donors (N=20) using flow cytometry and functional assays. Mechanisms which might serve as potential mediators of the immunosuppressive function of BM Treg were investigated using real-time PCR. *Results*. Following alloSCT, donor-derived $CD4^+CD25^+FOXP3^+$ Treg expanded faster than conventional $CD4^+$ T cells, leading to an accumulation of Treg in the BM of transplanted patients. Since patients post alloSCT are devoid of a relevant thymic function, Treg reconstitution was most likely based on peripheral expansion. This idea was supported by the fact that reconstituted BM $CD4^+CD25^+FOXP3^+$ Treg of MM patients post alloSCT consisted preferably of CD45RA-CCR7- memory T cells. BM-residing Treg of newly diagnosed and MM patients post alloSCT showed a strong inhibitory function and transforming growth factor (TGF)-β1 seemed to represent an important mediator of Treg function in the BM of MM patients post alloSCT and might also be involved in the expansion of BM-residing $CD4^+CD25^+FOXP3^+$ Treg. *Conclusions*. Our study demonstrates for the first time that BM-residing Treg expand outside the thymus and accumulate in the BM of MM patients post alloSCT. These Treg, which are donor-derived and lead to an efficient replenishment of Treg in the periphery, might be necessary for the prevention of GVHD. However, BM Treg might also contribute to the failure of an effective GVM effect in these patients.

PO-807

IMPACT OF HLA ON AVAILABILITY OF A DONOR, GRAFT VERSUS HOST DISEASE FREQUENCY, AND SURVIVAL AMONG 611 MYELOMA PATIENTS TRANSPLANTED AT THREE INSTITUTIONS FROM THREE CONTINENTS

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Aim. Although GVHD and mortality in allogeneic hematopoietic stem cell transplantion (HSCT) are more frequent among myeloma than among other HSCT patients, the underlying mechanisms have not been clarified yet. Patients: Locally HLA typed (611 AB, 444 ABDR typed) myeloma patients from Ankara (n: 91), Stanford (n:400) & Salamanca (n:120) Universities received either HLA identical sibling/unrelated donor(n:117) or autologous (n:370) HSCT, or were treated with chemotherapy alone (n:124). Most of the alloHSCTs were performed in a tandem autologous-allogeneic approach using a reduced intensity conditioning regimen and peripheral stem cells between 1986-2005. SPSS version 13.0 was used for Chi Square and Kaplan-Meier(univariate survival)(OS) analysis. P values were corrected for the number of antigens tested. *Results*. We have recently reported similarity and differences in HLA antigen frequencies between Spanish, Brazilian and Turkish myeloma patients, patients-controls. In the current analysis, HLA antigen frequencies were found to be similar between patients who had a donor and were allo-transplanted versus those not transplanted because no donor was found. Analysis of the HLA types observed most frequently among the 35 patients with acute GVHD revealed a trend to an increase in the frequency of HLA-A2 (n:21,OR: 1.91). All B51DR4 patients(n:5) developed chronic GVHD(5/57). HLA-DR11(n:19) was associated with less frequent cGVHD (8/57,OR:0.42). OS analysis in allo HSCT patients revealed a good prognosis effect for DR1 (p=0.08), DR7 (p=0.16), DR53 (homozygous versus heterozygous) (p=0.16) and B51DR4 (p=0.16). Antigens / combinations with unfavorable impact on OS were B8 (p=0.12), A1B8DR3 (p=0.022). A1B8DR3, decreased disease free survival (DFS) as well. A1 (p=0.2) and B35DR1 (p=0.00) were the other specities causing a shorter DFS. *Conclusion*. This is the first study evaluating the role of HLA as a susceptibility marker for GVHD and survival in a mixed population of myeloma patients of various ethnic backgrounds. Some of the combination/haplotypes analyzed here have been recently found to be important risk factors in HSCT for other hematological disorders and may be involved in the events leading to myeloma HSCT patients having survival less than that for most leukemia HSCT patients.

PO-808

HUMAN MESENCHYMAL STEM CELLS IN PATIENTS WITH MULTIPLE MYELOMA DO NOT CARRY THE 13Q14 DELETION AND REMAIN OF RECIPIENT ORIGIN AFTER SEX-MISMATCHED ALLOGENEIC STEM CELL TRANSPLANTATION

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Introduction. Human bone marrow contains mesenchymal stem cells (MSC) that can differentiate into various cells of mesenchymal or other origin. Recent studies in different haematological malignancies showed, that MSC remain of host origin even for some time after allogeneic transplantation in the great majority of the cases. Another investigation showed that in 2 of 19 cases MSC from donor origin can be observed in patients' bone marrow after allogeneic bone marrow transplantation. Interestingly both patients were multiple myeloma patients. It is well known, that these patients have an impaired bone marrow microenvironment and this fact could account for donors' MSC engraftment. Therefore we investigated whether MSC are of donor origin in a larger cohort of multiple myeloma patients after allogeneic transplantation. Another aim of this study was to determine whether the plasma cell specific deletion of the long arm of chromosome 13 (del 13q) could be found in the MSC. *Materials and Methods.* We investigated MSC derived from 13 myeloma patients' bone marrow aspirates who underwent allogeneic stem cell transplantation in the last 18 months with a sex-mismatched donor. Six of those patients carried a 13q deletion before transplantation. MSC were grown according to the method described by Caplan *et al.* The cells were analyzed by dual-colour- interphase *in situ* hybridization (FISH) analysis using centromere-specific DNA probes for X and Y chromososme and a probe specific for the region 13q14 (D13S25). *Results*. In all but one case the investigated MSC were of host origin. One patient showed the sex of his donor in 30% of the analyzed nuclei. None of the 6 patients with a known 13q14 deletion had evidence of this genetic aberration in his MSC. *Conclusion*. This data show that MSC in contrast to myeloma cells do not carry the 13q14 deletion. Furthermore, after allogeneic stem cell transplantation the MSC remain of recipient origin.

PO-809

REDUCED INTENSITY CONDITIONED ALLOGENEIC TRANSPLANT (ALLO-RIC) VERSUS A SECOND AUTOLOGOUS PROCEDURE IN CHEMOSENSITIVE PATIENTS WITH MULTIPLE MYELOMA (MM) NOT ACHIEVING COMPLETE REMISSION (CR) OR NEAR-CR WITH A FIRST AUTOLOGOUS TRANSPLANT. RESULTS FROM A SPANISH PETHEMA/GEM STUDY

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Background. It has been shown in non-randomized studies that tandem

transplant results in an increased CR rate. A randomized trial showed that tandem transplant resulted in a significantly longer EFS and OS in patients failing to achieve CR or near-CR with a single transplant. However, other studies failed to show survival benefit from a second transplant. *Aim*. To investigate the efficacy in terms of response up-grading and survival from a second transplant intensification in patients with chemosensitive disease who failed to achieve CR or near-CR with a first transplant. *Patients and methods.* Patients diagnosed with MM from Oct 1999 to Dec 2004 younger than 70 years received 6 courses of VBMCP/VBAD and responding patients were intensified with busulphan/melphalan or MEL-200 followed by stem cell support. Patients not achieving CR or near-CR were planned to undergo a second transplant (second auto with CVB - cyclophosphamide, etoposide and BCNU - or MEL-200 intensification or an allo-RIC with Fludarabine/MEL-140 conditioning, if sibling donor available). *Results*. Eighty-eight patients received a second autologous transplant while 26 underwent an allo-RIC. Thirty-seven percent of the patients given a second autologous transplant up-graded their response status (CR 11%, near-CR: 6%, PR: 9% and MR 11%) while 63% showed *no change*, progressive disease or early death. A response up-grade was observed in 45% of patients undergoing the allo-RIC (CR: 33%, PR: 4%, MR: 8%). The CR rate was significantly higher with allo-RIC (33% vs. 11%, p=0.02). There was a trend towards a higher TRM with the allogeneic procedure (5% vs. 16%, p=0.09). Although the median EFS (26 vs 19 m) and OS (57 m vs not reached in allo-RIC) from the second high-dose procedure were not significantly different, there is a plateau in the *allo-RIC* group beyond 3 years of the second procedure not observed in the autologous arm. *Conclusions*.1) an allo-RIC transplant after an autologous procedure results in a significantly higher CR rate than a second autologous transplant, and 2) although we found no significant differences in survival between the two transplant modalities, there is a plateau in the allogeneic group.

PO-810

A MATCHED CASE COMPARISON OF TANDEM AUTOLOGOUS STEM CELL TRANSPLANT TO AUTOLOGOUS-NON-MYELOABLATIVE ALLOGENEIC TRANSPLANT IN PATIENTS WITH MULTIPLE MYELOMA

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Tandem autologous peripheral stem cell transplant (PSCT) has been shown to improve overall survival (OS) and progression free survival (PFS) compared to single transplant in patients with multiple myeloma (MM). Tandem autologous-non-myeloablative allogeneic PSCT has also been reported to result in PFS in approximately half of MM patients with short follow up. Whether or not auto-non-myeloablative allogeneic PSCT (auto-allo) is superior to tandem autologous PSCT (auto-auto) in long-term disease control remains to be determined by ongoing phase III studies. A prospective French study (IFM-99) compared these 2 approaches in high-risk MM patients (β2M >3.0 and chromosome 13 deletion), and reported no advantage for auto-allo transplant. We performed a retrospective matched case analysis in 105 MM patients treated in IRB approved phase II studies at our institution to address this issue. Seventy patients underwent auto-auto PSCT (Mel 150 mg/m^2 in first and Bu 12.8-16 mg/kg, Cy 120 mg/kg in second PSCT followed by maintenance IFN + thalidomide) were compared to 35 patients who received auto (Mel 200 mg/m^2)-matched sibling allo PSCT (TBI 200 cGy with CSA + MMF) in 2:1 ratio. The 2 groups were matched for age, time from diagnosis and stage by Salmon-Durie. Subgroup analysis was performed to evaluate the influence of chromosome 13 deletion (data available in 80 patients) and B2M (data available in 100 patients). The probabilities of OS and PFS at 2 years were similar between the 2 groups with OS of 80% in the auto-auto group and 83% in the auto-allo group and PFS of 63% in the auto-auto group and 68% in the auto-allo group. However at 4 years the 2 groups separated with a trend for an OS advantage in favor of the auto-allo group with OS of 78% compared to 62% in the auto-auto group (p=0.07). Four-year PFS rates were 39% and 58% in the auto-auto and auto-allo groups respectively (p=0.14). Relapse rate was significantly lower in the auto-allo group (33%) than the auto-auto group (56%) at 4-years (p=0.05). Two-year non-relapse mortality rates were 5%and 10% in the auto-auto and auto-allo groups respectively (p=0.56). Subgroup analysis revealed no statistically significant association between chromosome 13 deletion and OS and PFS in the auto-allo group. No statistically significant correlation between B2M level and outcome was found in either group. *Conclusion*. Auto-allo PSCT is associated with statistically significant lower risk of relapse, which may ultimately translate to better long-term outcome as compared to tandem auto-PSCT in patients with multiple myeloma.

PO-811

REDUCED INTENSITY CONDITIONING (RIC) ALLOGENEIC STEM CELL TRANSPLANTATION (SCT) IS DISAPPOINTING IN MULTIPLE MYELOMA WHATEVER THE AGE OR THE STATUS OF THE DISEASE BEFORE SCT, A MULTICENTRIC BHS STUDY

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Introduction. Allogeneic stem cell transplantation (SCT) is still the only possible curative approach for multiple myeloma (MM); however this treatment is associated with high transplant related mortality (TRM). Therefore RIC regimens are used for older patients but their efficacy is still controversial in the setting of MM. Objectives. We prospectively studied the role of ATG in a fludarabine (F) phosphate based RIC regimen in pts with lymphoid malignancies not eligible for standard allogeneic transplantation. We analysed the subgroup of multiple myeloma pts and we compared pts 60 years old (y.o.) or more to the younger population in terms of TRM, event-free survival (EFS) and overall survival (OS). Population: A total of 46 MM pts were enrolled and 40 of those pts are evaluable. Pts were conditioned with F (30 mg/m^2 for 4 days) plus cyclophosphamide (1 g/m^2 for 3 days). In absence of GVHD, cyclosporine was tapered from day +100. All pts received cyclosporine (3-5 mg/kg IV daily) and ATG (40 mg/kg, n=9 or ATG 20 mg/kg, n=31) *Results*. The median age was 57 years (range 39-68) and 14 pts had 60 y.o. or more. The median follow-up was 33.76 months (6.7-66) for survivors. 25 pts were male and 21 female. The diseases status prior to transplantation was complete remission (CR) for 6 pts, partial remission (PR) in 21 cases and stable or progressive disease (PD) for 12 pts (8 PD). In the group of older pts there were 76% of PR and 23% of PD or SD. For pts younger than 60 years disease status was PR in 42%, CR in 25% and PD or SD in 33.3%. Engraftment rate was 100% of the evaluable pts. At d90, full T-cell (>90%) donor chimerism was achieved in 81% of the pts. We observed a low incidence of acute GVHD grade I-II (25%) no severe aGVHD and 50% of limited chronic GVHD. Overall TRM was 15% (6/40). It was higher in older pts (≥60 y.o.) 28.6% (p=0.029, HR 5.1) than in the younger population (8%). Median overall survival (OS) and median event free survival (EFS) were respectively 29.47 and 10.36 months. We observed a significant difference in term of OS according to age: 13, 3 months (≥60 y.o.) versus 33.5 months for the younger group (p=0.0293, HR 2.44) due to more toxic deaths. The median EFS was also reduced in the older population 5.28 months vs 22.23 months but the difference was not significant (p=0.098). In high risk pts (PD+SD) the median OS was 31.2 months with an median EFS of 7.8 months, for pts in CR the median OS was 33.5 months and the median EFS was 21.5 months and for patients in PR respectively 48.9 and 24 months. At this time only 5% of the pts are still in CR. *Conclusions*. In our experience, RIC allogeneic transplantation with ATG *in vivo* T-cell depletion results in low EFS without reaching a plateau. Patients older than 60 years had a higher TRM and lower OS. Absence of prolonged remission may be explained by the low level of GVHD.

PO-812

LONG-TERM OUTCOME OF RIC-ALLO SCT FOR MYELOMA

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Introduction. Reduced intensity conditioning regimens (RIC) for allogeneic stem cell transplantation (SCT) in MM can reduce regimen-related toxicity while retaining the graft-versus-tumor effect. Partial T-cell depletion can prevent GVHD, and establish a platform for subsequent immunologic manoeuvres. We have conducted a prospective phase II trial to investigate the efficacy of a partial T-cell depleted RIC-SCT followed by DLI in case of MM persistence or relapse. *Materials and Methods.* Twenty-four patients have been enrolled. The median age was 56 years (range 31-69), the median number of previous chemotherapy cycles was 2 (range 1-4), 20 patients (83%) received a previous autologous transplantation. Thirteen patients (54%) were chemosensitive.

Conditioning regimen consisted of thiotepa 5 mg/kg, cyclophosphamide 60 mg/kg, fludarabine 60 mg/ms. Six patients received ATG (5 mg/kg) and 12 alemtuzumab (7.5 mg/ms). All patients received cyclosporine and a short course of methotrexate. All patients received SCT from a sibling donor. Disease progression or relapse, in absence of active GVHD, were treated with cyclosporine withdrawal plus DLI. At last follow-up a quality of life (QoL) assessment was performed with the EORTC-MY24 and FACT-BMT scale. *Results.* All patients engrafted. With a median follow-up of 36 months (range 3-75) 16 (67%) patients are alive. Cumulative TRM at 100 days was 4%, and at 1 and 5 years was 13%. Acute GVHD grade I occurred in 5 (21%) patients, and one (4%) patient experienced grade III aGVHD. One patient (4%) had limited and 2 (8%) patients extensive cGVHD. In 5 (21%) cases cyclosporine was withdrawn early and 9 (37%) patients received one or more DLI. Nine (37%) patients had a clinical response. PFS at 4 years was 4%. OS at 5 years was 59%. Chemosensitive patients had a better OS than chemorefractory (82% vs. 21% at 3 years, p=0.02). Interestingly, QoL of surviving patients was satisfactory for all scales. *Discussion*. RIC-HSCT with partial T-cell depletion for MM can offer a long term survival, particularly for chemosensitive patients, despite an high rate of disease progression or recurrence. This approach seems a reasonable platform for subsequent immunologic manoeuvres, aimed to control the disease and maintain a satisfactory QoL.

PO-813

EXTRAMEDULLARY RELAPSES AFTER ALLOGENEIC STEM CELL TRANSPLANTATION IN MULTIPLE MYELOMA PATIENTS: FREQUENCY AND TREATMENT

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Several reports have suggested an increased incidence of extramedullary relapses after allogeneic nonmyeloablative stem cell transplantation (NMA SCT) and that these relapses have a poorer prognosis compared to systemic or local relapses. We retrospectively collected data on 172 patients, all treated by a tandem autologous, nonmyeloablative allogeneic SCT in transplantation centers in several academic hospitals. Fifty four (31%) patients relapsed following this procedure. There were 43 (79.6%) systemic relapses, including 6 (11.1%) with extramedullary localization of disease. Six patients (11.1%) had a local medullary and 5 (9.3%) had a local extramedullary relapse. Patients with deletion of chromosome 13 had a significantly higher incidence of extramedullary relapse. Treatment consisted of DLI in 21 patients (38.9%), radiotherapy in 19 patients (35.2%), reinduction chemotherapy in 40 patients (74.1%) and second line reinduction chemotherapy in 3 patients (5.6%), given as single line or in combination. One patient achieved MR, 10 patients PR, 3 patients VGPR, 11 patients CR, and 6 patients could not be evaluated because follow-up was less than 3 months. This results in a total response rate of 52.1% of patients. Response to treatment was not different in patients with local relapse or extramedullary disease, when compared with patients with systemic relapse or relapse without extramedullary disease. Median overall survival following relapse after allogeneic NMA SCT was 2.6 years (range, 0.1-3.7+). Overall survival and progression-free survival were not statistically different in patients with local relapse or relapse with extramedullary disease, when compared to patients with systemic relapse or relapse without extramedullary disease. In conclusion, the incidence of relapse with extramedullary disease following allogeneic NMA SCT was 20.4%, which is not different from the incidence in myeloablative SCT or autologous SCT. There was no negative effect of extramedullary disease on response rate, overall survival, and progression-free survival.

PO-814

IMPROVED OUTCOME WITH TANDEM TRANSPLANTATION IN PATIENTS WITH T(4;14) MULTIPLE MYELOMA. LONG-TERM RESULTS OF THE IFM99 TRIALS.

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Translocation (4;14) is associated with a poor outcome in multiple myeloma (MM) patients treated either with conventional or high-dose therapy (HDT). However the largest series of *de novo* t(4;14)-positive pts treated with HDT never exceed 25 pts and additional data are needed to assess the impact of such a therapy in this subset of pts. Between 04/2000 to 12/2003, 1064 pts under 66 years of age with *de novo* MM were enrolled into 3 prospective IFM therapeutic trials of double intensive therapy, IFM99-02 (Attal, Blood 2006;108:3289), IFM99-03 (Garban, Blood 2006;107:3474) and IFM99-04 (Moreau, Blood 2006;107:397). FISH analysis for t(4;14) was feasible in 716 cases and observed in 100 pts (14%). Pts were 52 males, 48 females, with a median age of 58 y. At the reference date of 15 may, 2006, the median follow-up time for living pts was 46 months. The median OS from diagnosis for the whole group of pts was 40 months, and the estimated 5-year survival was 38%. The median EFS was 21 months, and the estimated 5-year EFS was 6%. In univariate analysis, 4 factors were associated with an adverse outcome for both OS and EFS: initial b2-microglobulin > 4 mg/L, initial hemoglobin (Hb) level < 10 g/L, response to induction therapy (VAD regimen) less than partial response (PR), and overall response after HDT less than PR. Multivariate analysis revealed than initial β2-microglobulin and Hb level were statistically independent predictors for both OS and EFS. These 2 factors allowed the determination of a simple prognostic model and the selection of a group of pts with a better prognosis: those presenting at diagnosis with both low β2-m < 4 mg/L and high Hb > 10 g/L (low-risk: 46% of the pts) had an expected OS of 56% at 6 years, and a median EFS of 27 months; conversely pts with only one adverse prognostic factor (either high β2-m or low Hb, 39% of the cases) had a median OS and EFS of 29 and 21 months, respectively, and pts with both high b2mic and low Hb (15% of the cases) had a median OS and EFS of 19 and 12 months, respectively (OS, p=5.10-7 and EFS p=.0004, log-rank test). Our study, the largest ever reported of t(4;14) treated with HDT, indicates for the first time that a subgroup of pts with low β2-m and high Hb level at diagnosis experiences prolonged OS and EFS. This group of pts clearly benefits from intensive therapy. For other pts, especially those presenting with high β2-m and low Hb level, new treatment modalities should be explored.

PO-815

A PETHEMA STUDY OF HIGH-DOSE THERAPY/STEM CELL SUPPORT (HDT), INCLUDING TANDEM TRANSPLANT, IN PRIMARY REFRACTORY MULTIPLE MYELOMA (MM): IDENTIFICATION OF TWO POPULATIONS WITH DIFFERENT OUTCOME

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Background. It has been claimed that patients with primary refractory myeloma benefit from early HDT. However, the reported series have 2 shortcomings: 1) patients with *unresponsive-progressive disease* vs those *non-responding-non progressing* were not analyzed separately and 2) some included patients had MR or even PR according to the EBMT criteria. *Aim*. Response and survival after early HDT in the two populations of truly primary refractory MM. *Patients and methods.* From Oct 1999 to Dec 2004, 829 patients with MM received 6 cycles of VBMCP/VBAD and at least one transplant. 79 of the 829 patients were refractory to VBMCP/VBAD. These resistant patients were scheduled to receive a

tandem transplant, the first with Bu-12/MEL-140 or MEL-200 and the second *autologous* with CVB or *allo-RIC* (if donor available) with Fluda/MEL-140. Response and progression were defined by the EBMT criteria. *Results*. 32 of the 79 primary refractory patients had progressive disease under the initial chemotherapy while 47 were *unresponsive, non-progressive*. The prognostic features, including cytogenetics, were similar in both groups. 70% of the patients responded to the first HDT (CR/nCR 8%, PR 48%, MR 13%). 37 patients were given a second transplant (26 *auto*, 11 *allo*). 41% who received a second *auto* up-graded their response (CR 9%, PR 14%, MR 18%) while 42% who underwent *allo-RIC* increased their response (CR 28%, PR 14%). Median survival of the whole series was 3 years. Patients progressing while on therapy had a shorter survival than the *no-change* group (median 2 yrs vs not reached, p=0.00002). Finally, the 47 *non-responsive, non-progressors* patients had similar survival than the 718 with chemosensitive disease intensified with HDT. *Conclusions*. 1) HDT in patients with primary refractory MM results in a low CR rate, 2) patients progressing while on initial therapy have a short survival despite the intensive approach and 3) patients with *non-responding, non-progressive* disease have similar survival than chemosensitive patients. Whether this good outcome is due to the impact of HDT or to the natural history of a more indolent disease remains to be determined.

PO-816

AUTOLOGOUS TRANSPLANTATION IN 495 MYELOMA PATIENTS FROM THE CZECH REGISTRY

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Aims. Autologous stem cell transplantation (ASCT) has an important role in the treatment of symptomatic multiple myeloma (MM) patients. The aim of our study was to analyse retrospectively the results of ASCT in 495 MM patients (pts) from the Czech National Registry of Hematopoietic Stem Cell Transplantation. The data from 6 transplantation centres were evaluated in order to identify significant variables associated with progression free survival (PFS) and overall survival (OS). *Methods.* Patients were transplanted between 1994 and 2005, the median age was 56 years, clinical stages according Durie-Salmon were as follows: stage I - 8%, stage II - 29%, stage III - 63%. Transplantation was performed during the first year from diagnosis in 411 pts (83%). The median follow-up from ASCT was 33.3 months. *Results*. Transplant-related mortality to day +100 was 1%, the median time to neutrophil engraftment was 12 days. The treatment responses after ASCT were recorded at 455 (92%) pts, 91 pts (20%) were in complete remission (CR). Patients with progression of MM to one year after ASCT had poor prognosis, median PFS of this subgroup was 7.3 months and median OS was 21.6 months. Long-term outcomes of ASCT were evaluated in subgroup of 44 pts, who had been transplanted before 1997: 12 pts (27%) are alive, 4 pts (9%) are disease free after 10 years from ASCT. Median PFS and OS from transplantation at all 495 pts were 27.5 and 62.3 months, respectively. The significant prognostic parameters for both poor PFS and OS were as follows: IgA type of monoclonal immunoglobulin (p=0.003), renal impairment at diagnosis (p under 0.001), clinical stage III according to Durie-Salmon (p=0.005) and failure to achieve CR after ASCT (p under 0.001). The status of disease before transplantation and the age did not significantly affect PFS and OS after ASCT. *Conclusions*. ASCT in multiple myeloma is a safe and an effective treatment method with a low toxicity. The most significant prognostic factors for longer survival after transplant ASCT are lack of the renal impairment and achievement of CR after transplantation ASCT (p under 0.001).

PO-817

MELPHALAN PHARMACOKINETICS IN MYELOMA

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Aims. To examine the pharmacokinetics of melphalan in myeloma patients undergoing an autograft. *Materials and Methods*. A total of 51 patients (age range: 38 - 73 years) from 6 institutions received high dose melphalan as an intravenous infusion administered over median 0.57 h. The dose was 200 mg/m^2 for 29 patients, while 22 patients received lower doses (range: 115 to 193 mg/m^2) due to renal impairment or obesity. Melphalan concentrations were measured in 12 blood samples that were collected after the infusion end and melphalan pharmacokinetic parameters, including clearance and area-under-the-concentration-versus-time curve ($AUC_{0-\infty}$) were determined using the Kinetica software (Innaphase, USA). Patient weight and glomerular filtration rate (eGFR), estimated using an abbreviated MDRD equation [1], were tested for correlation with melphalan clearance. *Results*. The 200 mg/m^2 melphalan dose resulted in a 4.3-fold variation in exposure, with $AUC_{0-\infty}$ values ranging from 5.8 to 24.7 mg/L.h (median: 12.7 mg/L.h). Wide variability in exposure was also evident in the dose-adjusted group, with $AUC_{0-\infty}$ values ranging from 6.1 to18.4 mg/L.h (median:12.0 mg/L.h). Dose (mg/kg) ranged from 3.17 to 6.12 mg/kg and clearance ranged from 12.8 to 69.2 L/h (median: 30.4 L/h). There was a significant positive correlation between mg/kg dose and $AUC_{0-\infty}$ (r=0.28, p<0.05, n=51) showing a tendency for high mg/kg dose to be associated with high exposure to melphalan. Clearance correlated positively with both weight (r= 0.55, p<0.001) and eGFR (r=0.47, p 0.001). *Conclusions*. The 200 mg/m^2 dose results in widely variable exposure to melphalan. Lighter patients and those with poor renal function tend to have low clearance and high exposure to melphalan for a given dose but together these parameters account for less than 53% of interpatient variability. We are continuing to collect pharmacokinetic data as well as toxicity, efficacy and outcome data so that we can develop improved dosing strategies for melphalan that will provide myeloma patients with optimum levels of exposure.

Reference

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PO-818

OUTCOME AFTER SECOND STEM CELL TRANSPLANTATION FOR RELAPSED MULTIPLE MYELOMA

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Background. Autologous stem cell transplantation (ASCT) improves survival and remains the standard of care for patients (pts) with newly diagnosed myeloma considered eligible for transplant. However, ASCT is not curative and pts relapse after a median interval of 24-30 mos. While new therapeutic options have become available for relapsed MM, repeat ASCT remains a viable option for many of these pts, especially when previously collected stem cells are available. The outcome of pts going to a second SCT in the relapsed setting has not been studied extensively. *Methods.* We identified pts with MM who received a second SCT from a prospectively maintained database. A total of 56 pts received a second SCT for relapsed MM, including 11 allogeneic SCTs. Among the 45 second ASCTs, 5 were followed by reduced intensity allogeneic SCT and were analyzed with the allogeneic group. Planned tandem ASCTs were excluded from the analysis. *Results*. The median age at second ASCT was 60.2 yrs (range, 41.3-74.4) and 27 (68%) were males. The median time to second ASCT from diagnosis, first ASCT and relapse were 47.4 mos (22.8-158.6), 36.3 mos (13.5-129.8) and 7.2 mos (1-32) respectively. Among patients receiving second APBSC, 14 (35%) patients were alive with a median follow up of 10.5 mos (1-45 mos). All pts received melphalan conditioning followed by infusion of median CD34 cell dose of 4.7 million/kg (2.5-14.6). The median time to neutrophil engraftment was 15 days and platelet engraftment was 16 days. The median hospitalization was 4 days (range 0-33) and there was one transplant related death in the group. The best response included 13 pts with

CR (33%), 4 with VGPR (10%), and 19 with PR (48%). MM has relapsed in 22 (55%) pts with a median PFS of 12.5 mos from second ASCT. The median estimated OS from diagnosis, first ASCT and second ASCT were 100.5 mos, 65.9 mos, and 30.6 mos respectively. Among the 16 pts receiving allogeneic SCT, 8 pts (50%) were alive at analysis with a median PFS and OS from transplant of 17.9 mos and 33.5 mos respectively. *Conclusions*. Second ASCT as salvage therapy for relapsed MM is a viable approach and has a favorable outcome in this selected group of patients. The toxicity, engraftment kinetics, hospitalization and the response rates are comparable to patients undergoing initial ASCT. Allogeneic SCT with or with out a preceding ASCT results in comparable survival in selected patients with high risk disease.

PO-819

EARLY TRANSPLANT IS USEFUL FOR OLDER PATIENTS WITH MM

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Introduction. The value of early intensive therapy supported by autologous stem cells for patients with multiple myeloma older than 65 remains controversial so that until recently most oncologists have avoided such treatment. *Methods.* Between 1987 - 1999, only 10 of 171 patients aged 65 - 75 received intensive treatment at our center (6%), in contrast to 50% of similarly aged patients treated from 2000 - 2005. For the latter period, we evaluated frequencies of partial and complete remission, and survival from onset of therapy, for 59 consecutive, newly diagnosed patients between 65-75 years old who received a thalidomide-dexamethasone-based combination as primary treatment. All showed a myeloma protein marker; partial response (PR) required reduction of serum myeloma protein by >50% and of Bence Jones protein by >90%; complete remission (CR) required normal immunofixation. *Results*. The primary response rate was 78%, including 8% with CR. Within 12 months, 30 patients (51%) received intensive therapy with high-dose melphalan supported by autologous stem cells, with no treatment-related deaths. After intensive therapy, 5 of 6 patients with primary resistance (NR) achieved PR, 7 of 23 patients in PR achieved CR (30%), and 1 patient remained in CR, so that myeloma was in PR or CR in all but one patient. For 29 patients with similar age and disease features who received only standard therapy, 4 achieved CR, 18 reached PR, but 7 remained in NR, response outcomes inferior to those observed after intensification (p. 02 for NR to PR). The projected median survival of 30 patients aged 65 -75 who received intensive therapy (7 years) was longer than that of 29 patients who did not qualify for personal or medical reasons (4.0 years) (p<0.01), and similar to that of 394 younger patients (7.0 years) who received intensive therapy between 1987-2005. *Conclusion*. In view of the safety of the procedure for selected older patients, and the potential for longer survival with PR or CR in virtually all patients, early intensive therapy should be considered for most patients aged 65-75, especially those with primary resistant disease.

PO-820

MELPHALAN 200 MG/M² VERSUS 100 MG/M² IN NEWLY DIAGNOSED MYELOMA

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Introduction. The superiority of high-dose (usually 200 mg/m², MEL200) and intermediate-dose melphalan (100 mg/m², MEL100) to standard therapy has been demonstrated. In this prospective, randomized, phase III trial, we compared MEL200 with MEL100. The primary end points were complete remission (CR) rate, event-free survival (EFS) and incidence of gastrointestinal toxicity, infections and early deaths. *Materials and Methods.* Between January 2002 and July 2006, 298 patients were enrolled. Inclusion criteria were previously untreated myeloma, age < 65 and Durie and Salmon stage II or III. Exclusion criteria were abnormal cardiac, respiratory, liver or renal function, HBV, HCV, or HIV positivity, concomitant cancer or psychiatric disease. All patients received: 2 dexamethasone-doxorubicin-vincristine debulking courses, 2 cycles of cyclophosphamide plus G-CSF followed by stem cell harvest. The MEL200 group was conditioned with 2 cycles of melphalan 200 mg/m². The MEL100 group was conditioned with 2 courses of melphalan 100 mg/m². All MEL courses were followed by stem cell reinfusion. *Results*. At the present, 246 patients (median age 57) were evaluable: 124 were randomized to MEL200 and 122 to MEL100. Patient characteristics were similar in both groups. Forty-six patients did not complete tandem MEL200; 36 did not complete tandem MEL100. The very good partial response rate of MEL200 was superior to MEL100 (38% versus 22%, p=0.011), but CR was 17% versus 10% (p=0.2). The median follow-up for censored patients was 26.5 months. The 3 years EFS was 48% in the MEL200 and 31% in the MEL100 arm (p=0.31). The 3 years overall survival was 86% in the MEL200 and 71% in the MEL100 group (p=0.51). Grade 3-4 hematologic toxicity was comparable in two arms, but 84% of MEL200 patients received ≥4×10⁶ CD34⁺/Kg compared with 52% of MEL100 (p=0.0001). Grade 3-4 non-hematologic adverse events were more frequent in the MEL200 (52% versus 34%, p=0.01 in the 1st cycle and 39% versus 31%, p=0.9 in the 2nd cycle). The incidence of severe gastrointestinal toxicity was 51% after MEL200 and 21% after MEL100 (p<0.001). The incidence of grade 3-4 infections was similar in both group (54% of MEL200 versus 45% of MEL100 patients, p=0.25). Early deaths were 6% in the MEL200 and 4% in the MEL100 arm (p=0.9). *Conclusion*. MEL200 resulted in a significantly higher very good partial response rate but this did not translate in a superior EFS and overall survival.

PO-821

IGD MULTIPLE MYELOMA - THE THERAPEUTICAL RESULTS OF CMG

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Introduction. IgD multiple myeloma belongs to relatively rare types of plasmacytomas and affects less than 2% of all MM-patients. Survival of patients with IgD MM is generally believed to be shorter than that of patients with other types of M-protein. Different results of therapy in literature, sporadic occurrence of IgD MM and availability of new drugs were the reason of Czech Myeloma Group to process existing therapeutical results in our region. *Methods.* We carried out a retrospective analysis of 26 myeloma patients treated by 4 Czech and 3 Slovak hemato-oncological centers during the last decade. There were 14 (54%) males and 12 (46%) females aged 37 to 79 years, the median age was 61 years. 10 (39%) patients were treated by high-dose therapy (Melphalan 200mg/m²) with following autologous peripheral blood stem cell transplantation (APBSCT) as the first line therapy (= Group 1), 13 (50%) patients (= Group 2) were treated by conventional chemotherapy (mostly Melphalan+Prednison or any VAD-like regime). *Results*. Evaluation of therapy results was possible in 23 (89%) patients. It is very interesting that we observed 100% therapy response in Group 1 where 7 patients (70%) achieved CR and remaining 3 patients (30%) PR. The median progression-free survival in Group 1 was 18 months resp. 20 months in Group 2, this difference is not statistically significant. The median overall survival of whole analyzed group of patients is 34 months, hereat the median OS in group of patients after APBSCT has not yet been reached (it is still alive 70% patients) contrary to OS in Group 2 which is 16 months only. The difference in overall survival between both groups was found statistically significant when Fischer exact p test (p=0.04) and Coxs F-test (p=0.005) were used for the analysis. *Conclusion*. It is necessary to consider high-dose therapy with following APBSCT when treating patients with IgD MM under 65 years because it is possible to reach similar results compared to patients with other MM types. Further improvement of their outcome might be reached either using new drugs.

PO-822

PATIENTS WITH MULTIPLE MYELOMA, TRANSPLANTED WITH LYMPHOCYTE-RICH PERIPHERAL BLOOD AUTOGRAFTS HAVE MORE FAVORABLE OUTCOME

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Introduction. There is evidence that the number of infused lymphocytes in autotransplanted patients with multiple myeloma influences the outcome of transplantation. Patients and *Methods.* We investigated the impact of infused lymphocytes on the transplantation outcome in 135 patients (52 female, 83 male, median age 57.2 years, range 37.5-71 years) with multiple myeloma, undergoing autologous stem-cell transplantation (auto-SCT). The median number of infused lymphocytes was 1.05×10^9 per kilogram of body weight (range 0.05-5.37). Patients were arbitrarily split into two groups, those who received a lymphocyte-poor autograft ($<0.5\times10^9$ lymphocytes/kg: group A, N=46 patients) and those who, received a lymphocyte-rich autograft ($>0.5\times10^9$ lymphocytes/kg: group B, N=89 patients). The incidence of common and opportunistic infections in the post-SCT period, as well as Kaplan-Meyer's probability for Relapse-Free Survival (RFS) and Overall Survival (OS) were compared between the two groups, by using a log-rank test. *Results*. Time to neutrophil and platelet engraftment was comparable in both groups, however, group A patients had significantly longer post-SCT hospitalization period, as compared to those from group B (26.4±10.9 vs. 22±5.5 days, p=0.002). Common bacterial and viral infections were almost equally documented (group A: 13/46~28.3%, group B: 22/89~24.7%, p: n.s.). However opportunistic infections, including fungal, mycobacterial, varicella-zoster infection and PCP were more frequently encountered in group A (16/46 ~ 34.8%), as compared to group B (14/89 ~ 15.8%, chi square: p<0.05). One patient from group A developed malignant melanoma 64 months post-SCT. After a median follow-up time of 32 months, 17 patients from group A (37%) and 53 from group B (59.5%) are alive, while 8/46 from group A (17.4%) and 33/89 from group B (37.1%) are relapse-free. Median RFS was 12.3 months in group A and 27.5 months in group B (p=0.016). Similarly, median survival from SCT was 26.4 months in group A and 52.1 months in group B (p=0.033). *Conclusions*. Patients with multiple myeloma, transplanted with PBSC autografts containing $>0.5\times10^9$ lymphocytes/Kg of body weight require shorter post-SCT hospitalization, and they have less frequent opportunistic infections, as well as significantly longer RFS and OS, compared with patients transplanted with autografts containing $<0.5\times10^9$ lymphocytes/Kg.

PO-823

THE IMPACT OF AGE IN AN INTENSIVE TREATMENT OF NEWLY DIAGNOSED MULTIPLE MYELOMA (MM) PATIENTS: RESULTS OF THE PROSPECTIVE MULTICENTRIC SPANISH TRIAL GEM-2000

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Objectives. To analyze the impact of age (< 65 years vs ≥65 years) in newly diagnosed multiple myeloma (MM) patients included in a prospective national protocol (GEM2000). *Patients and Methods*. 1088 patients (< 65 years: 874 patients; ≥65 years: 314 patients) were included. Patients were treated with the VBCMP/VBAD protocol and consolidated with an autologous stem cell transplantation (ASCT). Those patients not achieving a complete remission after the first ASCT were treated with a second intensive procedure (an ASCT or a non-myeloablative allogeneic transplantation). Clinical and biological characteristics at diagnosis and at ASCT did not differ between both groups with the exception of β-2-m and ISS at diagnosis, which were higher in the older group. *Results*. Protocol compliance was significantly better in the younger group: 104 out of 296 patients (35%) did not reach the first ASCT in the older group vs 128 out of 792 in the younger group (16%) (p=0.005). Response rate at the different stages of the protocol and procedure related mortality (PRM) (after induction therapy, after the first ASCT and the second transplantation) did not differ between both groups of patients. With a median follow up from diagnosis of 68 (59 - 76) months, overall survival (OS) for the whole series at 2 and 3 years was of 79%±2% and 70%±2% at 2 and 3 years, respectively. Younger patients had a significantly better 2 and 3-year OS (80%±2% and 72%±2% vs 75%±2% and 67%±2%, p=0.0007). With a median follow up of 39 (35-43) months, event free survival (EFS) at 2 and 3 years was 70±1% and 53±1%, respectively. In the same way, EFS was better in the younger group than in the older group at both 2 and 3 years (70±1% and 54±2% vs 68±3% and 50±3%, respectively). *Conclusions*. Older patients were able to follow the protocol to a lesser extend than younger patients. Although PRM and treatment response did not significantly differ between both groups, OS and EFS were poorer in the older group. Adverse biological and clinical characteristics of the older group could account for these differences.

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PO-824

RELAPSE PATTERN AFTER AUTOLOGOUS TRANSPLANTATION IN MULTIPLE MYELOMA: CLINICAL RESULTS AND THERAPEUTICAL IMPLICATIONS OF 441 PATIENTS FROM THE MYELOMA SPANISH GROUP (GEM)

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Background and objectives. Autologous transplantation is widely indicated to treat patients with multiple myeloma (MM). However, only a fraction of patients remain free of disease in the long-term and most patients relapse. The clinical or biological presentation of relapses after intensification therapy is usually heterogeneous and some reports have analyzed this situation due to therapeutical implications. We report the different relapse patterns after autologous transplantation of 441 patients with MM included in the multicentric registry from the Myeloma Spanish Group. *Design and Methods*. The medical records of 860 patients with MM transplanted with autologous stem cells from 1999 to 2006 in different centres in Spain were reviewed. At diagnosis, 69 (8%) had stage I disease, 321(37%) stage II and 468 (55%) stage III. The median time from diagnosis to transplantation was 10.3 months (5-57). The median age was 62 years (37-86) Of the 777 patients assessable for response to intensification therapy after transplantation, 429 (55%) achieved a complete response (CR) and 264 (34%) a partial response (PR). The clinical characteristics of 441 patients (51%) who present relaps (EBMT criteria) after transplant were retrospectively assessed during long-term post-transplantation follow-up. *Results*. At a median follow-up of 19 months, 441 (51%) patients had relapsed or progressed after transplantation. The median overall survival was 54 months (SE 8), (CI 95% 37-68) and median estimated progression-free survival was 34 months (SE 2.2, CI 95% 26-398). The median period for relapse was 19 months (2-60) with an actuarial risk of progression or relapse at 60 months after transplantation of 72%. The relapse patterns were very heterogeneous: 12 cases (3%) presented extramedullary manifestations with multiple plasmacytomas as the main symptoms of relapse. 84 cases (19%) presented only insidious increase of MC protein in serum or urine without other clinical manifestations. In 8 cases (2%) relapse had criteria of plasmacytic leukemia. The remaining patients 337 (76%) presented progressive increase of MC associated with plasmacytic bone marrow infiltration and different clinical myeloma symptoms, mainly new osteolytic lesions. The therapeutical approach was also very heterogeneous mostly receiving high dose steroids and chemotherapy, and including thalidomide or bortezomib recently. The global antitumoral response was 35% with a median overall survival after relapse of 14 months (SE 1.4) (CI 95% 5-34). *Conclusions and comments*. Relapse patterns of MM after high-dose therapy are very heterogeneous. Most patients present classical, progressive and symptomatic disease. Some patients relapse only with stable biological data and insidious evolution. Another group has extraosseous plasmacytomas without systemic disease. Finally, some patients develop an aggressive transformed disease, including leukemic forms. These findings suggest the need of individualized approach during clinical follow-up after transplantation. The type and timing of relapse influence the outcome, with better results for less aggressive forms. At least one-third of patients respond to different therapy strategies with an acceptable median overall survival. New drugs, such as immunomodulators, bortezomib or experimental options applied sequentially or combined, probably will improve the response rate and survival of these patients.

PO-825

CR STATUS AT TRANSPLANT IS AN IMPORTANT PROGNOSTIC FACTOR IN MM PATIENTS RECEIVED UPFRONT SINGLE AUTO-TRANSPLANT

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Upfront high dose myeloablative chemotherapy followed by single autologous stem cell transplantation (ASCT) is a standard therapy for the newly diagnosed MM patients under 65 years. Disease status after the induction chemotherapy is variable, so we evaluated the prognostic effect of the disease status at ASCT in the initial chemo-sensitive MM patients (>PR) enrolled from KMMWP web based registration system. Response was assessed by EBMT criteria after 1998 and older criteria before 1998. Some patients with no detectable M-protein without testing immunofixation were classified as CR. Between November 1996 and January 2007, 197 patients (median age 53 years) received upfront single ASCT were enrolled. Median follow-up times were 27.4 months (5.4-103.8) from the day of diagnosis and 21.2 months (0.4-96.0) from the day of ASCT. CR and PR at ASCT were achieved in 63 (32%) and 134 (68%), respectively. The initial DS stage was not correlated with disease status at ASCT, but initial low stage according to the ISS & SWOG staging system were correlated with CR status at ASCT (p=0.017, p=0.044, respectively). Overall survival (OS) from the day of diagnosis and from the day of ASCT were significantly correlated with CR status at ASCT (p=0.0015, p=0.0013, respectively). OS from the day of diagnosis also correlated with ISS & SWOG staging system (p=0.046, p=0.017, respectively). CR status at ASCT significantly correlated with OS in patients with ISS stage III or SWOG stage III (p=0.0029, p=0.015, respectively), but CR status is not significant correlated with OS survival in the patients with the other stages. OS could be predicted by the ISS & SWOG staging system in the patients achieved PR status at ASCT (p=0.0007, p=0.0011, respectively), but there was no predictive role of these stage in the patents achieved CR. We concluded that CR status at ASCT was very important prognostic factor especially in the patients with advanced stage who received upfront single ASCT. So we should try more intensive induction chemotherapy regimens including new anti-myeloma agents to achieve maximum response status before ASCT especially in the advanced stage MM patients.

PO-826

PROGNOSTIC IMPACT OF GENETIC ABNORMALITIES IN NEWLY DIAGNOSED MYELOMA PATIENTS 60-70 YEARS OF AGE TREATED WITH HIGH-DOSE MELPHALAN (MEL140) AND AUTOLOGOUS STEM CELL TRANSPLANTATION

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Introduction. Chromosomal aberrations can predict outcome in patients with multiple myeloma (MM) but the prognostic heterogeneity of this disease is not fully mirrored by the currently known genetic markers such as deletion of chromosome arms 13q and 17p as well as translocation t(4;14). The prognostic relevance of most recurring genomic changes remains unknown. Moreover, there is scarce data on the significance of chromosomal abnormalities in elderly patients (pts.) receiving high-dose chemotherapy followed by autologous stem cell transplantation (ASCT). *Materials*. Between 05/2001 and 08/2006, 549 patients (pts.) 60-70 yrs. of age with newly diagnosed and symptomatic MM were enrolled in a multicenter trial of the Deutsche Studiengruppe Multiples Myelom (DSMM II) to receive two cycles of high-dose melphalan (140 mg/qm) followed by ASCT after 3-4 cycles of high-dose dexamethason-based induction chemotherapy, *e.g.* VAD (arm A1), or no induction chemotherapy (arm A2). cIg-FISH using a comprehensive DNA probe set for the detection of abnormalities involving the following chromosomal loci was applied to all pts. from that sufficient material has been sent to the genetic reference center of the DSMM: 1q21.2, 6q21, 8p11, 9q34, 11q25, 13q14, 14q32, 17p13, and 22q11. *Results*. As of 01/2007, bone marrow samples from 305 pts. were received at the genetic reference center of the DSMM. An interim analysis of the DSMM II trial will take place in 03/2007 enabling us to correlate clinical with molecular cytogenetic data. *Conclusions*. First results of the study will be presented at the meeting.

PO-827

MEGATHERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH MULTIPLE MYELOMA. THE EXPERIENCE OF A SINGLE HAEMATOLOGICAL CLINIC

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Megatherapy and autologous stem cell transplantation (ASCT) is the treatment of choice for patients suffering from multiple myeloma (MM) aged below 65 years. Purpose:The study of the toxicity of ASCT and its impact on the event free and overall survival of the MM patients *Patients and Methods.* From April of 1999 since November of 2006 37 ASCTs were done in a total of 38 scheduled.29 of them (78,3%) were male and 8 (21,7%) female. The median age was 55 years (38-71).8 patients were older than 60 years. Stem cell mobilization was achieved in 14 patients with G-CSF,in 20 with cyclophoshamide (2 gr/m^2) and G-CSF and in the remaining three with the DCEP regimen and G-CSF.The mobilization yielded a median value of CD34$^+$ cells of 3,22×10^6/kg (2,15-14,42×10^6/kg).The megatherapy consisted of 200 mg/m^2 melphalan. All patients prior to APSCT received the VAD regimen (4-6 cycles),except three(two treated with DCEP, one with vortezomib).Prior to the APSCT 3 of the patients were in CR,31 in PR and 3 of them were refractory to treatment according to the EBMT criteria. *Results*.Everyone tolerated megatherapy well enough. The median time interval for neutrophils to recover >500/il was 10 days (8-20 d) while the same value for PLTs (>20.000/il) was 14 days(9-23 d).Age or abnormal creatinine clearance did not adversely affect these values. Three months after the ASCT all patients in CR prior to it remained the same. From 31 patients in PR 17 achieved CR and the remaining 14 while still on PR exhibited further improvement. From the three patients who were refractory two exhibited PR and the third one remained unresponsive. With a median time interval of observation 40 months (2-81 months) 16 patients relapsed,6 of them in CR. The median time interval of relapse was 15 months (4-54 months). From the 16 patients that relapsed 7 of them died, while 2 are in CR,5 in PR and 2 with refractory disease. *Conclusions*.-Effective stem cell mobilization is totally feasible in MM -ASCT is safe and without mortality even in patients older than 60 years of age -CR after ASCT is the strongest predictive factor for the event free survival and overall survival of the MM patients.

PO-828

IMPROVED REMISSION STATUS AMONG MULTIPLE MYELOMA PATIENTS TREATED WITH TANDEM AUTOLOGOUS STEM-CELL TRANSPLANTATION

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Introduction. Although there is evidence that multiple myeloma patients, treated with tandem autologous stem-cell transplantation (auto-SCT) do better that those treated with a single procedure, the tandem auto-SCT approach has not been widely accepted as gold standard. *Patients and Methods*. We retrospectively analyzed transplantation outcome in 34 patients with multiple myeloma. Nineteen patients (group A) were treated with a single, and the remaining 15 (Group B) with a double tandem auto-SCT. Three patients from group A received a second autograft (2) or an allograft (1) following their relapse. Disease status at 3, 12, 18 and 24 months post-SCT was evaluated according to newly-established International response criteria for multiple myeloma. Relapse-free-survival (RFS) and overall survival (OS) were used as endpoints. *Results*. Two patients from each group were presented mainly with an osseous plasmacytoma. Age and sex distribution, chain isotype, initial stage, performance status, serum prognostic parameters and response to first-line treatment were comparable between the two groups. Disease status at SCT (1st SCT for group B) was also comparable (group A: CR: 1/19 patients, VGPR: 6/19, PR: 11/19, minor response 2/19, group B: CR 0/15, VGPR: 5/15, PR: 9/15, minor response 1/15). Melphalan 200 mg/m^2 was used as conditioning in 15/19 patients from group A and in 12/15 from group B. Engraftment was achieved following 18/19 procedures in group A and 29/30 procedures in group B. Nine patients from group B (60%) upgraded response category following second auto-SCT, and 5/15 achieved a CR, and 4/15 a VGPR. After a median follow-up of 22 months, 11/19 patients from group A are alive, 3 of them in CR, 2 in VGPR, 1 in PR (overall favorable response 31.6%) and 5 have

relapsed. Accordingly, 13/15 patients from group B are alive, 8 of them in CR, 2 in VGPR (overall response 66.7%) and 3 have relapsed. Median overall survival post-SCT was 33.5 months for patients of group A and has not yet reached for patients of group B. *Conclusions*. In our experience, double tandem auto-SCT is a safe treatment approach and improves quality of response in the majority of patients with multiple myeloma.

PO-829

HIGH CR RATE WITH MELPHALAL 280 MG/M² AND AUTOTRANSPLANTATION DOES NOT IMPROVE PROGRESSION-FREE SURVIVAL IN MYELOMA

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Introduction. Achievement of complete remission (CR) has become a goal of therapy in multiple myeloma (MM). Intensive therapy and autologous stem cell transplantation (ASCT) was the first treatment to reliably produce CR; the use of tandem ASCT represents one approach to further increase the CR rate. We evaluated an alternative single ASCT strategy in which an augmented dose of melphalan (MEL) was given with amifostine (AF) to ameliorate toxicity. *Materials and Methods*. MEL 280 mg/m² (MEL 280) (day -1) was preceded by 2 doses of AF 740 mg/m² IV over 5-15 minutes 24 hours and 15 minutes before MEL. *Results*. Between 5/1999 and 10/2003, 58 MM patients (pts) received this regimen after dexamethasone-based induction. Median age was 50 yrs (35-66), 33 were male, median initial β2-microglobulin level was 3.0 mg/L (0.8-22.4). The subtype included IgG in 32, IgA in 15, light chain in 8 and non-secretory in 3; 39 had Durie Salmon stage III disease and all but 6 were chemosensitive. Using the Bearman criteria for regimen-related toxicity (RRT), grade 1 mucositis was seen in 24, grade 2 in 8 and grade 3 in 1 pt. Other grade 3 RRT included lung (1 pt) and renal (1 pt); 1 pt (1.7%) died of respiratory complications. 4 pts experienced atrial fibrillation/flutter. Of 57 evaluable pts post-ASCT, immunofixation-negative CR was seen in 28 (49%), VGPR in 6 (11%), PR in 17 (30%), SD in 4 (7%), unknown response in 1 (1.5%) and progression in 1 (1.5%). At a median follow-up of 3.7 yrs, 38 have progressed and 22 have died due to MM (18) and non-relapse causes (4: 1 RRT, 1 lung cancer, 1 MI and 1 complications of alloSCT). The median acturarial overall survival (OS) has not been reached while the median progression-free survival (PFS) is 2 years. *Conclusions*: 1) MEL 280 + AF is well tolerated; 2) the overall response rate is 90% with 60% CR + VGPR; 3) an analysis of prognostic factors for OS and PFS will be presented; 4) in this trial a high CR + VGPR rate alone post ASCT did not confer an improved PFS.

PO-830

TOXICITY AND EARLY RESPONSE AFTER SINGLE DAILY DOSE OF INTRAVENOUS BUSULFAN AND MELPHALAN AS CONDITIONING REGIMEN FOR AUTOLOGOUS STEM CELL TRANSPLANTATION IN PATIENTS WITH MULTIPLE MYELOMA

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Introduction. Conditioning regimen with oral busulfan (BU) and melphalan (MEL) for patients with multiple myeloma (MM) undergoing autologous transplant (ASCT) has been associated with a high incidence of liver complications, especially severe sinusoidal occlusive syndrome (SOS). *Materials and Methods*. We conducted the current study to assess the toxicity profile and outcome associated with ASCT after intravenous (iv) BU-based conditioning in 29 patients (19 M/10 F; median age 61 years, range 38-68) with MM. Front-line chemotherapy consisted of VBMCP/VBAD (20 cases), VAD (3 cases), and other regimens (3 cases). At transplant, 5 patients were in complete response (1 with negative immunofixation), 19 in partial response, 3 with progressive disease, and 2 with minimal response. Median interval from diagnosis to transplant was 9.5 months (range 5-19). Conditioning regimen consisted of iv BU (3.2 mg/kg in a single daily dose, days -5 to -3) and MEL (140 mg/m², day -2). In 26 patients this was the first ASCT and the remaining 3 had relapsed after a previous ASCT conditioned with MEL alone. *Results*. Median number of CD34⁺ cells administered was 3.06×10^6/kg (range 1.02-5.09). Hematopoietic recovery was fast with median (range) time to 0.5 PMN and 20 platelets $\times10^9$/L of 11 (11-35) and 15 (9-64) days, respectively. The toxicity profile was favourable. No patient developed SOS. Mucositis was the non-hematopoietic toxicity most frequently seen (22 cases). Other toxicities observed were uncommon (increase of liver enzymes, 1 case and mild cardiac failure, 1 case). Fever was observed in 22 patients: fever of unknown origin in 13 cases, microbiologically documented infection in 7 cases and clinically documented infection in the remaining two cases. Only one patient (3%) died due to transplant-related toxicity (pneumonia, day 51). With a median follow-up of 7.5 months, 20 patients had been evaluated for response: 12 are in complete response (7 with negative immunofixation), 7 in partial response and 1 with stable disease. The 1-year actuarial overall and progression-free survival rates are 88 and 90%, respectively. *Conclusions*. These preliminary results show that iv BU-containing regimen for MM is associated with an acceptable toxicity profile and with a high anti-myeloma effect.

PO-831

MATCHED PAIR ANALYSIS OF INTRAVENOUS BUSULFAN AND MELPHALAN VS. MELPHALAN ALONE AS CONDITIONING REGIMEN FOR PATIENTS WITH MULTIPLE MYELOMA UNDERGOING AUTOLOGOUS STEM CELL TRANSPLANTATION

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Introduction. Autologous stem cell transplantation (ASCT) with Melphalan 200 mg/m² (Mel200) conditioning regimen is considered standard consolidation therapy for patients with multiple myeloma (MM). However, Mel200 results in response rates of 40% with nearly all patients ultimately relapsing. A double alkylating regimen of oral Busulfan (Bu) and Mel has been suggested as an effective pre transplant conditioning regimen for these patients. Recently, a new intravenous formulation of Bu (ivBu) has been developed and introduced into clinical use. This iv formulation has less pharmacokinetic variability, and results in less toxicity than the oral drug. *Material and Methods*. To assess the efficacy and toxicity of an ivBu and Mel conditioning regimen we conducted a multicenter, retrospective trial comparing the toxicity and outcomes of 28 patients with MM undergoing ASCT after ivBu administered in a single daily dose and Mel with 56 matched controls receiving ASCT after conditioning with standard Mel200. ivBuMel conditioning regimen consisted of a single dose of 3.2 mg/kg over three hours once a day on days -5 to -3 followed by Mel at a dose of 140 mg/m² on day -2. Patients in the Mel200 group were transplanted from 2002 to 2005, while patients in the ivBuMel group underwent transplant between 2005 and 2006. Controls were selected to match on sex, age, Durie-Salmon and ISS stage at diagnosis, and disease status at transplant. Pretransplant chemotherapy consisted of VBMCP/VBAD or VAD in every case. Median time form diagnosis to transplant was similar between groups, and every patient received ASCT as front-line therapy. *Results*. There were no differences in the CD34⁺ cell dose administered and in the hematopoietic recovery after transplant in both groups. Mild or moderate mucositis was the toxicity most frequently observed (89% in the ivBuMel and 90% in the Mel200). Fever was seen in 22 cases in the ivBuMel group an in 43 cases in the Mel200 group, but bacteremias and clinically documented infections were more frequently observed among controls (74% vs. 41%, p=0.008). No cases of Bu-associated seizures were seen and no patient developed sinusoidal occlusive syndrome. Median duration of hospitalization was similar in both groups. One patient died due to transplant-related complications in each group. Three months after ASCT, 50% of patients were in complete remission in the ivBUMel group vs 62% in the controls (p=ns). One-year progression free survival was 96±4% and 85±5%in the ivBuMel and Mel200 group, respectively. *Conclusions*. These preliminary results suggest that the use of ivBu-based regimen for ASCT in patients with MM is a well tolerated conditioning regimen associated with a low transplant-related morbidity and mortality that compares favourably with Mel200. More patients and further follow-up is necessary to confirm these findings and to ascertain whether ivBu will favourably influence time to progression and overall survival in this setting.

PO-832

VELCADE AND HIGH DOSE MELPHALAN: A NEW PREPARATIVE REGIMEN

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Introduction. The achievement of Complete Response (CR) or Very Good Partial Response (VGPR) is the main prognostic factor for survival after Autologous Stem Cell Transplantation (ASCT) in Multiple Myeloma (MM). High Dose Melphalan (HDM) is the recommended conditioning regimen before ASCT. However, the rate of CR+VGPR is only 40% to 50%. Velcade (VEL) has demonstrated a significant activity in relapsed/refractory patients, a synergistic effect with Melphalan (MEL) and a lack of haematological toxicity. The combination of VEL and HDM was a logical approach to improve the rate of CR+VGPR after ASCT. *Methods*. Between 06/2005 and 06/2006, 35 patients with stage II or III Durie Salmon were enrolled to receive VEL and HDM as conditioning regimen before ASCT. VEL (1 mg/m^2) was delivered on days - 6, - 3, + 1, + 4 and MEL (200 mg/m^2) on day - 2. Peripheral blood stem cells (median 3,3×10^6 CD34/kg) were infused on day 0. *Results*. Main characteristics of the patients were: median age = 56 years; ISS= I in 18, II in 12 and III in 5 cases; chromosome 13 deletion in 9 on 18 assessable. Twenty six patients were in frontline therapy (1CR, 7 VGPR, 8 partial response (PR) and 10 stable disease (SD) before ASCT). Nine patients were in SD or with a progressive disease (PD) after a first course of classical HDM. VEL did not increase the haematological toxicity observed with HDM. Median duration of neutropenia (<0.5×10^9/L) and thrombocytopenia (<20×10^9/L) was 7 and 1 days respectively. Extra-haematological toxicities were limited: grade 3/4 mucositis in 5 cases, erythrodermy in 10 cases and cardiac arrhythmia in 5 cases. No toxic death was observed. All patients were assessed for early response at 3 months after ASCT. Eleven patients (31,4%) achieved a CR and 11 (31,4%) a VGPR. One patient was non responder. Among the 9 patients receiving a second ASCT, 6 CR+VGPR were observed after VEL+HDM. *Conclusions*. These preliminary results strongly suggest that VEL (1 mg/m^2) and HDM is a safe and highly effective conditioning regimen with more than 60% of RC+VGPR rate.

PO-833

MELPHALAN-200 MG (MEL) VERSUS BUSULPHAN PLUS MELPHALAN (BUMEL) AS CONDITIONING REGIMENS FOR MULTIPLE MYELOMA (MM): A COMPARATIVE STUDY OF EFFICACY AND TOXICITY

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Introduction. Autologous stem cell transplant (ASCT) has become the standard of care in young newly diagnosed MM patients. In order to obtain the maximum benefits from ASCT, the conditioning regimen should combine the maximum anti-MM effect with the minimum toxicity. Few studies have been prospectively conducted in order to evaluate different conditioning regimens, and in fact Melphalan 200 mg/m^2 is universally used as a standard. *Materials and Methods.* We have evaluated the efficacy and toxicity of two conditioning regimens in newly diagnosed MM patients included in the Spanish Pethema/GEM 2000 trial. 591 patients were conditioned with MEL 200 mg/m^2 and 256 with oral Busulphan at dose of 12 mg/Kg in combination with MEL 140 mg/m^2. All patients had previously received six cycles of alternating vincristine, BCNU, melphalan, cyclophosphamide, prednisone/vincristine, BCNU, Adriamycin, dexamethasone (VBMCP/VBAD). *Results*. Baseline characteristics were similar in both cohorts of patients, as well as the response rate to induction VBMCP/VBAD chemotherapy. Time to engraftment after transplant was also similar in both groups. Overall grade 3-4 non-hematologic toxicities were higher in the BuMel arm (30% vs 20%; *p*: 0,005); hepatic toxicity, reported as sinusoidal obstruction syndrome (SOS) was the most relevant non-hematologic toxicity (observed in 8% of patients in the BuMel arm as compared to 0,4% in MEL arm; *p*:0,0001). Mortality directly associated to SOS was 2% and 0,2% in BuMel and MEL arms, respectively (*p*:0,026). Early deaths, occurring during the first two months after transplantation, were significantly higher in the patients' group conditioned with BuMel as compared to MEL arm (7,4% vs 2,5%; *p*:0,001). There were not differences in the response rate (≥ partial response) at 100 days after ASCT: 84% in the MEL arm (33% CR with negative Immunofixation) vs 77% for BuMel (35% CR IF-). The median event free survival (EFS) was similar in both arms (42,5 vs 41,5 months for BuMel and MEL, respectively; p: 0,6). However, when early deaths were excluded the EFS of BuMel was slightly superior to that of MEL (45,2 vs 42,6; p: 0,1). With a median follow-up of 45 months (range: 8-91), median overall survival (OS) has not been reached in MEL arm and the 8-year estimated overall OS in this cohort was significantly better than in the BuMel arm (57% vs 47%; *p*:0,001). *Conclusions*. Although these two conditioning regimens showed similar overall response rate and EFS, the overall survival is longer with MEL, which could be due to the higher toxicity, including early deaths, observed in patients conditioned with BuMel. The longer EFS observed with BuMel after early deaths exclusion is intriguing and since we have used oral Busulphan without monitorization of serum levels and appropriate dose adjustment, it could be argued that the use of intravenous Busulphan would reduce the toxicity and eventually lead to a higher efficacy in MM patients.

PO-834

PHASE I TRIAL OF ESCALATING DOSES OF TOTAL MARROW IRRADIATION (TMI) WITH HELICAL TOMOTHERAPY AND PERIPHERAL BLOOD PROGENITOR CELL RESCUE (PBPC), FOLLOWING HIGH-DOSE MELPHALAN AND PBPC AS PART OF TANDEM HIGH-DOSE THERAPY (THDT) FOR PATIENTS WITH MULTIPLE MYELOMA (MM)

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Introduction. Attempts to combine total body irradiation (TBI) with high-dose melphalan (Mel) resulted in substantial organ toxicities and precluded optimal dosing of Mel. Helical tomotherapy may allow delivery of total marrow irradiation (TMI), while avoiding collateral toxicities. *Materials and Methods*. We designed a phase I/II study for patients (pts) with responding or stable stage I-III MM. Following PBPC mobilization, pts receive THDT first with Mel 200 mg/m^2 and PBPC, and, > 6 weeks later, escalating doses of TMI (starting dose: 200 cGy daily x 5 [1000 cGy], up to 200 cGy twice daily x 5 days [2000 cGy]) and PBPC. Maintenance consists of dexamethasone 40 mg/day x 4 days every 28 days and thalidomide 50-200 mg/day. *Results*. The median number of prior chemotherapy regimens is 2 (1-4). The median duration from diagnosis to the HDT is 8 mos (4-13). Median age of pts is 53 (35-66). Sixteen pts with stages II (6) and III (10) MM have received Mel; 15 of 16 pts (8F/8M) have received treatment at dose levels 1-5 of TMI (1000 cGy through 1800 cGy); 1 pt is about to start TMI at 1800 cGy. The median time between the first and second THCT cycles is 74 days (47-125). Hematopoietic toxicities were independent of TMI dose levels: granulocyte recovery to >1000/microliter following Mel required 12 days (11-38) versus a median of 10 days after TMI (range; 9-12). Platelet (excluding 6 pts not needing plt transfusion) independence was seen by day 10 (8-13) versus 8 (6-11) following TMI. In the first 15 pts the estimated median radiation dose to normal organs was 15-60% of the targeted bone marrow dose. Reversible grade 3 non-hematologic toxicities by TMI dose levels included fatigue and febrile neutropenia (FN) (level 1: 1 pt each), FN (level 2: 1 pt); none (level 3); fatigue (level 4: 2 pts); anorexia and stomatitis (level 5: 1pt; anorexia: 1 pt). The median follow-up is 9 mos (4-22); 1 pt progressed at 8 mos. Final data on toxicities including patients already in screening for treatment at TMI 2000 cGy, tolerability of maintenance, and response rate will be presented. *Conclusion*. TMI is feasible and could potentially be useful as part of THDT for patients with MM.

PO-835

BORTEZOMIB/MELPHALAN FOR AUTOLOGOUS SCT IN MULTIPLE MYELOMA

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Introduction. Autologous stem cell transplantation has been used as a treatment for multiple myeloma. However, the relapse is still frequent in patients with multiple myeloma even after autologous stem cell transplantation. Thus, more effective approach is required to improve the efficacy of autologous stem cell transplantation in multiple myeloma. Bortezomib, a proteosome inhibitor is an effective agent for relapsed or refractory multiple myeloma. A previous study suggested bortezomib might be safely added to high-dose melphalan as a conditioning regimen. Herein, we reported the preliminary results with bortezomib-melphalan conditioning regimen for autologous stem cell transplantation in patients with multiple myeloma. *Materials*. Five patients were enrolled:

3 newly diagnosed patients, 2 relapsed patients. The conditioning regimen was as follows: bortezomib 1.0mg/m^2 and melphalan 50 mg/m^2 IV on day-4, and bortezomib 1.0 mg/m^2 and melphalan 150 mg/m^2 IV on day-1. *Results*. Three newly diagnosed patients received 4 cycles of VAD (vincristine, adriamycin, dexamethasone) before transplantation. Their disease status at transplantation was as follows: 1 CR, 2 PR. Two relapsed patients received two kinds of treatments including VAD, thalidomide/dexamethasone before transplantation, and their disease status at transplantation was PR. In all patients, peripheral blood stem cells were mobilized with G-CSF infusion. Median number of collected CD34$^+$ cells was 4.5×10^6/kg (range, 3.5-7.8). Four patients showed adequate hematologic recovery after autologous stem cell transplantation. Median day for absolute neutrophil count over 500/mm^3 was 14 (range, 13-22). One patient died due to pneumonia on day+11. After stem cell transplantation, four patients showed CR. Among them, CR was maintained in one newly diagnosed patient (disease-free duration: 449 days), but the other three patients showed relapse (disease-free duration: 222 days, 79 days, and 124 days, respectively). *Conclusions*. Conditioning regimen with bortezomib and melphalan may be effective for autologous stem cell transplantation in multiple myeloma, however, its feasibility should be further evaluated with larger study populations.

PO-836

THALIDOMIDE POST-AUTOLOGOUS STEM CELL TRANSPLANT (ASCT) IMPROVES PROGRESSION-FREE SURVIVAL (PFS) IN MYELOMA WITH NORMAL BUT NOT UPREGULATED FGFR3 EXPRESSION, AND MAY OVERCOME THE POOR PROGNOSTIC EFFECT OF T(4;14)

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The t(4p16; 14q23) translocation occurs in 15-20% of myeloma and confers a poor prognosis. FGFR3 is one of several candidate genes, but its prognostic significance is unknown. Since thalidomide is an inhibitor of bFGF, a FGFR3 ligand, we assessed whether changes in FGFR3 expression may affect the response to thalidomide, and whether thalidomide may affect FGFR3 expression. The ALLG MM6 trial is a randomised study of maintenance thalidomide and prednisolone (T+P) vs. prednisolone (P) following ASCT. We flow-purified CD38hi CD138$^+$ plasma cells from 176 marrow samples; 96 were collected following induction chemotherapy and before ASCT, and 80 after 12 months' maintenance therapy. Translocation t(4;14) was assessed by RT-PCR for IgH-MMSET transcripts; FGFR3 expression was quantitated by FGFR3-specific Taqman probes, using beta-actin as internal control. FGFR3 upregulation was found in 29%, both pre-transplant and post-maintenance. There was no significant difference in the incidence of FGFR3 overexpression post-maintenance between the T+P (28%) and P (25%) cohorts. However, post-maintenance FGFR3 expression had a significant influence on PFS. Patients with normal FGFR3 expression on T+P had improved PFS (median not reached) compared with those on P (median 23 months; p=0.01). There was no significant difference in PFS in patients with upregulated FGFR3. For T+P, those with upregulated FGFR3 post-maintenance had an inferior median PFS (18.5 months) compared with normal FGFR3 (not reached), p=0.002; no significant difference was seen in P. We confirmed the poor prognostic significance of t(4;14). Of 106 patients examined, 27% were positive. PFS for t(4;14)+ patients post-maintenance was significantly inferior (median 17 months) compared with t(4;14)- patients (median not reached; p=0.003). Thalidomide overcomes the poor prognostic effect of t(4;14) as the median PFS for t(4;14)+ T+P group (35 months) is similar to t(4;14)- P group (36 months), although not as good as t(4;14)- T+P (median not reached), which is significantly superior to the cohort with the worst prognosis, t(4;14)+ and not on thalidomide (17 months) (p=0.008). Similar significant differences were seen in OS (p=0.008). In summary, (1) FGFR3 overexpression is found in ~30% of myeloma, (2) thalidomide does not affect FGFR3 expression, (3) the therapeutic benefit of thalidomide on PFS was seen only in patients with normal FGFR3 expression, (4) thalidomide maintenance overcomes the poor prognostic effect of t(4;14) post-ASCT.

PO-837

CONSOLIDATION TREATMENT WITH CYCLOPHOSPHAMIDE, DEXAMETHASONE AND THALIDOMIDE FOLLOWING AUTOLOGOUS STEM CELL TRANSPLANTATION IS SAFE, WELL TOLERATED AND IMPROVES DEPTH OF RESPONSE

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High dose therapy (HDT) supported by autologous stem cell transplantation (ASCT) has improved outcomes for newly diagnosed patients with multiple myeloma (MM). Thalidomide in combination with chemotherapy is effective in newly diagnosed and relapsed patients, and may improve depth of response and impact on PFS when used as single agent maintenance treatment. This study investigated whether consolidation therapy with the combination of cyclophosphamide, dexamethasone and thalidomide (CDT) improves outcomes following HDT. Twenty-one newly diagnosed patients without disease progression were eligible for CDT consolidation, between 3 and 6 months following melphalan HDT (200 mg/m^2) and ASCT. A monthly cycle of weekly oral cyclophosphamide (400 mg/m^2), dexamethasone (40 mg) days 1-4, and daily thalidomide (50 mg increasing to 200 mg) was continued for up to 6 months until maximal response. Patient characteristics were as follows; 13 male, 8 female; median age 58 years (range 41 to 70 years); isotype (9 IgG, 7 IgA, 4 light chain only, 1 non-secretory), and Durie-Salmon stage (16 stage III, 3 stage II, 2 stage I). Comparison was made with 21 patients receiving the same induction (VAD/ZDex) and HDT protocols without receiving thalidomide maintenance. CDT treated and control groups had similar isotype and Durie-Salmon stage. Importantly, both had similar responses to induction treatment (15(71%) PR and 4(19%) VGPR/CR). Median duration of CDT consolidation was 4 months (range 2 to 6 months). Cyclophosphamide dose was reduced in 13(62%) patients due to grade 2 neutropaenia. Median thalidomide dose was 100 mg daily. Nine (43%) patients developed grade 1 neuropathy, which resolved in all but 2(10%) cases on stopping treatment. There were no incidences of febrile neutropenia or DVT. Median follow up from HDT is 30 months (range 18 to 36 months) in CDT treated patients, and 49 months (range 32 to 84 months) in the control group. CDT treated patients showed improved depth of response at 12 months following HDT (13(62%)VGPR/CR vs 6(29%) in control group). Median PFS was longer in CDT treated patients (29 months vs. 24 months control group), although this did not reach statistical significance in this small group of patients. *Conclusion*. CDT consolidation following HDT is safe, well tolerated and improves depth of response.

PO-838

IDIOTYPE VACCINATION IN MM USING MATURE DENDRITIC CELLS

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Introduction. Dendritic cell (DC)-based vaccination is a therapeutic option for a number of malignancies, including MM. Although the idiotype protein (Id) seems an ideal tumorspecific antigen (TAA), previous vaccination trials were not successful. This may have been due to weak immunogenicity of Id or to insufficient antigen presentation because of DC immaturity. We used mature Id-pulsed DCs in patients with minimal residual disease, conditions most optimal to induce an immune response. *Materials and Methods*. Eight patients with a (near) complete remission after high dose Melphalan (HDM) participated in this pilot study. The HDM-to-vaccination interval was 6-28 months. Mature DCs were generated from PBMCs and pulsed with a conjugate of purified Id and keyhole limpet hemocyanin (KLH) as an adjuvant. Patients were vaccinated 3 times using 15-60*10e6 DCs at 2 week intervals. Four patients were vaccinated intradermally and 4 were vaccinated both intradermally and intravenously. Cellular immune response against KLH was measured by proliferation and cytokine-release assays. KLH-antibodies were measured by ELISA. Two weeks after the last vaccination DTH-testing was performed and skin biopsies were taken for T-cell culturing from positively indured sites. Disease activity was monitored using an ASO-PCR assay. *Results*. Vaccination was tolerated well with limited toxicity. A strong cellular immune response against KLH was seen in all patients. In one patient, vaccinated 7 months after HDM, the response to KLH was limited but when re-vaccinated 9 months later a strong cellular immune

response to KLH was measured. KLH-antibodies were present in 3 patients. No Id-specific immune response was observed. DTH was positive for DCs+Id or Id alone in all patients. However, positive induration was also seen with DCs alone and no Id-specific T-cells were identified among the DTH-infiltrating lymphocytes. *Conclusions*. Vaccination after HDM using mature DCs is safe and feasible. Functional mature DCs can be generated after HDM, inducing an adequate immune response against KLH after an interval of more than 6 months. Idiotype-protein seems only weakly immunogenic, despite the use of mature DCs and a setting of MRD. Based on these results we now started a vaccination program using mature DCs expressing different TAAs, after RNA-electroporation.

PO-839

BORTEZOMIB AS CONSOLIDATION AFTER ASCT, AN INITIAL SAFETY REPORT FROM NMSG

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Introduction. High-dose melphalan followed by autologous stem cell transplantation (ASCT) prolongs event free and overall survival (EFS and OS) for myeloma patients < 65 years of age. No consolidation regimen have so far resulted in undisputable benefits. Bortezomib is a new promising agent, showing a clear anti-myeloma effect in heavily pre-treated patients. The hypothesis, guiding the present study, is that consolidating treatment with bortezomib post ASCT, can prolong the time to relapse. *Materials and Methods.* In 2006 NMSG initiated a prospective, open-labelled, randomized, multicentre study of bortezomib consolidation in myeloma patients receiving ASCT as part of first line treatment. Patients are included at the time of ASCT and three months later randomized to receive no consolidation (standard) or bortezomib. The drug is given in a dose of 1.3 mg/sqm twice weekly for 2 weeks followed by one weeks rest and this schedule is repeated on day 22 for a total of two cycles. From day 43, bortezomib is given in weekly injections for 3 weeks followed by 1-week rest, and this 4-week cycle is repeated 4 times. In total, 20 injections over a period of 21 weeks are given. Patients in both arms are evaluated monthly for response and events the first year and every second month from year two and onwards. The study is designed to comprise 400 patients accrued during a three year period. The primary endpoint is EFS. Secondary endpoints are OS, toxicity, quality of life and health economy. Findings. Up to February 1st , 123 patients have been included, 89 are randomized and 45 have started bortezomib treatment. For those patients who have completed their bortezomib treatment, none has developed neuropathy greater than grade 2 or neuropathic pain greater than grade 1. So far, thirteen SAEs have been reported, 7 due to infection or infection related symptoms and 3 to neurotoxicity. The haematological toxicity has been limited to grade 1 thrombocythopenia and neutropenia.. *Conclusion*. Our preliminary results indicate that myeloma patients can well tolerate bortezomib as consolidation treatment after ASCT and that the toxicity in this stage of the disease is limited.

PO-840

PEGYLATED INTERPHERON ALPHA2B (PEGINTRON) AS MAINTENANCE TREATMENT AFTER AUTOLOGOUSPERIPHERAL BLOOD STEM CELL TRANSPLANTATION (PBSCT) IN MULTIPLE MYELOMA (MM): PRELIMINARY CLINICAL RESULTS IN 30 PATIENTS WITH A UNIQUE WEEKLY DOSE

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Introduction. Intensification therapy in MM with autologous PBSCT is widely indicated for younger patients with favourable results regarding response, OS and PFS. However, there is no clear plateau in the survival curve. To improve these results and to prolong remission duration, various maintenance treatments have been proposed with minimal and discrepant benefits. Interpheron $\alpha 2\beta$ s.c, at low dose, steroids on alternate days and most recently low dose oral thalidomide have demonstrated modest improvements in EFS and OS times but these results needs confirmation. Recently a new formulation of interpheron $\alpha 2\beta$ (Pegintron) is available conjugated with polietilenglicol with the advantage of being administered only once a week. This new formulation of Interpheron alpha has been tested scarcely in MM. We present the preliminary results of a spanish, phase II, non comparative, multicentric study (PI-MM-01) with Pegintron (Schering-Plough) as maintenance treatment after autologous PBSCT. *Patients y Methods*. 30 patients with MM, 64% female and 36% male with a median 56 years, received Pegintron once a week subcutaneously as maintenance treatment after favourable response post-HDT with autologous PBSCT when the engraftement was stable and complete. The initial dose was 15 g/week x 2 week and this dose was escalated to 25 g/week and then 35 g/week. The final dose was adopted according clinical and hematological tolerance. The maintenance treatment was continued at least 5 years postransplant or until toxicity, relapse or progression. *Results*. 30 patients were evaluable for this preliminary analysis. The median time from transplant to treatment was 3.8m. The median dose of Pegintron was 15 g/week. 9 patients have suspended the treatment (30%). 5 cases (16%) due to progression , 2 (4%) for toxicity and 2(4%) for other reason. The remainder 21 patients (76%) continue the treatment with clinical response, with median duration of 16 months (2-42). All patients are alive. Astenia, *pseudo-flu* symptoms and grade I thrombopenia and neutropenia were the most common adverse effects observed. One patient suspended the treatment due to dermatological reaction with pruritus. The best tolerated dose was 15 g/week. *Conclusions and Comments*. Although these results need completion and further analysis, maintenance treatment with a weekly dose of Interpheron- 2b conjugated with Polietilenglicol (Pegintron) is well tolerated after autologous PBSCT in MM. No major adverse effects were observed and no relevant negative impact was observed on the autologous graft. Pegintron sc could be an alternative to standard interpheron sc with the main advantage of a therapy simplification with only one dose weekly. More experiences and longer follow up are needed to evaluate the role of this strategy in the global treatment of MM and new approaches for maintenance have to be investigated , including association with steroids or new drugs as thalidomide, bortezomib or lenalidomide.

PO-841

THALIDOMIDE MAINTENANCE ERADICATES MINIMAL RESIDUAL DISEASE WITHOUT AFFECTING NORMAL PLASMA CELLS

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Introduction. Thalidomide is effective as a single agent in depleting myeloma cells and is under investigation as maintenance therapy. Many patients start thalidomide maintenance in a complete remission and conventional response assessment may be uninformative. The aim of this study was to determine the effects of thalidomide on minimal residual disease levels detected by multi-parameter flow cytometry in comparison to patients treated with interferon or no maintenance after high-dose melphalan (HDM). *Methods*. The effect of thalidomide maintenance (50-250mg) was assessed in 8 patients with 12 patients in the control group. Bone marrow samples were analysed at day 100 post-HDM and at routine assessment a median 6 months (range 4-11) later. Thalidomide treatment continued for a median 15 months (minimum 2 months, 3 patients remain on treatment); cessation was due to peripheral neuropathy (n=3), thrombocytopenia (n=1) or depression/lethargy (n=1). Plasma cells were identified by flow cytometry using CD38/138/45 expression. Cases in which abnormal ($CD19^-$ and/or $CD56^+$) plasma cells represented <10% of total plasma cells and <0.01% of total leucocytes were classified as MRD-negative. *Results*. In the control group, 6/12 patients showed increasing disease levels; 6/12 remained MRD-negative but no patients showed decreasing disease levels during follow-up. In the thalidomide maintenance group, no patients showed increasing disease levels; 4/8 patients remained MRD-negative and 4/8 showed decreasing disease levels with 2/4 becoming MRD-negative. Depletion of neoplastic plasma cells was significantly greater in the Thalidomide maintenance group compare to control, Fisher's exact test two-tailed p=0.04. Normal plasma cell levels were stable in all patients on thalidomide maintenance. In the control group, normal plasma cells became undetectable in 2 patients with increasing neoplastic plasma cells and were stable in the remaining 10/12 patients. *Conclusions*. This study highlights the importance of using direct measurements of tumour burden for assessing response as immunofixation and sFLC do not reveal responses in a minimal disease setting. Thalidomide is clearly active in depleting minimal levels of neoplastic plasma cell levels in a situation where disease levels would steadily increase without intervention. Thalidomide activity is specific to neoplastic plasma cells as normal plasma cells are unaffected.

PO-842

BORTEZOMIB LEADS TO HIGH YIELD CD34+ COLLECTIONS IN MM

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Standard stem cell mobilization regimens for multiple myeloma patients include G-CSF alone or in combination with high dose cyclophosphamide. Given the known *in vitro* and *in vivo* synergy between alkylating agents and proteosome inhibitors, we sought to optimize the potential for concurrent cytoreduction by adding bortezomib to the mobilization regimen. Nine evaluable patients, whose prior therapy consisted of six cycles of a 21-day treatment with bortezomib/dexamethasone ± pegylated liposomal doxorubicin, were mobilized. They received IV push bortezomib at 1.3 mg/m^2 on days 1, 4, 8, and 11 in combination with high-dose cyclophosphamide at 3 g/m^2 on day 8. G-CSF was given for 10 consecutive days starting on day 9. Stem cells were easily harvested from each of the nine patients. The number of CD34$^+$ cells collected far exceeded the study goal of 10×10^6 cells/kg, a collection result typical of standard mobilizations using cyclophosphamide and/or G-CSF alone. Six out of nine patients had more than adequate collections in a single day. Median CD34$^+$ collection was 21×10^6 cells/kg in 1-5 collection days (range of 9.3-294.2×10^6). All patients who began mobilization with less than a CR continued to respond positively to treatment, including one transition from nCR to CR and one from SD to PR. No patients progressed or moved to lower response categories. Though expected grade 3 and 4 toxicities did occur, none of them required discontinuation of protocol treatment. As planned, all patients were able to high dose melphalan supported by autologous stem cell transplantation post protocol mobilization. All nine showed typical and adequate engraftment after CD34$^+$ infusions. The median number of days to ANC recovery was 11 days (range 10-17). Platelet recovery median 18 days (range 13-24). Bortezomib in addition to high dose cyclophosphamide followed by G-CSF is a novel, well-tolerated and efficacious combination for stem cell mobilization in patients with multiple myeloma. This regimen not only yields a high number of stem cells within a short collection time, but also provides the potential for further cytoreduction.

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PO-843

FAILURE TO MOBILISE IS NOT A POOR PROGNOSTIC FACTOR IN MYELOMA

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Aim. 12-20% of myeloma patients fail to mobilise adequate peripheral blood stem cell (PBSC) numbers (<2×10^6 CD34/kg) at first mobilisation. It is less clear what is the fate of those who fail to mobilise or undergo autologous transplantation (ASCT) with suboptimal CD34 dose. *Methods*. We performed 155 ASCTs for myeloma between 1993-2006. Cyclophosphamide /G-CSF was used as first line mobilisation regimen and high dose G-CSF (16-24mcg/kg) second line. In patients who failed a repeat attempt at PBSC collection after 6 weeks delay and/or a bone marrow harvest (BMH) was performed. *Results*. 35/ 155 (22.5%) failed to achieve 2×10^6/kg CD34 positive cells from first mobilisation. 14/35 patients were successfully mobilised at second attempt, BMH was performed on 9/35 patients and 4/35 patients needed > 2 lines of mobilisation to augment cell dose. 9/35 patients had previous ASCT and median lines of prior therapy was 2 (Range 0-4). 15/35 patients had previously received melphalan and 5/35 thalidomide therapy prior to mobilisation. Of the failed mobilisers, 23/35 underwent ASCT; 11/23 achieved >2×10^6/kg CD34 with further mobilisation and 12/23 were transplanted with (median cell dose 1.23×10^6/kg CD34; (range: 0.5-1.75). Patients with cell dose < 1.0×10^6/kg CD34 had 25%-50% melphalan dose reduction. 3/35 underwent MRD allogeneic transplantation. Median time to neutrophil engraftment, in those receiving <2×10^6/kg CD34 was 15.5 days (10-31) versus 16 days (10-46) in all other patients. 7/23 were platelet transfusion dependent 2 months post-transplant. Median OS post transplant in patients receiving ASCT was 34.3 months (95% CI 28.5- 40), and 9 months in the 9 patients who were not transplanted. Median OS in ASCTs with optimal cell dose was 23.9 months versus 34 months in ASCT with suboptimal CD34 dose, not statistically significant. 2/3 allografted patients are alive, but relapsed 2 years post-transplant. *Conclusion*. This data (34.3 months) compares favourably with the 54 months OS in untreated patients treated with intensive therapy (MRC Myeloma VII). This data supports further attempts at PBSC mobilisation and BMH and ASCT with suboptimal cell dose in heavily pre-treated patients who failed first mobilisation.

GROUP 9: Other plasma cell dyscrasias

PO-901

IGM MULTIPLE MYELOMA: REPORT OF TEN CASES

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Of 600 patients with the diagnosis of multiple myeloma treated at the Department of Haematology Warsaw Institute of Haematology and Transfusion Medicine in 10 cases (=1,6%) proliferation of plasma cells was associated with synthesis of a monoclonal IgM. These 10 cases are subject of this study. *Results*. One patient was at age 25 years, the other one-41 years, all remaining patients were older than 60 years. In 2 patients diagnosis of multiple myeloma was preceded by a 3 -year period of monoclonal gammopathy of undetermined significance. In 4 patients severe hyperviscosity syndrome was observed. Bone marrow plasma cell rate ranged from 35-90%, osteolysis was found in 8 patients, hypercalcemia in 4, kidney failure in 2, and amyloidosis in 1 patient. Survival time in 6 cases amounted respectively 6,11,14,14,22,>35 months. In 3 cases without organomegaly, in whom in the bone marrow there was found plasmacytosis CD138+ exceeded 35% and also simultaneous presence of CD20+ cells up to total rate of both type of cells equal 90% (what also suggested a low grade non-Hodgkin's lymphoma with plasma cell differentiation) survival time was longer than 90 months. In 2 of those cases osteolysis was revealed and in one there was observed a disease -associated renal insufficiency. In one case with proliferation of plasma cells and lymphoid cells in the bone marrow no M -protein was detected in serum and urine. Plasma cells with appearance of flaming cells constituted 17,6% of all nucleated bone marrow cells. Their cytoplasm was completely filled with acidophilic substance displacing nucleus into the edge of the cytoplasm. Ultrastructural and immunoelectronoscopic examinations proved, that those cells accumulated IgM in their cytoplasm, but were not able to release this immunoglobulin. *Conclusion*. IgM myeloma display clinico-pathologic features intermediate between multiple myeloma and Waldenstrom's macroglobulinemia.

PO-902

FAMILIAL CLUSTERING OF LYMPHOPROLIFERATIVE DISEASES

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Backgound. The etiology of lymphoproliferative diseases remains unclear although reports of familial clusters indicate that genetic factors may play a role. In Iceland eight families have previously been idendified with multiple cases of monoclonal gammopathies and other lymphoproliferative diseases (Ogmundsdottir *et al.* 2005). *Aim*. The aim of this study was to trace these families further and to screen family members above 20 years for the presence of paraproteins by protein electrophoresis and serum light chain analysis. *Methods*. Two probands from each family were chosen and traced two generations back and from there forward. The resulting list of persons was compared with the Icelandic cancer registry (ICR) to find all cases of hematological malignancies and monoclonal gammopathies of unknown significance. First degree relatives of affected persons and their descendants above 20 years were selected for screening for paraprotein (N=350). Serum protein electrophoresis has been done and light chain analysis is in progress. *Results*. The complete pedigrees of the eight families contain 4370 persons. Comparison with the ICR records revealed 20 previously unknown cases of lymphoproliferative diseases. Protein electrophoresis on serum from 350 relatives idendified additional 6 cases of monoclonal gammopathy, 4 and 2 being 1° and 2° relatives of affected family members, respectively. A total of 55 cases of lymphoproliferative diseases were idendified within the families, 32 of those cases being monoclonal gammopathy, 10 B cell NHL, 6 Hodgkin's disease, 4 ALL and 3 T cell NHL. All the T cell NHL cases and 3 of the ALL cases were within the same family. One family with 5 affected siblings, 1 with MM, 1 with MGUS and 3 with WM, and two cases in the next generation, 1 MM, 1 MGUS, has been previously described (Ogmundsdottir *et al.* 1999). *Conclusion*. The occurrence of multiple cases of lymphoproliferative diseases in some families strongly implies a genetic predisposition. Further studies on *in vitro* B cell responses, including phenotypic and gene expression analyses to reveal more clues on pathogenesis are in progress.

PO-903

A NEW CASE OF MU-HEAVY CHAIN DISEASE

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Introduction. The heavy chain disease represent a lymphoplasma cell proliferative process involving B cells and are characterized by the production of incomplete heavy chains devoid of light chains. *Methods*. We report the new case of mu-heavy chain disease. 63-year old female successively was ill of carcinoma vulvae, Hodgkin lymphoma, mu-heavy chain disease and acute myeloid leukemia. We determined monoclonal heavy chain mu in serum of patient with method immunofixation electrophoresis on agarose (HYDRAGEL 4IF, SEBIA, Paris) and with Capillarys Immunotyping (Capillarys 2, SEBIA, Paris). *Results*. Concentration of mu-heavy chains in our observation was very high - 34.9 g/L. Molecular weight of heavy chain mu was determined by 2-dimensional gel electrophoresis and it was 53 kD. Monoclonal mu-heavy chains and light chains Ig were not determined in urine in this case of mu-heavy chain disease. *Conclusion*. We describe an interesting case of mu-heavy chain diasease. Our observation is unusual by its clinical and also biochemical findings.

PO-904

IDENTIFICATION OF ANTIGENS WITH INDUCED IMMUNE RESPONSE IN MGUS

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Introduction. Monoclonal gammopathy of undetermined significance (MGUS) is a benign condition with 1,5% of the patients developing symptomatic multiple myeloma (MM) per year. Importantly, the majority of patients do not progress to MM. Several evidences have supported the idea that the immune system, in patients with MGUS, may play a role in controlling the progression to MM and the identification of antigenic targets, recognized by the immune system, could open the way for future immunotherapeutic approaches to delay or prevent such progression. *Methods*. We have screened a cDNA expression library, constructed from purified CD138+ bone marrow myeloma cells, by Serological Analysis of Recombinant cDNA Expression Library (SEREX) using high dilution (1:500) of serum from 3 MGUS patients with stable disease for 1 to 4 years. *Results*. Antibody response appeared to be directed against intracellular proteins involved in apoptosis (SON, Hip1), DNA and RNA binding proteins (KIAA0530, GPATC4), signal transduction regulators (AKAP11), developmental proteins (OFD1), transcriptional co-repressors (IRF2BP2), proteins of the ubiquitin-proteosome pathways (PSMC1). We have further analyzed frequency of antibody response against these antigens in additional 26 MGUS sera, 11 newly diagnosed MM patients, 11 MM patients in remission after auto-transplant, 7 refractory MM patients and 25 normal donors. We have observed antibody response against OFD1 (20.6%); KIAA0530 (10.3%); AKAP11 (10.3%); and GPATC4 (6.8%) in patients with MGUS. Antibody response against these antigens was not observed in newly diagnosed or refractory MM patients. Interestingly, 3/11 (27%) patients in remission after auto-transplant had an antibody response against OFD1 with evidence of increase in antibody titer in one patient after transplant suggesting its importance as a target. Significant antibody responses were not observed against any of these antigens in the sera of 25 health donors. We have further focused our studies on OFD1, a developmentally expressed Hedgehog (Hh) pathway-related protein that co-localizes with ã-tubulin in the centrosome. We have confirmed, by western blot analysis, that MM cell lines and primary MM cells selectively express OFD1 protein. Specific T cell responses directed at OFD1 and its role in cell signaling are under investigation. *Conclusions*. These data open the possibility to identify target antigens that are important in the disease process of MGUS and may allow us to design future vaccines and immunotherapeutic approaches targeting these antigens in MGUS as well as in MM.

PO-905

INCREASED MGUS PREVELANCE IN CHRONIC KIDNEY DISEASE PATIENTS

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Introduction. Renal impairment is a common complication of plasma cell dyscrasias. Predominately this relates to the well described nephrotoxicity of free light chains (FLC). Hypothetically, monoclonal sFLC in patients with monoclonal gammopathies of undetermined significance (MGUS) may result in renal injury. To date, the prevalence and importance of MGUS in patients with chronic kidney disease (CKD) has not been described. *Methods*. A prospective single centre study was undertaken to determine the prevalence of MGUS in patients with CKD. Samples from 386 patients were assessed by serum protein electrophoresis, immunofixation electrophoresis (SPE and IFE; Serbia, UK) and FREELITETM (The Binding Site, UK). Immunoglobulin concentrations were measured in serum samples using nephelometric immunoassays. The Cockcroft-Gault equation was used to estimate glomerular filtration rate (eGFR) and assign CKD stage. sFLC ratio, serum M Protein and Ig subtype was used in MGUS risk stratification. *Results*. The mean age of the CKD patients was 62.5 (17-96), male (57%), mean Cystatin C 2.79 mg/L (0.2-7.4), mean serum kappa 51 mg/L (3.12-686), mean serum lambda 44.4 mg/L (4.19-621). 10% of patients with CKD, over 50 years of age, had a monoclonal protein confirmed by IFE. 50% were low risk, 40% low-intermediate risk and 10% intermediate to high risk. The prevalence of MGUS increased non-significantly with CKD stages (p<0.089). *Discussion*. The prevalence of MGUS in patients with CKD is three times higher than published age matched normal prevalence of 3.2%(Kyle R *et al.*, NEJM'06). In addition polyclonal FLC levels are increased in the absence of monoclonal disease. Further work must now be undertaken to determine if these abnormalities are mechanistically involved in the progression of CKD.

PO-906

PLASMA CELL IMMUNOPHENOTYPE IS HIGHLY PREDICTIVE OF THE LEVEL OF CHROMOSOMAL ABNORMALITIES IN PATIENTS WITH MGUS

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Introduction. Myeloma plasma cells are phenotypically distinct from normal plasma cells. MGUS patients with predominantly myeloma-phenotype plasma cells are at risk of progression to myeloma whilst those with normal-phenotype plasma cells have predominantly stable disease. Chromosomal abnormalities typical of myeloma are often detected in MGUS plasma cells by fluorescence *in situ* hybridisation (FISH). The aim of this study was to determine the relationship between plasma cell phenotype and chromosomal abnormalities in MGUS. *Methods*. Plasma cells were assessed from 51 patients with MGUS and 4 patients with a CD5- B-lymphoproliferative disorder associated with a paraprotein. Plasma cells were identified by flow cytometry using CD38/138/45 expression. Cases in which myeloma-phenotype ($CD19^-/CD56^+$) plasma cells represented >90% of total plasma cells and/or >1% of total leucocytes were classified as having a high-risk phenotype. FISH was carried out on CD138-purified plasma cells using numerical probes for chromosomes 3,4,5,6,7,8,9,11,13,14,15,16,17 and 20. The level of chromosomal abnormalities was classified according to the proportion of plasma cells with an aberrant FISH result: low <50%, moderate 50-75% and high >75%. *Results*. Patients with CD5-negative B-lymphoproliferative disorders had normal phenotype plasma cells and normal FISH results. MGUS patients with a low-risk phenotype (n=15, median 0.9% plasma cells) showed a low level of chromosomal abnormalities in 47% (7/15) with 53% (8/15) showing a moderate/high level of abnormalities. In contrast, MGUS patients with a high-risk phenotype (n=36, median 1.2% plasma cells) showed a low level of chromosomal abnormalities in 11% (4/36) whilst 89% (32/36) had a moderate/high level of abnormalities. There was no difference in the total plasma cell level (p=0.18, Two-sample unequal variance T-test) but a highly significant difference in the proportion of cases with moderate/high levels of chromosomal abnormalities (p=0.0004, Fisher's exact test) between the two MGUS groups. *Conclusion*. The data indicate a close association between plasma cell immunophenotype and the presence of genetic abnormalities that is independent of total tumour burden. This further supports the use of plasma cell immunophenotyping for risk-stratification in MGUS and indicates that simultaneous analysis of chromosomal abnormalities by FISH will provide additional prognostic information in this setting.

PO-907

PLASMA CELL PHENOTYPE & SFLC PROVIDE INDEPENDENT PROGNOSTIC INFORMATION IN MGUS

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Introduction. An abnormal serum Free Light Chain (sFLC) ratio predicts an increased risk of disease progression in patients with MGUS independent of the paraprotein level and isotype (Rajkumar *et al.*, Blood 2005: 106(16), 812-7). Abnormal bone marrow plasma cell immunophenotype is also an independent predictor of outcome (Rawstron AC *et al.*, Blood 2003: 102(11), 116). The aim of this study was to determine the relationship between sFLC and plasma cell immunophenotype in MGUS patients. *Methods*. Serum, bone marrow aspirate and trephine were assessed from 67 patients (median age 70, range 39-92) with a paraprotein (median 10 g/L range 4-29 g/L; isotype: 36 GK, 25 GL, 7 AL, 1 AK) with a final diagnosis of MGUS by International Myeloma Working Group criteria. Median follow-up is 23 months (range 11 - 38). sFLC levels were calculated using the FREELITE assay. Plasma cells were identified by flow cytometry using CD38/138/45 expression. Cases in which myeloma-phenotype ($CD19^-/CD56^+$) plasma cells represented >90% of total PC and/or >1% of total leucocytes were classified as having a high-risk immunophenotype. *Results*. There was no association between either plasma cell immunophenotype or sFLC ratio and paraprotein level. An abnormal sFLC ratio was present in 30/67 cases of which the majority (25/30) had a high-risk immunophenotype. Patients with a normal sFLC had a high-risk immunophenotype in 11/37 and a low-risk immunophenotype in 26/37 (Fisher's exact test two-tailed p=0.0001). Patients with both normal sFLC ratio and low-risk immunophenotype had stable disease. Disease progression occurred in 3/67 cases: two developed myeloma and one plasmacytoma at 11, 27 and 40 months after diagnosis. The patient developing myeloma at 11 months had a high-risk immunophenotype and abnormal sFLC; the other two patients had a high-risk immunophenotype but a normal sFLC. *Conclusions*. Identifying a population of MGUS patients at risk of progression to myeloma is critical for developing preventative therapeutic approaches. This initial data indicates that sFLC and plasma cell immunophenotype provide complementary information about the risk of progression and both are independent of the paraprotein level. This study indicates that both plasma cell immunophenotype and sFLC should be assessed for risk stratification in MGUS.

PO-908

PYDERMA GANGRENOSA WITH MGUS RESPONDING TO THALIDOMIDE

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Introduction. Pyoderma gangrenosum is a rare neutrophilic dermatosis. Although it is more commonly associated with inflammatory bowel disease, 20% of cases are associated with haematological disorders especially IgA monoclonal gammopathies. The aetiology of pyoderma gangrenosum is unknown as is the mechanism for its association with IgA gammopathy. *Methods*. We report a 55-year-old man who had a 15-year history of extensive pyoderma gangrenosum affecting his trunk and lower limbs associated with an IgA lamda monoclonal gammopathy of undetermined significance (MGUS). *Results*. Over the years, he was treated with numerous courses of prednisolone and other medication including minocycline, ciclosporin, tacrolimus and mycophenolate mofetil. With each systemic medication, he experienced an initial partial response, but after a few months on the medication, his disease would flare-up and no longer be controlled. He also suffered from various adverse effects of these systemic agents including osteoporosis, hypertension and renal impairment. Over the course of his disease, his paraprotein levels continued to rise, reaching a peak of 11 g/L as his pyogerma gangrenosum progressed. He was then treated with thalido-

mide and dexamethasone to target the plasma cell clone. His paraprotein level decreased to 5 g/L and he had the best response to treatment of his lesions of pyoderma gangrenosum. *Conclusions*. Thalidomide has anti-inflammatory, immunomodulatory and anti-angiogenic properties and has been reported previously to have successfully treated recalcitrant cases of pyoderma gangrenosum; these cases of pyoderma gangranosum, however, were not associated with MGUS. Our patient was started on thalidomide to treat the plasma cell dyscrasia, and interestingly, his pyoderma gangrenosusm has also responded well and is now in remission for 6 months.

PO-909

PROGNOSIS IN AMYLOIDOSIS WITH RENAL INVOLVEMENT

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Introduction. More than 50% of patients with AL amyloidosis have renal involvement. Elevation in serum creatinine is often not prominent at presentation, but end-stage renal failure can occur during the course of the disease. In the present study we evaluated the parameters predicting organ survival in patients with AL amyloidosis and renal involvement. *Materials*. One-hundred fifty-nine consecutive patients with biopsy proven AL amyloidosis with renal involvement at diagnosis referred to our center between April 1998 and April 2004 were included in the study. Amyloid typing was confirmed by immunoelectron microscopy and hereditary amyloidoses were also excluded by DNA analysis. Renal involvement was defined according to the International Society for Amyloidosis guidelines (24 hour urine protein >0.5 g/24 hours). *Results*. Median urine protein loss was 5.2 g/24h (range: 0.5-56.4 g/24h) and median serum creatinine was 1 mg/dL (range: 0.34-8.3 mg/dL). Proteinuria was >3 g/24h in 110 patients (69%) and serum creatinine was ≥ 2 mg/dL in 27 cases (17%). Twenty-five patients (16%) progressed to end-stage renal disease and dialysis after a median time of 15.2 months (range: 0.1-61.5 months). A Cox multivariate analysis showed that progression to end-stage renal disease was independently affected by proteinuria (p=0.005), serum creatinine (p<0.001) and younger age (p=0.01) at diagnosis and by hematologic response to chemotherapy (p<0.001, protective). Sixty-one patients (38%) died and median survival was 5.8 years. The cause of death was cardiac in 80% of cases. Overall survival was independently affected by cardiac involvement (p<0.001) and hematologic response to therapy (p<0.001, protective), and not by proteinuria or serum creatinine at diagnosis. *Conclusions*. In AL amyloidosis with renal involvement, serum creatinine, proteinuria and young age at diagnosis predict the progression to dialysis. The most frequent cause of death in these patients is progression of cardiac involvement, independently from the stage of the renal disease. Response to chemotherapy prolongs both renal and overall survival.

PO-910

TREATMENT OF AL AMYLOIDOSIS GUIDED BY BIOMARKERS

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Introduction. Serum N-terminal natriuretic peptide type-B (NT-proBNP) is a marker of cardiac involvement in AL amyloidosis and a powerful prognostic determinant. Effective chemotherapy induces a simultaneous reduction of circulating amyloidogenic free light chain (FLC) and NT-proBNP, improving survival. Cardiac amyloidosis carries a considerable risk of treatment-related death. To optimize the risk/benefit ratio, treatment should be limited to the minimum associated with organ response and extended survival. *Methods*. One-hundred consecutive patients with cardiac AL amyloidosis were evaluated before and after chemotherapy. Patients in dialysis were excluded. Fifty-nine patients were treated with melphalan and dexamethasone, 22 with thalidomide and dexamethasone, 10 with dexamethasone alone and 9 with melphalan and prednisone. Patients were divided into 4 groups: A, no response (43 cases); B, ≥50% FLC reduction without organ response (15 patients); C, ≥50% FLC reduction and ≥30% NT-proBNP reduction (22 patients); D, complete remission at immunofixation irrespective of organ response (20 patients). *Results*. Twenty-nine patients died (24 in group A, 4 in group B, 1 in group D) after a median time of 5.3 months. The median follow-up of living patients is 21.1 months. Median survival in group A was 11.7 months, whereas it was not reached in the other groups. There was a significant survival advantage of group D over groups A (p<0.001) and B (p=0.05), of group C over groups B (p=0.03) and A (p<0.001) and of group B over group A (p=0.04). There was no significant difference between groups D and C. Cox univariate analysis showed that absolute value (p=0.001) and percent reduction (p<0.001) of NT-proBNP and absolute value (p=0.008) and percent reduction (p=0.003) of FLC after chemotherapy were prognostic determinants. Baseline values of NT-proBNP and FLC did not influence survival. Cox multivariate analysis indicated that both NT-proBNP (percent reduction p<0.001 or absolute value p=0.002) and FLC (percent reduction p=0.015 or absolute value p=0.006) after chemotherapy independently affected prognosis. *Conclusions*. Response to chemotherapy can change the natural history of AL cardiomyopathy irrespective of its severity. Complete remission does not grant short-term survival advantage over partial response associated with cardiac response.

PO-911

M-DEX GRANTS DURABLE RESPONSE IN AL AMYLOIDOSIS

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Introduction. Autologous stem cell transplantation (ASCT) has long been considered the most effective therapy for AL amyloidosis, but is associated with significant mortality. Oral melphalan and dexamethasone (M-Dex) grants a response rate comparable to that achievable with transplantation and is well tolerated. To investigate the durability of response and its effect on survival, we extended the follow-up of the original cohort of 46 patients ineligible for ASCT treated with M-Dex (Blood 2004;103:2936-8). *Methods*. Forty-six patients with AL amyloidosis ineligible for transplantation due to advanced disease, were treated with oral melphalan (0.22 mg/Kg on days 1-4) and dexamethasone (40 mg on days 1-4), repeated every 28 days, between 1999 and 2002. Patients relapsing from complete remission (CR) repeated M-Dex. Second-line treatment with thalidomide and dexamethasone (T-Dex) was offered to non responders and patients in partial hematologic response (PR) at the investigators' discretion. *Results*. Thirty-one patients (67%) obtained a hematologic response (CR 33%). Median follow-up of living patients is 5.0 years (range: 3.5-6.7 years). Twenty-one patients (46%) died after a median time of 1.6 years (range: 0.1-5.7 years). Overall median survival is 5.1 years. Hematologic response improved survival (median: 2.1 years vs. not reached, p<.001). Of the 15 patients in CR, 2 died due to unrelated causes and in 9 CR is maintained after a median follow-up of 4.8 years (range: 3.2-6.1 years). Four patients relapsed after a median time of 2 years (0.8-2.5 years), but regained CR after 3 (4 in 1 subject) cycles of M-Dex and CR is maintained in all of them after a median time of 3.0 years (range: 2.1-5.1 years). Twelve patients (7 non responders and 5 in PR) received T-Dex. Response to T-Dex was observed in all the patients in PR (2 obtained CR) after M-Dex and in 2 non responders to M-Dex. One patient developed a myelodysplastic syndrome after 2 cycles of treatment. No other late toxicity was observed. *Conclusions*. Hematologic response to M-Dex is durable, can be easily restored in relapsing patients and prolongs survival. Second-line treatment with T-Dex improves response to M-Dex. Oral M-Dex is a viable alternative to ASCT performed with reduced-intensity conditioning.

PO-912

CLONAL RESPONSES TO BORTEZOMIB PLUS DEXAMETHASONE IN PATIENTS WITH SYSTEMIC AL-AMYLOIDOSIS

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Background. Amyloid light-chain (AL) amyloidosis has remained a therapeutic challenge. Various anti-myeloma regimens have been tested, with high-dose melphalan and autotransplantation being most active, but only a minority of patients is eligible for such therapeutic interventions. Treatment with novel agents is therefore of growing interest in the treatment of AL amyloidosis. *Methods*. Bortezomib (1.3 mg/m^2 on days 1, 4, 8, and 11) plus dexamethasone (20 mg per os; days

1, 2, 4, 5, 8, 9, 11, and 12) was used to treat two patients with newly diagnosed AL amyloidosis. Cycles were repeated every 3 weeks. *Results*. Patient 1 (age: 37 years) had involvement of lymph node and bone marrow involvement and presented with an IgG-lambda paraprotein. Four cycles of bortezomib/dexamethasone resulted in a >50% reduction of the paraprotein including lambda light-chains in the free-light-chain assay. The size of enlarged cervical lymph nodes has remained unchanged to date. No toxicity of bortezomib/dexamethasone (grade 2 or greater) was observed. Bortezomib/dexamethasone was followed by a successful harvest of G-CSF primed peripheral blood stem cells, and autologous transplantation is now being performed. In patient 2 (62 years), AL amyloidosis was diagnosed after work-up of proteinuria (lambda light-chains detectable only by the free-light-chain assay). Within 3 cycles of bortezomib/dexamethasone, free lambda light-chains were reduced to >90%, which was also associated with a significant decrease in the 24-hour protein excretion. Due to rapidly evolving peripheral neuropathy, bortezomib was temporarily stopped. *Conclusion*. Bortezomib/dexamethasone can induce major hematological responses in patients with AL amyloidosis. Longer follow-up is needed to see whether or not these clonal responses are followed by organ responses.

PO-913

TREATMENT OF LIGHT CHAIN (AL) AMYLOIDOSIS WITH THE COMBINATION OF BORTEZOMIB AND DEXAMETHASONE

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Background. Primary systemic amyloidosis (AL) is a clonal plasma cell dyscrasia characterized by widespread deposition of abnormal amyloid fibrils derived from abnormal light chains, leading to multisystem organ failure. Aggressive treatment of AL amyloidosis with high-dose melphalan and autologous stem cell transplantation (HDM-ASCT) is currently the treatment of choice for selected patients, while the combination of melphalan and dexamethasone is used for patients who are not eligible for HDT. Bortezomib is a proteasome inhibitor with proven activity in relapsed/ refractory Multiple Myeloma, alone or in combination with dexamethasone. *Aims.* To evaluate the activity and feasibility of the combination of Bortezomib and Dexamethasone (BD) in patients with primary systemic amyloidosis. *Methods*. We treated consecutive patients with histologically proven, symptomatic AL with the combination of Bortezomib 1.3 mg/m^2 on days 1, 4, 8 and 11, and Dexamethasone 40 mg on days 1 to 4, every 21 days, for up to 6 cycles. Dose modifications were made based on toxicity. All patients received prophylaxis with Valacyclovir 500 mg daily and trimethoprim-sulfamethoxazole 3 times weekly. Omeprazole was given in all patients during at least dexamethasone administration. For the assessment of hematologic and organ response we followed the recommendations of the 10th International Symposium on Amyloid and Amyloidosis (Gertz *et al.*, Am J Hematol (2005) 79: 319-328). *Results*. seventeen consecutive patients have started treatment with BD since September 2005. Their characteristics are shown in Table 1. Eight patients (47%) had at least one prior therapy (median 1, range 1 to 3). Eleven (73%) patients had two or more organs involved; kidneys and heart were affected in 14 patients each respectively. The majority of patients had impaired performance status, high BNP values and serum creatinine was elevated in 7 patients. Among 15 evaluable patients, three had a complete hematologic response (CR) and 11 had partial hematologic response (PR) for an overall response rate of 87% (95% CI 60-98%). Among evaluable patients, 80% achieved a normal FLC ratio including five patients who had not responded to prior high dose dexamethasone based treatment and one patient under dialysis. Hematologic responses were rapid: median time to hematologic response was 1.2 months (95% CI 0.8-1.8). So far, four patients (27%, 95% CI 8-55%) had a response in at least one affected organ. Median time to organ response was 4.3 months (range 2-8). Median follow-up after initiation of treatment for all patients was 6.3 months (range 1-17) and 9 months (range 2-17) for living patients. Median overall survival for all patients has not been reached with 13 (76%) patients alive 2 to 17 months after initiation of treatment. Peripheral neuropathy, fatigue, peripheral edema, constipation and exacerbation of postural hypotension were manageable although necessitated dose adjustment or treatment discontinuation in most patients. *Conclusions*. The combination of BD is feasible for patients with AL amyloidosis. Patients achieve a rapid hematologic response and toxicity can be managed with close follow-up and appropriate dose adjustment. This treatment may be a valid option for patients with severe heart or kidney impairment. Updated results will be presented at the Workshop.

Table 1. patient characteristics.

Male / Female	7/ 10
Age (median /range)	59 (42-80)
Untreated / Pretreated	9 / 8
Previous treatments	
p.o Melphalan/ High Dose Dexamethasone	3
VAD	5
IV Melphalan/ High Dose Dexamethasone	1
HDM	1
Light chain type κ/λ	2 / 15
Heavy chain (by immunofixation)	
IgG / IgA / Light chain only	4 / 2 / 6
Involved FLC, mg/L (median/range)	216 (41-2580)
Bone marrow Plasma cells	
(Median / range)	20% (<5-80%)
Organ involvement	
Heart	14
Kidney	14
Peripheral nerve	3
GI	3
Number of organs involved	
1	6
2	5
>2	6
Ejection fraction (median / range)	74% (47-84%)
EF<55%	1
LV septum mm (median/range)	15 (10-20)
NYHA class >1	9
Baseline biochemical measurements	
BNP pg/mL(Median/Range)	294 / 59-4790
BNp>120 pg/mL	12
β2-microglobulin>3.5 mg/dL	7
Creatinine >2 mg/dL	7
Albumin<3.5 g/dL	11
Urine protein (mg/24hrs)	2800 (900-7600)

PO-914

CALRETICULIN, AL-AMYLOIDOSIS AND HIGH-DOSE MELPHALAN

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Introduction. High-dose melphalan is an effective therapy for selected patients with systemic AL-amyloidosis. Melphalan can eliminate the indolent clonal plasma cells that cause AL and the achievement of a complete response is associated with extended survival while lack of response is not. The biologic basis of response to melphalan in AL patients is unknown. *Materials and Methods*. In order to identify factors specific to clonal AL plasma cells, gene expression profiles (Affymetrix U133 Plus 2.0) were obtained using FACS-sorted $CD138^+/DAPI^-$ plasma cells (>95% pure) from 17 untreated AL patients before SCT. Stringent supervised analyses were then performed based on responses at 3 months post-SCT, comparing the complete and the no response groups. In validation studies we used cells from 25 AL patients for real-time PCR, and formalin-fixed marrow biopsies from 30 AL patients to assess protein expression. *Results*. Gene-expression studies identified calreticulin as having significantly higher expression in the purified pre-treatment plasma cells of patients who then went on to have a complete response to high-dose melphalan. The basic analysis was a t-test, filtering genes for differential expression at $p<0.01$. A secondary filtering selected only genes with two-fold difference in expression between complete and no response groups, average expression of at least 1000, and inclusion in an EASE group that had a $p<0.05$. Real-time PCR showed that AL plasma cells were indolent by Ki-67 expression compared to myeloma cells and cell lines. Real-time PCR showed that $CD138^+$ plasma cells from responders and complete responders expressed significantly higher levels of calreticulin mRNA than non-responders ($p<0.05$ in both cases). Immunohistochemical staining showed that expression of calreticulin was significantly higher in the plasma cells of those with a complete response. Patients with a calreticulin staining index of 0-6 had a lower likelihood of achieving a response than those with an index of 9. *Conclusions*. Calreticulin is a pleiotropic calcium-binding protein found in the endoplasmic reticulum and the nucleus whose over-expression is associated with increased sensitivity to apoptotic stimuli. These data support further investigation of calreticulin and its association with response to melphalan in patients with systemic AL-amyloidosis.

GROUP 10: Prognostic factors, response assessment, staging, changes in prognosis epidemiology

PO-1001

EARLY MORTALITY IN MM: CORRELATION WITH DISEASE PARAMETERS

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Introduction. Induction therapy in multiple myeloma (MM) often cannot achieve rapid tumor load reduction on the one hand, while on the other treatment related toxicity may further compromise immune system, renal, cardiac and bone marrow function. Consequently, up to 10% of patients die within the first 2 months from diagnosis. The aim of the present study was to assess the rate and causes of early mortality in a large number of MM patients and to evaluate possible correlations with clinical and laboratory parameters at the time of diagnosis. *Materials and Methods.* Between January 1989 and October 2006, 484 patients with MM were diagnosed and treated in our department. Early mortality was defined as death within 60 days from diagnosis. Patients' characteristics at the time of diagnosis (gender, age, stage, ECOG status, MM type, extent of bone disease and bone marrow infiltration, β-2-m, CRP, laboratory parameters) were compared between patients with early death and the rest, using chi-square tests. *Results*. Early death was observed in 36 (7.4%) patients, 16 (44.4%) during treatment with melphalan-prednisolone and 20 (55.6%) under VAD. Causes of early mortality included infection in19 (52.8%) cases, renal failure in 10 (27.8%), cardiovascular complications in 4 (11.1%) and hemorrhage in 3 (8.3%). Characteristics that demonstrated the highest correlation ($p<0.0001$) with early death were ECOG status IV (50% vs. 8.9%), β-2-m >6 mg/L (69.4% vs. 29.9%) and CRP > 20 mg/L (61.1% vs. 21.7%). Early death was also significantly ($p<0.05$) associated with age >70 years (55.6% vs. 34.4%), Durie-Salmon stage IIIB (41.7% vs. 17.6%), extensive bone disease (69.4% vs. 47.5%), bone marrow infiltration >80% (36.1% vs. 18.8%), Hb < 7.5g/dL (41.7% vs. 19.2%), neutropenia/thrombocytopenia (33.3% vs. 13.4%), albumin <3 g/dL (44.4% vs. 23%) creatinine >2.5 mg/dL (30.6% vs. 11.6%), uric acid >7mg/dL (63.9% vs. 44.2%), calcium >12mg/dL (33.3% vs. 14.7%) and LDH >350 U/L (58.3% vs.37.5%). Gender and MM type did not show any correlation with early mortality ($p>0.05$). *Conclusion*. Infections and renal failure were the major causes of early mortality in our MM patients. ECOG status IV, β-2-m >6 mg/L and CRP > 20 mg/L at diagnosis were the most important adverse factors.

PO-1002

PREVALENCE OF PREVIOUS APPENDECTOMY IN COHORT OF PATIENTS WITH MULTIPLE MYELOMA

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Introduction/Aims. Previous epidemiological studies have examined the associations between various medical conditions and the subsequent development of cancer. Of particular interest to researchers has been the hypothesized link between autoimmune conditions, infection/inflammation and the development of cancer. Studies examining the relationship between appendectomy and risk of cancer have reported mixed results. A Swedish study in 2003 identified increased risk for the development of NHL and stomach cancer occurring after remote appendectomy though failed to find associations between appendectomy and other types of cancer. The pathophysiology is thought to be due to infection/inflammation of lymphoid tissue in addition to surgical removal of such tissue at a young age. The appendix is laden with lymphoid tissue that develops early in life and continues to grow into early adulthood. It is possible that the appendix contributes to immune function via gut-associated lymphoid tissue (GALT). *Materials and Methods.* Fifty-six patients with multiple myeloma were prospectively accrued from outpatient oncology clinics at the Tom Baker Cancer Center for a study examining biologic correlates of fatigue in patients with multiple myeloma. Past medical history was collected to allow for identification of potential correlates between comorbid medical conditions, fatigue and quality of life. *Results*. The sample was comprised of 56 patients. The mean age was 61.96 years (SD 10.027) with a range of 41-84. Several patients had a history of gastrointestinal conditions including diverticulosis (8.9%), diverticulitis (3.6%), GI bleed (10.7%) and cholecystecto-

my (5.4%). Of interest, 17.9% of the sample had a history of appendectomy. *Conclusions*. The finding that 17.9% of the study participants had a history of appendectomy is intriguing. An epidemiological study in 1990 reported a lifetime risk of appendicitis to be 8.6% for males and 6.7% for females. The sample size in this study was small and without an age-matched group control group these findings may not be clinically significant. Further study is required to examine the association between appendectomy and the development of multiple myeloma.

PO-1003

EYE COLOR: IS THERE ANY IMPLICATION IN THE DEVELOPMENT OF MULTIPLE MYELOMA?

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Introduction. Eye color is determined by the distribution and content of the melanocyte cells in the uveal tract of the eye and is inherited as a polygenic trait. Several enzymes are known to have major effects on pigmentation including dopachrome tautomerase (DCT), which is involved in the catalytic formation of melanin. The implicated gene for DCT is located on 13q32. Monoallelic loss of 13q sequences is one of the most frequent abnormalities (50% with Fluorescence *In Situ* Hybridization) in multiple myeloma (MM). Based on the clinical observation that many of the MM patients presented in our center had blue or green eyes we evaluated the incidence of different eye colors in MM patients compared to healthy individuals. *Patients and Methods*. Ninety MM patients and 180 healthy individuals of the same origin (Greek) were studied. The median age of MM patients and healthy individuals was well balanced (62,3 and 64 respectively). Patients and healthy individuals were classified into four groups according to the eye color (blue, green, hazel and brown). Pearson Chi-Square test was used for comparisons of proportions across levels of categoric variables. A level of 1% was used to denote statistical significance. *Results*. In the group of MM patients 21 (23,3%) had blue eyes, 13 (14,5%) green 27 (30%) hazel and 29 (32,2%) brown. In the control group of 180 healthy individuals 27 (15%) had blue eyes, 13 (7,2%) green, 24 (13,3%) hazel and 116 (64,5%) brown eyes. According to the analysis of the adjusted residuals, the proportion of blue and green eye color among the MM patients was significantly higher (p<0.001) compared to healthy individuals. *Conclusions*. Our results confirmed the observation that, the incidence of blue or green eye color in MM patients of Greek origin is higher compared to the typical brown eye color of the Mediterranean origin. Further epidemiological data concerning the incidence of different eye colors among the Greek population, are needed in order to verify these results.

PO-1004

CRP IS PREDICTIVE OF FATIGUE AND QOL IN PATIENTS WITH MULTIPLE MYELOMA

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Introduction/Aims. Fatigue is one of the most commonly reported symptoms in patients with cancer and is almost certain to occur at some point along the illness trajectory in patients with multiple myeloma. Fatigue is a multifaceted symptom whose etiology remains to be elucidated. Although most research has focused on the role of anemia, there is growing evidence that other processes, such as inflammation, may contribute to the development of fatigue. A greater understanding of the pathophysiology of fatigue in multiple myeloma may provide important information about individual experience and lead to improved symptom management and quality of life in these individuals. The purpose of this study was to begin an exploration of factors related to disease and treatment in multiple myeloma that contribute to the development of fatigue in these individuals. The objective of this study was to examine the relationships between clinical variables (hemoglobin, C-reactive protein), fatigue and quality of life in individuals with multiple myeloma. *Materials and Methods.* This study employed a descriptive exploratory design. Fifty-six patients with multiple myeloma (stage I, II and III) were prospectively accrued from outpatient oncology clinics at the Tom Baker Cancer Center. Instruments used to assess quality of life included the EORTC-QLQ-C30 and MY20 (a myeloma specific QOL module). Fatigue was assessed using the FACT-F and the fatigue subscale of the EORTC-QLQ-C30. Laboratory and demographic information was collected from patient charts. *Results.* As expected, significant bivariate relationships emerged between hemoglobin and fatigue (r=-0.352, p=0.008) and hemoglobin and QOL (r=0.406, p=0.002). Additionally, significant bivariate relationships emerged between CRP and fatigue (r=0.503, p=0.000) and CRP and QOL (r=-0.524, p=0.000). Of interest, multivariate regression analysis identified that CRP was a significant predictor of fatigue (p=0.004) and QOL (p=0.010), while hemoglobin was not predictive of fatigue (p=0.28) or QOL (p=0.328) once the effect of CRP was accounted for. *Conclusions.* These findings suggest a possible role for inflammation in the development of fatigue and QOL and support the need for further research into the mechanisms underlying cancer related fatigue with the greater goal of improving symptom management and patient quality of life.

PO-1005

PRE-TREATMENT CLONOTYPIC VDJ% PREDICTS REMISSION LENGTH

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Background. Predicting remission duration in multiple myeloma is difficult. Conventional measures of disease burden including bone marrow plasmacytosis (BMPC) and serum monoclonal protein (M-protein) levels do not correlate well with outcomes such as time to disease progression or survival. *Methods*. We used real-time quantitative clonotypic VDJ PCR, compared to β2 microglobulin, to estimate the proportion of clonotypic cells in blood or bone marrow prior to, during and following drug therapy. A standard curve was generated to estimate the percentage of clonal cells in the sample based on the relative VDJ DNA quantitation (termed VDJ%). VDJ quantitation was correlated to BMPC, M-protein levels and time to progression (TTP). *Results*. To date, 22 pretreatment (nine relapsed myeloma and 13 previously untreated) and 22 post-remission (10 relapsed and 12 previously untreated) BM samples have been evaluated. All patients received a course of therapy, including 12 and 14 respectively treated with newer therapies (bortezomib or lenalidomide containing regimens). Pretreatment BM VDJ% below the median predicted longer TTP (HR=0.31, p=0.03). BMPC and M-protein levels failed to predict TTP (p=0.3 and 1.0, respectively). Modest but statistically significant correlations between pretreatment VDJ% and BMPC (r2=0.29, p=0.0001) or M-protein levels (r2=0.49, p<0.0001) were found. Remission BM were analyzed as above and neither the remission VDJ% nor log kill predicted TTP (p=0.6 and 0.5). PB from 24 patients (15 relapsed and nine previously untreated) were analyzed for VDJ% at pre-treatment, approximately 30, 60, 90 and 120 days after initiation of therapy. The log kill values and VDJ% from the four treatment samples compared to the pre-treatment samples did not predict TTP. However, survival analysis indicated that VDJ% below the median in pre-treatment PB samples predicted TTP (HR=0.335, p=0.02). VDJ% in BM and PB were significantly correlated (r2=0.62, p=0.006). *Discussion*. The proportion of clonotypic cells in the BM and PB prior to initiation of treatment predicted time to progression whereas remission values, log kill, BMPC or M-protein levels did not. This suggests that molecular monitoring immediately prior to initiating treatment is a superior method of quantifying disease burden that correlates well with clinical outcome.

PO-1006

RENAL RECOVERY IS A FAVORABLE PROGNOSTIC MARKER IN PATIENTS WITH NEWLY-DIAGNOSED MULTIPLE MYELOMA

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Introduction/aims. Serum creatinine value in MM at diagnostic is used for staging and assessment of prognosis. As many as 50% of patients with MM may experience some degree of renal insufficiency, in the majority renal function will improve in response to simple measures (hydration, correction of hypercalcaemia, etc), but a proportion of patients have persistently compromised renal functions. *Methods*. We reviewed the outcome of 37 patients with serum creatinine >200 micromole/L at start of chemotherapy with VAD or Dexametazone high dose (Dex). *Results*. 18,9% patients died early (1-9 weeks, median 3) after starting therapy due to renal failure, infections or other medical complications. Of the remaining 81,1% patients, 13,33% attained CR, 56,56% PR, and 16,16% did not respond after median 4 course of therapy. On an intent-to-treat basis, overall response rate to induction therapy was 59%. Creatinine declined with therapy in 84% of 27 evaluable patients, and the change in creatinine from cycle 1 to 2 was on an average of 32%. The extent of the change in creatinine was not predictive of eventual survival suggesting that intensive therapy should be continued even if renal function does not

improve after 1 cycle of chemotherapy. 6 patients had creatinine >200 micromole/L (including 2 on dialysis). Median survival of the whole group was 21 months, with one patient alive with disease at 4 years. Not surprisingly, 62,5% patients who died had creatinine values >200 micromole/L at death vs 2/9 living patients at last follow-up (p=0.03) suggesting that kidneys are compromised and regardless of cause of death, they fail as terminal event. *Conclusions*. While renal dysfunction at initiation of induction therapy is indicative of relatively poor outcome, it's largely due to early mortality - which can be reduced with intensified supportive therapy. In patients surviving the first 2-3 months, long-term outcome is comparable to those with normal renal function if treated similarly.

PO-1007

IS HYPERCALCEMIA AN INDEPENDENT PROGNOSTIC FACTOR IN BRAZILIAN PATIENTS WITH MULTIPLE MYELOMA?

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Aims. In a previous retrospective study, we characterized the demographic and clinical features of multiple myeloma (MM) patients treated at tertiary care centers in Brazil, validating the predictive accuracy of the International Staging System (ISS) in that sample. We now examine the prognostic role of baseline hypercalcemia. *Materials and Methods.* 16 hematology centers provided information on 1,112 patients with MM diagnosed between 1998 and 2004, whose data were obtained from institutional charts and entered on a web-based system designed for the study. Survival was analyzed by the Kaplan-Meier method and log rank tests, with multivariate analysis using Cox model. *Results*. 49.7% of patients were female, with median age 60.5 years (range, 28 to 94). Durie-Salmon staging (DSS) was I/II/III in 6.4/17.1/76.5%, and ISS was I/II/III in 20.1/48.7/31.2% of cases. High-dose chemotherapy (HCT) was administered to 25.5% of patients. Hypercalcemia was absent in 76.2% of patients, who had a median overall survival (OS) significantly longer than those with hypercalcemia (63.9 vs 21.3 months; p<0.001). Other significant factors for OS in univariate analyses (p<0.001 for all variables) were ISS, DSS, age, and receipt of HCT. In a multivariate model including these variables, the hazard ratio (HR) for mortality was 2.35 (95% CI, 1.75-3.17; p<0.001) for patients with hypercalcemia (using no hypercalcemia as reference). Other significant HR were 0.39 for HCT (vs no HCT, p<0.001), and 1.80 for ISS stage III (vs. I, p=0.010). *Conclusions*. This retrospective study suggests that baseline hypercalcemia is among the most important prognostic factors in Brazilian patients with MM, even when the ISS is considered. Prospective validation of these findings seems warranted. In addition, it is possible that hypercalcemia might further categorize patients within ISS groups, a hypothesis also worthy of further study.

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PO-1008

MONITORING OF MINIMAL RESIDUAL DISEASE IN MULTIPLE MYELOMA: FLOW CYTOMETRY VS PCR-BASED TECHNIQUES

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Highly sensitive methods are essential to an accurate diagnosis of minimal residual disease (MRD) in multiple myeloma (MM). Recent studies have indicated that nested polymerase chain reaction (PCR) using patient-specific primers to identify rearrangements of immunoglobulin-heavy-chain sequences (ASO-primers) and multi-parametric flow cytometry (FCM) to detect phenotypic aberrations of plasma cells (PC) are equally reliable techniques in this case. Here we performed a comparative analysis of those two and a third method which assesses lineage-specific PC chimerism by real-time PCR based on bi-allelic sequence-nucleotide polymorphism regarding their sensitivity to detect MRD. We investigated 66 bone marrow (BM) samples from 42 MM-confirmed patients at different time points after allogeneic stem cell transplantation. All samples were analysed by real-time PCR for chimerism analysis and by FCM using a panel consisting of $CD56^{+++}/CD19^{-}/CD45^{-}$ following the $SSClo/CD38^{+}/CD138^{+++}$ gating strategy. For FCM an MRD threshold value of 0.01% of the total white blood cells (WBC) for PC with phenotypic aberrations (aPC) was used. Fifteen samples were tested with patient-specific PCR using ASO-primers. FCM and ASO-primer PCR results correlated very well in all 15 samples tested (6 positive and 9 negative MRD cases. Also, all twenty samples which were positive for MRD using FCM (0.01% - 1.41%) demonstrated a PC chimerism of <99.8% (0.00-9.7%). In contrast, only fourteen of the 46 samples with no detectable MRD (by FCM) showed a PC chimerism below or equal 99.9% (96.1-99.9%), but 32 of the 46 samples had a full donor chimerism of >99.9% in the PC department. Notably, the kinetics of MRD measured simultaneously by FCM and PC chimerism revealed high correlations in all 18 from a total of 42 patients.The present report summarizes the comparison of 3 different methods in the evaluation of MRD in MM. It shows that FCM was a comparable with the patient-specific ASO-PCR. We have seen a correlation between FCM and PC chimerism data in all MRD cases, but mixed chimerism was not predictive for MRD. Obviously, chimerism kinetics would be much more reliable. In conclusion, we suggest that multi-parametric FCM can be regarded as an effective surrogate method in addition to PCR (supposed to be the most sensitive tool) in monitoring of MRD in MM patient after allogeneic stem cell transplantation.

PO-1009

MINIMAL RESIDUAL DISEASE MONITORING IN MULTIPLE MYELOMA: HOW SPECIFIC IS THE FLOW CYTOMETRIC ANALYSIS?!

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Recent studies have indicated that flow cytometry (FCM) is a sensitive tool to detect minimal residual disease (MRD) in the bone marrow (BM) of patients with multiple myeloma (MM). Although this approach has been shown to allow a reliable prediction of MRD, its specificity has not been proven yet. To detect aberrant plasma cells (aPC) based on their phenotypical characteristics, we applied a panel consisting of CD56/CD19/CD45 following the $SSClow/CD38^{+}/CD138^{+++}$ gating strategy described by Almeida *et al*. (1999). We investigated 21 BM samples from healthy donors as control and 16 BM samples from 14 MM-confirmed patients. Surprisingly, in normal control subjects we detected a cell population mimicking the phenotypic aberrations ($CD138^{+++}CD38^{+}$ $CD56^{+}CD19^{-}CD45^{-}$) characteristic for myelomatous cells. Indeed, we found that low amounts of cells in all of the 21 BMs from healthy donors displayed the immunophenotype typical for MM. The percentage of these *positive* cells from healthy donors ranged from 0.001% to 0.06% as related to the entire white blood cell (WBC) population. This corresponded to 0.11% - 9,76% of the entire plasma cell (PC) population. This data is in conflict with the broadly used threshold value for MRD, which is, based on a proposal of Rawstron *et al.* (2002), set at 0.01%. In fact, in 12 of our 21 healthy donors we detected between 0.02% to 0.06% *aberrant cells*. This was not only higher than the above mentioned threshold for MRD but equalled or exceeded the values for five of the 16 samples from MM-confirmed patients. The presence of cells in the bone marrow mimicking a pathological phenotype is not unusual. Kern *et al.* (2003) reported the observation of immunophenotypic aberrations characteristic for AML cells in the BM of healthy donors. The presence of cells with pathological markers typical for CLL in normal blood has also been reported (Bottcher *et al.* 2004). The frequency of those pseudo-malignant cells in the samples of healthy individuals has been considered in the definition of cut-off values allowing a reliable diagnosis of MRD in both types of leukemia.

PO-1010

THE RELATIONSHIP BETWEEN SERUM FREE LIGHT CHAIN ASSAY AND SERUM IMMUNOFIXATION ELECTROPHORESIS

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The serum free light chain (SFLC) assay enables detection of an abnor-

mal protein in patients who secrete no/trace monoclonal protein. Thus, the serum free kappa:lambda ratio (SFKLR) may be abnormal (normal 0.26-1.65) in patients who have no detectable M protein on serum immunofixation electrophoresis (IFE). However, the value of SFKLR in patients with positive serum IFE has not been studied adequately. The new international response criteria propose that negative IFE and normalization of SFKLR be used to define *stringent* complete remission (CR) in myeloma, and that in the absence of SFKLR or with abnormal SFKLR, negative IFE be used to define *non-stringent* CR. The obvious implication of this proposal is that SFKLR is the most sensitive indicator of residual disease. Thus, one would expect to see abnormal SFKLR with normal IFE but not vice versa. As these criteria have not been validated yet, our aim was to correlate serum IFE and SFKLR. Results on 326 samples from myeloma patients with IgG or IgA disease where simultaneous serum IFE and SFKLR were available were analyzed. Samples with >1 monoclonal band (oligoclonal bands or biclonal disease) were excluded. IFE was positive in 227 (151 IgG kappa, 33 IgA kappa, 30 IgG lambda, 13 IgA lambda), and negative in 99. SFKLR was normal in 116 (51%) and abnormal in 111 (49%) of cases with positive IFE. SFKLR was normal in 71 (72%) and abnormal in 28 (28%) of cases with negative IFE. Thus, 22% of the cases had normal SFKLR + negative IFE, 34% had abnormal SFKLR + positive IFE, 9% had abnormal SFKLR + negative IFE, and 36% had normal SFKLR + positive IFE (p=0.001). The extent of discordance between serum IFE and SFKLR in our study suggests that additional work is required before the place of SFLC estimation in assessing response in myeloma can be determined, and serial data are needed to see if normalization of SFKLR is invariably followed by IFE negativity and if an abnormal SFKLR always heralds IFE positivity.

PO-1011

SERUM FREE LIGHT CHAIN LEVELS IN MYELOMA PATIENTS WITH OLIGOCLONAL BANDS

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The detection of multiple restricted bands on immunofixation in myeloma patients on or after therapy (*oligoclonal bands*) is thought to represent immune recovery with no adverse prognostic significance. We studied 52 serum free light chain (SFLC) levels and serum free kappa:lambda ratios (SFKLR) in 23 patients with oligoclonal protein bands (defined as >1 monoclonal heavy and/or light chain bands on serum and/or urine immunofixation). 10 (19%) samples did not show the original monoclonal protein isotype whereas 42 (81%) did. 17 (33%) samples came from patients in CR/near-CR based on stringent conventional criteria. Based on clinical and treatment history, 23 (42%) samples were classified as being from patients with unresponsive disease (stable or progressive on current therapy) and 29 from patients with responsive disease (CR/near-CR or declining disease burden on current therapy). SFKLR was abnormal in 32 (61%) samples. 45% of samples where the original monoclonal protein isotype was one of the bands detected had abnormal SFKLR compared to 10% when it was absent (p=0.068). 18% of samples from patients in CR/near-CR had abnormal SFKLR compared to 49% when the disease was not in CR/near-CR (p=0.038). 48% of samples with responsive disease had abnormal SFKLR compared to 26% when the disease was unresponsive (p=0.10). The level of monoclonal protein on serum protein electrophoresis did not correlate with SFKLR: 9 of 22 samples with monoclonal protein ≥0.2 g/dL had abnormal SFKLR compared with 11 of 30 samples with monoclonal protein <0.2 g/dL (p=0.76). We conclude that in myeloma patients showing oligoclonal bands on serum and/or urine immunofixation on or after therapy, the presence of normal SFKLR suggests a significantly greater likelihood of CR/near-CR

PO-1012

FREE LIGHT CHAINS IN URINE - AN ADDITIONAL DIAGNOSTIC ADVANTAGE?

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Background. The examination of free light chains (FLC) in serum is a very useful diagnostic tool in patients with monoclonal light chain (MLC) disease. About 30% of patients with multiple myeloma have renal involvement at the time of diagnosis increasing to 50% during the course of disease. But different kinds of renal involvement have different prognostic value. Cast nephropathy usually progress very rapidly to end stage renal disease, whereas nephrocalcinosis can disappear completely with treatment. At the time the kind of renal involvement can be diagnosed correctly only by kidney biopsy. We investigated, if the analysis of FLC in urine may be helpful to predict the kind of renal involvement. *Methods*. We analysed the excretion of FLC in urine in patients with MLC associated disease who underwent kidney biopsy because of unclear proteinuria or renal insufficiency. Patients were grouped according their histological findings: 1:cast nephropathy (CN), 2:light chain deposit disease (LCDD), 3:AL amyloidosis (ALA), 4:other renal disease (ORD). Urine FLC were determined by immune electrophoresis and immunoessay. *Results*. Kidney biopsies of 35 patients with multiple myeloma (MM n=26), immunocytoma (IM n=2), AL-amyloidosis (ALA n=2) and monoclonal gammopathy of undeterminated significance (MGUS n=5) were available. In 33 patients we had data about light chain excretion in urine. The findings in kidney biopsy were CN n=13(37.1%), LCDD n=3(8.6%), ALA n=8(22.8%) and ORD n=11(31.4%). The mean FLC concentrations [mg/dL] were 329.5±334.5(CN); 17.37±21.6(LCDD); 11.2±13.2(ALA) and 117.1±171.5 (ORD) (p<0.5 CN vs.LCDD and ORD; p<0.1 CN vs.ALA) If the critical FLC concentration was defined with >25 mg/dL the positive predictive value (PPV) was 0.61 for having a CN, sensitivity was 100%, specificity 60%. With FLC concentration >50 mg/dL PPV was 0.68, the sensitivity was 85% the specificity was 75%, respectively. *Discussion*. This data demonstrate that the examination of FLC in urine may be helpful to find patients with cast nephropathy. Patients with LCDD or ALA had significant lower light chain concentrations in urine and are clearly to distinguish from CN. This might be important, because CN decreases very often rapidly with renal function and the correct diagnosis of renal involvement is important for treatment decisions.

PO-1013

FLC AS A MARKER OF RESPONSE IN A MATURE DATASET: ECOG E9486

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Introduction. Questions remain about the best uses of the quantitative nephelometric assay for serum immunoglobulin free light chains (FLC) in patients with multiple myeloma: can it replace 24 hour urinary protein electrophoresis measurements and is an early decrease in FLC prognostic? *Methods*. E9486 randomized patients to receive one of 3 treatments: VBMCP, VBMCP plus high dose cyclophosphamide, or VBMCP plus interferon (Oken *et al.* Cancer 1999). Patients had research blood samples stored pre-treatment and after 2 months of therapy. 408 patients had stored sera which was used to run the serum FLC assays (The Binding Site, U.K.), but 7 were excluded because they were non-secretors. For the analyses, patients were divided into 3 groups irrespective of treatment group: those with *serum M-spike only* (n=103); *urine M-spike only* (n=55); and *both serum and urine M-spike* (n=243). The FLCs were evaluated in terms of involved absolute values, ratios of involved to uninvolved, and differences of involved and uninvolved. Changes in FLC measurements and M-spikes were based on absolute changes and percent changes. A FLC response was analyzed using 2 definitions: 1) a 50% decrease in the difference between involved and uninvolved FLC levels; and 2) a 50% decrease in the level of involved FLC AND a 50% decrease in the ratio of involved/uninvolved FLC. *Results*. The median involved FLC was 38.7 mg/dL (range 0.25-3370). For the 33 *urine M-spike only* patients with urine samples at baseline and at 2 months, the correlation coefficient of urine M-spike change and involved FLC ratio change at 2 months was only 0.60 (p=0.0003) while the respective value for the 79 serum and urine patients was 0.23 (p=0.0001). The FLC response at 2 months was associated with overall ECOG hematologic response (p<0.0001). Of 288 ECOG responders, 189 (66%) were detected at 2-month as FLC responders. This early FLC response did not predict for either progression free survival or overall survival. *Conclusions*. The correlation between serum FLC and urine M-spike is not adequate for the former test to replace the latter in myeloma patients. However, a drop in free light chain two months into alkylator based therapy does associated with eventual response.

PO-1014

SERUM FLC AS A MEASURE OF TREATMENT OUTCOME IN MM

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Introduction. Preliminary data suggest that serum Free Light Chains (sFLC) could be used not only as a close measure of tumor burden but also as a surrogate marker of treatment outcome in MM. *Patients and methods*. We evaluated sFLC (FREELITE(r), The Binding Site LtD) and FLC ratio (FLCr: pathological FLC/non pathological FLC) at baseline and after 1-2 therapy cycles in 51 MM patients with the following features: median age 66 yrs, 16 *de novo* MM, 35 relapsed. Twenty seven were IgG, 12 IgA and 12 BJ MM, 49 in stage 2-3 ISS, median β2-microglobulin 4.0 mg/L (range 1.3-20). Median sFLC l 854 mg/L (range 50-13800) and median FLCr 106.5 (range 3-13000). Twenty patients received thalidomide, while the other 31 were treated with bortezomid, in all of them chemotherapy was associated: 36 patients (70.5%) had a response VGPR and 13 patients (25.5%) progressed during a median follow-up of 12 months. *Results*. Patients with baseline FLCr 60, β2-microglobulin level 4.0 mg/L and stage 1 ISS achieved a significantly higher response rate VGPR versus those presenting higher levels of the above parameters (85% vs 56%, p=0.025; 90% vs 60%, p=0.028; 87% vs 45.5%, p=0.003; 93.5% vs 61%, p=0.021, respectively). On the contrary standard MC, sFLC absolute values, bone marrow plasma cells, cytogenetics and therapy were not predictive of response. Moreover, patients who had at least 5-fold FLCr decrease after 1-2 courses of therapy achieved a significantly higher rate of VGPR response (94.5% vs 57%; p=0.027). We observed a trend for better TTP in patients with lower values of baseline FLCr, post therapy FLCr and β2-microglobulin value (NR vs 12.5 months, p=0.0974; 18.2 vs 12.6 months, p=0.1154; 18 vs 12.5 months, p=0.1892, respectively). *Conclusions*. Our results suggest that baseline FLCr, as some other standard prognostic factors and not standard MC or sFLC absolute values, is predictive for response and TTP in MM patients treated with new drugs. Moreover, decrease of FLCr seems to be an early indicator of resistant diseases needing dose increases or alternative therapy.

PO-1015

EVALUATION OF MM RESPONSE BY FREE LIGHT CHAIN ASSAY

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Background. The serum free light chain assay(FLC) provides a useful parameter for monitoring non-secretory MM, but the role of FLC in response criteria remains unclear. FLC criteria have been proposed by the IMWG, but questions of validity of FLC assays compared with electrophoretic(EP) and immunofixation(IF) have also been raised. We performed a retrospective analysis of patients with previously untreated MM who underwent induction therapy followed by intensive therapy with blood stem cell support (SCT). *Methods*. Data from 76 consecutive patients treated with induction therapy/SCT who had an abnormal pretreatment FLCratio(λ:<0.26, n=25; κ:>1.65, n=51) and available data for monoclonal serum and urine EP/IF was reviewed. EBMT criteria for response>PR were used for EP data and FLC response was analyzed by the following (IMWG criteria): abnormal FLCratio and either 1. 50% decrease in involved FLC and 50% decrease (or normalization) of involved/uninvolved FLC or 2. 50% decrease in involved - uninvolved FLC levels. Analysis of *paraprotein CR* was defined by serum and urine IF negativity for EBMT criteria or normalization of FLCratio. *Results*. 75/76 pts responded by EP and 70 of these responding patients had FLC response by criteria 1 (sensitivity 93%) and 74 by criteria 2 (sensitivity 99%). Conversely 70/71(99%) pts who had FLC response by criteria1 and 74/75(99%) by criteria 2 also responded by EP. Twenty-seven pts achieved IF negativity, and the FLCratio remained abnormal in only 15%(4pts), but among 59pts with normalization of FLCratio, 36pts(61%) still had positive EP/IF studies. 21/27(78%)pts with CR by EBMT paraprotein criteria and 43/59(73%)pts with FLCratio normalization had <5% marrow plasmacytosis(BMPC). Among 18pts with BJP only, 18(100%) achieved response by criteria 1, 2, and EP; 12(67%)pts had normalization of FLCratio, and only 4 of these 12(33%) had negative IF, conversely 7(38%)had negative IF and 4(57%)of these had normalization of FLCratio. *Conclusions*. EP/FLC criteria are useful to determine response; like Kumar *et al.* (ASH#3479,2005), this study suggests a trend towards criteria 2 being more sensitive. A combination of negative IF, FLCR normalization and <5%BMPC appears necessary for CR, and for pts with BJP alone, normalization of FLCR should be used as an adjunct, not replacement, for IF.

PO-1016

USE OF FREELITE ASSAY TO MONITOR MYELOMA WITH RENAL FAILURE

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Introduction. Renal failure is a common and serious complication of multiple myeloma. An important cause of renal impairment in these patients is the excessive secretion of light chains, which leads to tubular damage with cast nephropathy (myeloma kidney). Free light chains (FLC) are not only produced by Bence Jones myeloma cells but also those producing intact immunoglobulin. As these FLC may be nephrotoxic, it is essential to monitor the amount of FLC in all patients with myeloma, especially those with renal disease, in order to assess disease response to treatment. In patients with myeloma producing only light chains, there is difficulty in monitoring the response to treatment as urinary protein measurements become less reliable with decreasing urine output. The introduction of a sensitive assay for serum free light chains (Freelite™ The Binding Site) has meant that we now have a sensitive, rapid and accurate way of assessing responses to anti-myeloma treatment in patients with renal impairment. *Methods*. 7 patients, 3 with intact IgG immunoglobulin and 4 with light chain myeloma were monitored using this assay. *Results*. The excess light chain production was lambda in 4 and kappa in 3. The mean serum FLC was 6573 mg/L (range 355-15880 mg/L). 6 patients had renal impairment during the course of their disease and 4 required dialysis. In three of these there was a marked rise in FLC levels preceding the onset of renal failure. All 4 were responsive to treatment with a rapid reduction in FLC which was followed by dialysis independence. The three other patients were not dialysed, one had considerable renal impairment and showed no response to treatment with worsening renal impairment, another had sudden and progressive terminal relapse and the third did not have renal impairment despite very high levels of FLC. *Conclusions*. The value of using serum FLC assays to monitor these patients to predict need for and to monitor response to treatment, especially in the presence of renal impairment, is discussed.

PO-1017

IMPACT OF THE IMMUNOPHENOTYPICAL AND MOLECULAR RESPONSE EVALUATED BY FLOW CYTOMETRY AND FLUORESCENT PCR IN THE FOLLOW UP OF MULTIPLE MYELOMA PATIENTS ACHIEVING CONVENTIONAL COMPLETE RESPONSE AFTER AUTOLOGOUS STEM CELL TRANSPLANTATION

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Aim. To standardize an easy-to-perform PCR methodology with enough sensitivity to help in the response evaluation in most MM patients. Patients: 72 patients enrolled in GEMM2000 protocol designed by the Spanish GEM-PETHEMA group, were analyzed in this study. All cases were in Complete Remission (CR, with negative immunofixation, n=30), Electrophoretic CR (ECR, n=21) or VGPR (90% reduction of the M-component, n=20); 1 patients was non-secretor myeloma. MFC data were available in 71/72 patients, and PCR data in 51/72 patients. *Methods*. The molecular analysis of Ig rearrangements was carried out with Fluorescent PCR (F-PCR) according to the Biomed-2 protocols. The rearrangements amplified included: Ig heavy DH-JH, Ig Light (IgL) Kappa V-J, KDE and IgL Lambda V-J. Multiparametric Flow Cytometry (MFC) was employed as the reference technique. Survival curves were plotted according to the method of Kaplan & Meier, and compared using the Log-Rank test. At present, the median follow-up of the series was 55.3 months. *Results*. Applicability: The study at remission could be carried out by PCM in 90% of patients and by F-PCR in 88%. Molecular evaluation was performed in 51 cases. All 16 patients analyzed in VGPR yielded positive PCR results while 25 out of 35 (71%) at least in ECR had negative PCR results. Progression free survival (PFS) at 4 year was 80%

and 0% for negative and positive F-PCR patients, respectively (p=0,005). Within the 21 cases in CR, 16 were F-PCR negative and 5 were positive, which translated into a 4 year PFS of 83% vs. 0%, respectively (p=0.01). MFC evaluation was carried out in 71 patients. 20 of them were analyzed in VGPR and all were MCF positive. By contrast, 20/51 patients at least in ECR were MCF positive and 31/51 MCF negative, with a PFS of 83% and 36% at 4 years, respectively (p=0.0115). In addition, within the 30 patients who achieved CR, 20 (67%) were MCF- and 10 MFC$^+$; within these groups, PFS was 79% and 62% at 4 years, respectively (p>0.05). Multivariate analysis showed that a negative PCR result was the only variable associated with a superior PFS (p=0.04, OR=13.1). *Conclusions*. this study shows that the achievement of an MRD$^-$ result by F-PCR after APBSCT identifies a group of multiple myeloma patients with an excellent prognosis irrespective to the immunofixation result.

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PO-1018

SERUM FREE LIGHT CHAINS IN MM: COMPARISON OF 2 ASSAYS

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Introduction. Monoclonal immunoglobulin free light chains (FLC) are present in serum and/or urine of many patients with plasma cell proliferative disorders: lately over the years, serum tests for free kappa and free lambda have been developed opening the door to new applications.FLC usefulness relies on the evaluation of patients at presentation, in monitoring disease progression and response to treatment. Since each immunoglobulin light chain folding and aminoacid sequences are unique, accurate measurements are difficult to obtain and thus, comparison of different tests will be useful. This work aims to define the ability of different methods to assess serum FLC concentration and its correlation with clinical diagnosis. *Patient and Methods*. 32 serum samples were collected and tested. Of those 8 were of patients with undetermined renal failure with/without monoclonal component of uncertain significance on serum protein electrophoresis (SPE), 7 patients with diagnostic evidence of amyloidosis, 7 patients with lymphoproliferative disease (Multiple Myeloma, Micro molecular Myeloma, Solitary Plasmacytoma) and 2 patients with a double monoclonal component on SPE. Samples were processed for immunofixation, sFLC quantification and SPE. The sFLC were performed on Delta analyzer (RADIM), using Freelite™ (The Binding Site Ltd, Birmingham, U.K.) or on BNII nephelometer (Dade Behring) using New Scientific Company reagents (NSC). Our data shows that 9 patients out of 24 resulted in the normal range for the κ/λ ratio (reference range: 0.26 - 1.65) with both immunological tests. Of the 15 patients with abnormal ratio revealed with Freelite™, only 5 were assessed as pathologic with the NSC test. Interestingly all amyloidosis patients were positive with the Freelite™ while only 2 were positive with NSC. The selected patients group (renal failure but no haematological malignancy) were all negative to both tests except in 1 case (cryoglobulinemia). As per plasma cell disorders, all patients presented an altered κ/λ ratio with Freelite™ (100%) while only 50% were positive with NSC. In some cases we could compare data before and after treatment: no modifications were observed when samples were tested with NSC, indeed post-therapy remission was evident with Freelite™. *Conclusion*. our data suggest a good relationship between the Freelite™ values and disease diagnosis.

PO-1019

FREE LIGHT CHAIN ANALYSIS IN PATIENTS RECEIVING BORTEZOMIB

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Introduction. In multiple myeloma patients, measurement of serum free light chains (FLC) can provide an earlier indication of response to therapy than intact immunoglobulin (Ig). This is due to the shorter serum half-life of FLC (2-6 hours versus 20 days for IgG). This study assessed the utility of FLC monitoring during Bortezomib treatment. *Patients and Methods*. 17 patients with multiply refractive myeloma received 2-8 cycles of Bortezomib with doses of 1.3 mg/m^2 on days 1, 4, 8 and 11. Serial serum samples were analysed for FLC (Freelite, The Binding Site Ltd, Birmingham, UK) and intact Ig. 12/17 patients had intact Ig myeloma (IIM), 4/17 had nonsecretory myeloma (NSM), 1/17 had light chain myeloma (LCM). *Results*. One IIM patient had a tumour FLC concentration <100 mg/L (too low to assess response according to the international uniform response criteria), while for 5 patients (4 NSM, 1 LCM) FLC measurement was the only serologic marker available. 7/17 patients (all IIM) achieved a partial response (>50% fall in intact paraprotein). In 4/7 of these patients the FLC results indicated a partial response 1 cycle earlier than the intact Ig results. 3/17 patients (all NSM) showed progressive disease (>25% increase in FLC). 7/17 patients (5 IIM, 1 NSM, 1 LCM) had stable disease (defined as not meeting the criteria for response or progression). However, there was a conflicting assessment of response in 2/5 IIM patients. In these patients, intact Ig levels indicated stable disease, but their FLC levels increased before death. 3 patients showed a distinctive pattern of response with tumour FLC concentrations falling after each cycle of Bortezomib but recovering before the next cycle. This oscillation was seen in 2 patients (1 IIM, 1 LCM) with stable and 1 patient (NSM) with progressive disease. *Conclusions*. Serum FLC measurement can provide a valuable indication of response to treatment earlier than intact Ig. Oscillating FLC concentrations may indicate temporary inhibition of protein synthesis, rather than tumour kill, but this is not reflected in Ig levels because of their longer serum half-life. This oscillating response is potentially an early indication of tumour resistance.

PO-1020

QUALITY OF RESPONSE AND SURVIVAL IN MYELOMA PATIENTS UNDERGOING CONVENTIONAL CHEMOTHERAPY

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Background. The relationship between response and survival in multiple myeloma has been discussed in several studies with conflicting outcomes. In particular, it is unclear whether the magnitude of M-protein reduction apart from a complete remission is relevant for patients undergoing conventional chemotherapy. *Methods*. We retrospectively analyzed data from a study evaluating continuous versus intermittent prednisone and polychemotherapy with VMCP in 292 myeloma patients. Response was determined according to EBMT criteria and correlated with survival. In addition, we conducted a Medline literature review on studies analyzing the relationship of response and survival in myeloma patients treated with conventional chemotherapy. *Results*. In our own cohort, response was significantly associated with survival in 260 evaluable patients (p 8.91×10^{-8}). If patients with PD were excluded, only those reaching a CR did significantly better than all other patients (p 0.0425). In a multivariate analysis including response, stage at diagnosis, age, gender and M-protein type, only response, stage and age were prognostically relevant (p-values 6.3×10^{-6}, 0.014, and 0.0038, respectively). In the literature review, we identified 15 trials published since 1967, including 2252 patients which fit to the criteria described above. The trials showed significant heterogeneity regarding treatment setting, treatment regimens, and length of treatment. The magnitude of paraprotein reduction required for a response ranged from 25% to 75%. In all studies, patients with progressive disease were included in the non-responder category. Overall, 11 of the 17 analyses (2 studies included 2 analyses each) showed a significant relationship between response and survival, while in 5 of the 6 other analyses the association did not reach statistical significance. In one study, the survival of responders was 26.5 months versus 29 months in non-responders (n.s.). A comprehensive table including trial characteristics and results will be presented. *Conclusion*. When response defined by paraprotein reduction is compared to non-response including SD and PD, it is associated with prolonged survival in myeloma patients undergoing conventional chemotherapy. The magnitude of paraprotein reduction required to translate into longer survival remains undefined by the available data.

PO-1021

EVALUATION OF SERUM FREE LIGHT CHAINS AND OUTCOME IN MULTIPLE MYELOMA PATIENTS WITH AN INTACT MONOCLONAL IMMUNOGLOBULIN TREATED WITH AUTOLOGOUS STEM CELL TRANSPLANTATION

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Introduction. The serum free light chain (FLC) assay has been shown to be a useful tool in diagnosing and monitoring multiple myeloma

(MM) patients (pts) with non-secretory and light chain only disease. In addition, the detection of an abnormal serum FLC ratio is an adverse prognostic factor in pts with monoclonal gammopathy of undetermined significance. However, the relationship of the FLC assay to the outcome of patients with an intact monoclonal immunoglobulin following a single autologous stem cell transplantation (ASCT) has not been studied. Thus, the objective of this single centre, retrospective review study was to evaluate the usefulness of the FLC assay as a predictor for response rate and progression free survival (PFS) in this category of pts. *Materials and Methods.* We identified in our Princess Margaret Hospital MM database a total of 290 pts who underwent a single ASCT between September 2003 and June 2006. Of these, 59 had an intact monoclonal immunoglobulin (IgG in 37, IgA in 13 and IgD in 2) detected at diagnosis plus FLCs measured at referral. Based on calibration from our laboratory, the normal range for FLC measurements is as follows: kappa ≤ 13.1 mg/L, lambda ≤22.7 mg/L, and kappa/lambda ratio of 0.4-1.0. *Results*. The median time from diagnosis to ASCT was 8.7 months (range, 5.0-24.4), with a median follow-up time of 13.7 months (range, 1.1-55.9). Assessment of best response following ASCT revealed that 25 pts achieved CR/nCR, 14 VGPR, 16 PR, 2 MR and 2 stable disease. No prognostic factors for response were identified. To date, only 6 pts have died and the median overall survival is 54.8 months. The median PFS is 24.6 months, with 19 patients progressing after ASCT. An elevated kappa and lambda light chain was detected in 36 (61%) and 16 (27%) of the 59 pts, respectively. Additionally, 50 (85%) of the 59 pts were found to have an abnormal kappa/lambda ratio. The 1 and 2-year PFS probability for the normal versus abnormal ratio group is 100% vs. 78% and 100% vs. 48%, respectively (p=0.15). A decreased PFS was also associated with elevated levels of serum free lambda light chains (p=0.01), β-2m (p=0.006) and LDH (p=0.0004). *Conclusions*. 1) The majority of pts with an intact monoclonal immunoglobulin also have an abnormally high level of the corresponding serum FLC and an abnormal FLC ratio; 2) an elevated serum free lambda level as well as increased β-2m and LDH levels, as previously described, were identified as adverse prognostic factors for PFS in this population; 3) longer follow-up is needed to evaluate if the trend for a decreased PFS observed in pts with an abnormal baseline serum FLC ratio becomes significant.

PO-1022

THE ISS SHOWS HIGHER PREDICTIVE ACCURACY IN PATIENTS WITH MULTIPLE MYELOMA (MM) UNDERGOING ABSCT THAN THE DURIE AND SALMON SYSTEM

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Introduction. For the last 30 years the Durie and Salmon (DS) staging system was the commonly used staging system for patients with MM. In the 1980's Sß2M became an accepted prognostic factor. The combination of Sß2M and Albumin in the ISS allows a more precise prognosis in MM patients. Here we describe the higher predictive accuracy of the ISS compared with the DS system for patients undergoing high-dose chemotherapy and ABSCT. *Materials and Methods.* To evaluate if a statistically significant prognostic factor leads to improved predictability, we have to quantify its predictive accuracy. The Integrated Brier score (IBS) as a function of time was shown to be a valuable tool for assessing the predictive performance of prognostic classification schemes for survival data. We collected data of 620 patients with previously untreated MM from our center. All patients underwent at least one high dose chemotherapy and ABSCT. Data for event-free survival (EFS) and overall survival (OAS) were analysed by Cox proportional hazards regression comparing ISS and DS. Their predictive accuracy was evaluated by the IBS. *Results*. Of 620 pts. 71 were in DS I, 61 in DS II and 488 in Stage III. Concerning the distribution of the ISS 321 had stage I, 194 stage II and 105 stage III. The median follow-up was 56 months, the median EFS was 44.2 months and the median OAS was 94.3 months. Using the Cox proportional hazards model for the OAS only the ISS was statistically significant with p<0,001. Computing the IBS over a follow-up time of 9 years, ISS explained 7.0% whereas DS explained only 1.2% of the residual variation. In case of the EFS both, ISS (p<0,001) and DS (p<0,008), were statistically significant. Computing the IBS over the same follow-up time explained residual variation was 3.8% for the ISS and 1.8% for the DS system. *Discussion*. For patients with MM undergoing ABSCT the ISS showed a higher predictive accuracy for EFS and OAS than the DS system. We therefore recommend to apply the ISS in prospective trials evaluating transplantation for patients with newly diagnosed MM.

PO-1023

PROGNOSTIC IMPLICATION OF THE COMBINATION SFLCR + ISS IN MM

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Introduction. We have shown that serum free light chain ratio (sFLCR) provides independent prognostic information in patients with newly diagnosed MM (Kyrtsonis *et al.*, BJH, in press). *Aims.* To extend our previous observations in a multicenter setting and investigate the potential additive effect of sFLCR to ISS in MM patients' prognostication. *Patients and Methods*. We analyzed 127 MM patients (78 κ-, 49 λ-). sFLC were measured in sera drawn at diagnosis by immunoassay (The Binding Site, Birmingham, UK). sFLCR was calculated, accordingly as kappa/lambda or lambda/kappa, depending on the monoclonal light chain type of the patient. Based on our previous study *high* sFLCR was defined as ratios ≥3.57 and ≥45.09 for κ- and λ- MM respectively. *Results*. Patients' median age was 69y, 28%, 39%, and 33% had Durie-Salmon stages I, II, and III. ISS stage was 1, 2, or 3 in 35%, 27%, and 38% respectively. Median sFLCR was 5.04 in kappa-MM, and 58.23 in lambda-MM patients. With a median follow-up of 18 months (1-98), 53 patients with *low* sFLCR had a 3- and 5-year disease specific survival (DSS) of 94% and 82% vs 61% and 31% for 73 patients with *high* sFLCR (p=0.0001). The 3- and 5-year DSS rates were 97% and 90% vs 94% and 61% vs 46% and 29% for patients with ISS stage 1, 2 and 3 respectively (p<0.0001). In multivariate analysis, *high* sFLCR provided prognostic information independent of ISS. Based on that, we tested if the combination of ISS and sFLCR could increase the prognostication of ISS. Patients with *Low* sFLCR and ISS <3, had 3- and 5-year DSS rates of 100% and 93%, while for those with either *High* sFLCR or ISS=3, the 3- and 5-year DSS rates were of 86% and 53% and for patients with *High* sFLCR and ISS=3, the corresponding DSS rates were of 32% and 16% (p<0.0001). sFLCR was still significant if only patients requiring treatment at diagnosis were analyzed. *Conclusion*. Baseline sFLCR combined to ISS appears to be a powerful and promising novel prognostic model for survival in MM. Establishment of the optimal cutoff and prospective validation is needed.

PO-1024

CLINICAL TRIAL DESIGNS FOR MULTIPLE MYELOMA

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Introduction. The development of new anti-myeloma agents with different mechanisms of action from conventional chemotherapy has necessitated a new look at clinical trial designs. We discuss design issues for cytostatic agents for phase I, phase II and phase III clinical trials pertaining to myeloma. *Methods*. The success of phase I trials with cytotoxic agents is predicated on the dose toxicity curve being strictly monotone. With cytostatic agents, however, the shape of the dose-toxicity curve is not necessarily monotone, and traditional phase I trial designs may no longer be applicable. The distinction between cytotoxic and cytostatic agents also highlights the importance of thinking of endpoints other than tumor response (or shrinkage) with agents whose success depends more on keeping the tumor stable. The appropriateness of different endpoints in phase II myeloma trials is discussed. The goal of phase III clinical trials for cytostatic agents is typically twofold: to determine efficacy of the new agent for all patients and for the subset of patients with a certain biomarker. In this context it is important to distinguish prognostic and predictive markers. *Results and Conclusions*. We present different trial designs that can address both aims and discuss their advantages and disadvantages in different scenarios. In practice the underlying marker distribution and the response distribution as a function of the marker value are often continuous. In that case it is advantageous to take the actual marker distribution into account when designing the trial and we present possible methods to do that.

PO-1025

MYELOMA INTERNATIONAL STAGING SYSTEM RETAINS ITS PROGNOSTIC VALUE AT DISEASE RELAPSE

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Background. The international staging system is a simple, but powerful staging system that is based on two simple and easily available laboratory measurements. The system was developed and validated on a large group pf patients from across the world and has become the standard for patients with newly diagnosed myeloma. While it's prognostic value is clear in patients with newly diagnosed myeloma, its value at relapse has not been explored. *Methods*. We examined a uniform cohort of patients relapsing after an autologous stem cell transplant to assess the utility of ISS determined at the time of documented relapse. Relapse was defined using standard EBMTR response criteria. β-2m and albumin values from within 30 days of the date of relapse were extracted from the medical records. ISS was determined based on the published cut offs. *Results*. Of the 389 patients evaluated, 132 had values available for β-2m and 276 patients had the serum albumin available. Only 131 patients had both the data available and the ISS stage could be determined. Of these patients, 64 patients (49%) were ISS stage I, 50 (38%) were stage II and 17 (13%) were stage III. An albumin <3.5 mg/dL was prognostic for overall survival post relapse with a median survival of 10.5 months for those with albumin < 3.5 mg/dL compared to 26.3 months for the rest of the group (p<0.0001). Similarly the median OS from relapse was 15 months for those with β-2m > 3.5 mg/dL compared to 23.3 months for those with lower β-2m (p=0.014). The ISS stage predicted overall survival for this group of patients with the OS from relapse estimated at 27.3 mos, 17.8 mos and 12.3 months for ISS stage I, II and III respectively. *Conclusions*. In a uniform group of patients relapsing after autologous stem cell transplantation, the β-2m and albumin maintain their prognostic value and allows the use of the International Staging System for determining prognosis. Results should be validated in a larger dataset that will allow comparisons to other known prognostic factors.

PO-1026

ARE RANDOMIZED CONTROLLED TRIALS (RCT) IN MULTIPLE MYELOMA ADEQUATELY POWERED

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Introduction. Sample size is one of the critical components in the design of a RCTs. The findings generated in the underpowered RCTs is most often inconclusive, typically false-negative. In our analysis of myeloma RCTs published between1966-1998, we found that majority of these trials were underpowered [Ann Oncol 2001;12:1611-17]. The last decade has witnessed significant advances in treatment of myeloma. We hypothesized that improvement in myeloma treatment has been associated with increase in sample size in myeloma RCTs. Accordingly, we compared the sample size in trials published from 1966-1998 with those from 1999-2006.*Methods*. Our group maintains a database of all phase III myeloma RCTs published from 1966 to date (Lancet Oncology 2003;4:293-304). Data were extracted on sample size, and the source of funding from all RCTs. Sample size of the trials published from 1999-2006 was compared with those published from 1966-1998. We also assessed if the source of funding (industry vs public) had any association with the sample size of RCTs.*Results*.Our database consists of 195 RCTs, out of which 147 were published from 1966-1998 and 48 from 1999-2006. Overall, the median sample size in trials published after 1998 increased to 168 compared to 116 in RCTs published before 1999(p=0.003). Three RCTs (6%) published after 1998 had adequate sample size to detect a 10% survival difference at 5 years compared to one (1%) published before 1999 (p=0.625). Twenty three (48%) RCTs published after 1998 had adequate power to detect a 20% survival difference at 5 yrs compared to 47 (31%) before 1999 (p=0.006). The increase in sample size was significant across all RCTs regardless of the source of funding (p=0.001). An average sample size of RCTs published after 1998 was 228 which is adequate to detect 20% of survival advantage at 5 years. *Conclusion*.Overall the sample size in myeloma RCTs published after 1998 have increased. Given that advances in therapeutics against cancer are typically incremental, this may be one of the reasons why scientific community succeeded in discovering new important treatments in myeloma during the last decade. Currently, myeloma trials are, on average, powered to detect improvement in survival ≥20% (at 5 yrs).

PO-1027

LONGITUDINAL ASSESSMENT OF CLINICAL CHANGES IN TREATMENT AND OUTCOME OF MULTIPLE MYELOMA IN AUSTRIA; THE LACCITO SURVEY 2006/07

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Introduction. Practice does not always adhere to approved indications or published clinical guidelines. Neither do treatment schedules prevail. Routine effectiveness of medical treatments in the *real life* often differs from the efficacy based on best evidence derived from randomized clinical trials. Concerning drug safety, approval studies are notoriously underreporting rare toxicities and long-term sequelae. This can be addressed by representative population-based pharmacoepidemiologic surveys. Providing a comprehensive view on treatments, they could serve as an instrument for quality assurance and generate benchmark values. As of today there is an urgent need for such analysis in the rapidly changing world of MM treatment options. *Methods*. All Austrian care givers are invited (under the auspices of the Austrian haematological society OEGHO) to take part in LACCITO. Documentation Data will be collected retrospectively for all myeloma pts. receiving any treatment in the 4th quarter of 2006 (T 0) and the 1st quarter of 2007 (T 1). Six months onward (T 2) from T 1, an inquiry on all pts. identified at T0 and T1 will be invited, as well documentation of additional pts. At 12 months (T 3), a reappraisal on the cohort will be performed, using standard descriptive statistics and survival analysis using the Kaplan–Maier method. The basic data set optimized on multiple myeloma (MM) treatments, risk factors, adverse events, and health-economic variables will be presented. Forms are offered as paper questionnaire or internet-based. *Conclusions*. LACCITO is designed to generate a *real time* picture of MM treatment in Austria and could be developed into a model system for highlighting rapidly changing oncological strategies in times of targeted therapies. Data will be further exploited regarding their long-term clinical and health-economic consequences using the approach of systematic health technology assessment (HTA).

PO-1028

ANALYSIS OF SURVIVAL DIFFERENCES OVER TIME AND BETWEEN COUNTRIES IN THREE TRIALS WITHIN THE NORDIC MYELOMA STUDY GROUP. IMPACT OF INTERNATIONAL CLINICAL COLLABORATION ON TREATMENT AND OUTCOME

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Background. The development and widespread interest in new treatment strategies has led to the evolution of a number of small but excellent research groups throughout Europe with little interaction between them. Individually each of these groups is producing good quality basic and clinical research but each lacks a critical mass. It is evident, that in the field of MM, coordinated activities are needed to improve the clinical research design and protocols as well as organisation and patient resources. For a proper understanding of the impact that an international joint clinical program would have, we here present a retrospective analysis of data generated from 3 consecutive clinical trials within the Nordic Myeloma Study Group (NMSG) documenting a major national benefit from coordinating and integrating a common new clinical activity in Denmark, Norway and Sweden. *Aims*. The present report focuses on the national survival differences observed in NMSG high dose therapy trial #5/94, which was eliminated in the subsequent NMSG trial #7/98. It was hypothesized that a detailed analysis of 1) patient accrual, characteristics and prognostic factors 2) risk for selection bias and 3) differences in clinical practice outside the protocol should reveal important information and explains the differences observed between Denmark, Norway and Sweden. *Results*. The analysis revealed no detectable differences in disease stages, prognostic variables or patient selection.

However, clinical practice outside the protocol including treatment intensity in refractory or relapsed patients documented a difference as the number of initial treatment failure were reduced and post relapse survival superior in Swedish compared to Danish patients. *Conclusions*. The broad range of analysis described in this report illustrates the impact of non-protocol practice on outcome, which makes the use of overall survival a difficult end point to be used in evidence based modern medicine. Most likely the improvement of non-protocol practice of relapse therapy is a consequence of clinical guidelines prepared by the Nordic Myeloma Study Group in the mid nineteenth recently updated in collaboration with UK Myeloma Forum. This should be considered a first step to European guidelines with focus on the common use of novel therapeutic approaches to improve patient survival.

PO-1029

RELATIONSHIP BETWEEN URBANIZATION AND DEVELOPMENT OF MM

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Introduction. Until today, the etiology of multiple myeloma (MM) remains a mystery. One of the suspected causes of MM is chemical exposure. We therefore undertook a study of MM patients in Singapore to determine whether patient's homes and workplaces were clustered in particular areas in Singapore, and whether chemicals could potentially be causal factors. *Materials and Methods*. Newly-diagnosed MM patients (n=10) were interviewed either in person or via telephone. Their occupations, as well as locations of their homes and workplaces were recorded. Particular attention was paid to the period that was spent in a certain job, as well as the period spent at workplace or at home. Moreover, patients were asked specifically about chemical exposure, both at work as well as in and around their homes. A best-fit hypothesis was used to select the work and home locations with the greatest likelihood of exposure to carcinogens. These locations were then plotted on a map of Singapore to determine if there was clustering. *Results*. Ten patients have so far been interviewed, and 6 patients had admitted to being exposure to chemicals (smoke and/or fumes) at their workplace and/or homes. Specifically, these patients had been exposed for years to various types of pesticides (including mosquito coils), petroleum and diesel fuels, lubricants, paints, furniture varnishes, leather treatment chemicals, joss sticks and incense, and passive cigarette smoke. In fact, very prolonged (up to 10 years), as well as day and night exposure to chemicals was reported by 3 patients; and 1 patient even reported a persistent smell of chemicals in his breath after returning from work. Workplaces appeared to cluster around the Kallang Basin area of Singapore, where the largest gasworks (Kallang Gasworks) is located. Moreover, patient's homes appeared to cluster around the Queenstown area of Singapore, which is the oldest public high-rise complex in Singapore. *Conclusions*. Our preliminary data suggests a strong relationship of chemicals to the development of MM. Since many patients had been living and working for many years in and around gas and chemical industries during Singapore's period of rapid industrialization in the 1960's and 1970's; these data suggest that MM could be a disease related to urbanization.

PO-1030

IMPACT OF NOVEL THERAPIES ON PATIENT OUTCOME IN MULTIPLE MYELOMA

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Background. The median survival from diagnosis for patients with myeloma has usually been estimated at 4-5 years based on data from clinical trials of alkylating agents and autologous stem cell transplants. The past 6 years have seen a dramatic change in the available treatment options for patients with myeloma. Previously meta-analysis of multiple trials had demonstrated that combination chemotherapy holds no survival advantage over MP. It is not clear how the introduction of the novel therapies may have altered the survival for patients with myeloma. *Methods*. We examined the outcome of patients with multiple myeloma relapsing after autologous stem cell transplantation. The patients were divided into two groups based on their date of relapse; one group who relapsed after Dec 31, 2000 and the other who relapsed before this date. The overall survival from relapse was compared between the groups. This cutoff date was used based on wider availability of thalidomide and bortezomib as well as timing of initiation of early lenalidomide trials. *Results*. A total of 387 patients relapsing after autologous stem cell transplantation were included in the study. Among these 97 patients (25%) had relapsed prior to Dec 31, 2000 and the rest had relapsed after this date. The median overall survival from relapse was 11.8 mos for those relapsing in the early time period compared to 23.9 months for those relapsing in the later time period. The 2 year estimated survivals were 24% and 49% in the two groups respectively. In a multivariate analysis it was independent of the time from diagnosis to transplant. *Conclusions*. This time based analysis of patient outcomes following relapse from transplant, from a single academic institution where access to novel therapies are likely to be higher than in the community, a clear improvement in the overall survival of patients is demonstrated with time. The results suggest that the novel therapies that have become available in the recent years have indeed had a favorable impact on the outcome of patients with relapsed myeloma.

PO-1031

SURVIVAL IN MYELOMA-POSSIBLE IMPACT OF THALIDOMIDE

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Introduction. the median survival in myeloma is quoted in various trials as approximately 3 years for patients treated with conventional therapy and 5 years in patients treated with high dose therapy. Thalidomide was introduced in the treatment of myeloma in the late 1990s and it is still not known as to what impact the use of this and other newer agents will have on patient survival. *Methods*. A retrospective analysis of survival of 103 patients diagnosed with myeloma from 1997 to 2006. Data was obtained from computer records. 40 patients were diagnosed in the first five years and 63 in the subsequent 5 years. *Results*. Of the 103 patients with myeloma, 80 were actively treated (median age 66 yers range 35-91 years), 8 died (7 prior to 1998) within two months of diagnosis without treatment of myeloma, 13 patients chose to be treated elsewhere and two refused treatment. 13 patients had high dose therapy with stem cell rescue and 59 were treated with thalidomide containing regimens (TCR) at some stage of their illness. 45 are alive, 24 have died and 9 have been lost to follow up and two newly diagnosed patients have not started treatment. Of the treated patients, 22 (27.5%) have survived more than 36 months, 11 (13.8%) more than 60 months, 5 (6.25%) more than 92 months. Of those who survived more than 60 months, all had treatment with TCR, 4 had previous autografts, and two had TCR as salvage after failure of response to VAD and one after MP. *Conclusions*. This study of unselected patients with myeloma presents more truly the natural history of multiple myeloma in a mainly elderly population. There is a high rate of initial mortality prior to 1998 which improved due to more vigorous initial support treatment of patients. This study highly suggests that at least in some patients, thalidomide has increased the chances of survival.

PO-1032

A SINGLE-CENTRE EXPERIENCE OF 20 YEARS OF MULTIPLE MYELOMA

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Introduction. Evidence from clinical trials is difficult to apply to general populations due to bias introduced by selection criteria. Investigation of wider populations of Multiple myeloma (MM) patients has been limited. This project characterised the demographic features and survival of an unselected population in a single centre over a period in which new therapies were introduced, autologous stem cell transplants (ASCTs) in 1992 and thalidomide in 2000. *Materials and Methods.* A retrospective analysis of all patients diagnosed with MM at Wellington Hospital between 1986 and 2006 was conducted. Patients were identified from a register of bone marrow aspiration procedures and verified using hospital clinical records. Death data were obtained from the New Zealand Health Information Service. *Results*. 286 patients were identified. Males comprised 56.7% of patients, and median age at diagnosis was 65.7 years. The mean number of cases of MM increased by 0.7 per year (95% confidence interval (CI) 0.31-1.09). Median overall survival was 31 months (95% CI 24-40 months). Median survival of patients diagnosed before 2000 was 25 months, compared to 43 months for those diagnosed after 2000 (p=0.045). 64 patients received thalidomide, 41 received at least one ASCT, 5 underwent allogeneic stem cell transplantation. 5 received bortezemib. No patient received lenalidomide. Median survival of patients undergoing ASCT was 113 months from diagnosis compared to 29 months in historical controls aged <65years (p<0.0001). Median survival of patients who received thalidomide was 66 months from diag-

nosis compared to 22 months in those who did not receive this agent (p<0.0001). *Conclusions*. This study represents a complete record of a single-centre experience of treating patients with MM over 20 years. Median survival has improved since 2000 in this population. The observed increase in rate of MM diagnosis together with the increased survival of our patients has significant implications for future service planning. A possible explanation for improved survival includes the introduction of two new therapies, thalidomide and ASCT.

PO-1033

MULTIPLE MYELOMA IN THAILAND: COMPARISONS OVER TWO EXTENDED PERIODS

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There is currently no published data on the management of multiple myeloma in Thailand. We therefore examined the records of patients treated for multiple meyloma at the Ramthiobodi hospital in Thailand during the period 1998-2004 (n=44) and compared them with patients treated during 1970-1985 (n=62). Records were compared with respect to the symptoms and initial findings, medication used, and outcomes. The number of patients presenting for treatment has increased from an average of 4.1 per year during 1970-1984 to 7.3 per year during 1998-2004. The patients in the latter series were slightly younger (93% over 40 years of age compared with 96.5% in the earlier series) and contained a greater proportion of females (50% male compared with 68% in the earlier series). Anemia and bone pain remain the most common presenting features, but there has been a substantial fall in the number of patients with renal dysfunction at the time of presentation (15.9% vs 73%, see Table). During the period of the study, first-line treatment continued to be based on melphalan and prednisolone, as in the earlier series. However, there have been marked changes in second- and third-line treatment with greater reliance on combination chemotherapy as opposed to monotherapy (see Table). Infection remains the principal cause of death in multiple myeloma patients. Dates 1998-2004 1970-1985 Number of patients 44 62 Cases per year 7.3 4.1 Age > 40 years (%) 93 96.5% male 50 68 Findings at presentation (%): Anemia (Hb<10g/dL) Bone pain Fatigue Renal failure/insufficiency Hypercalcemia Bence-Jones proteinuria Abnormalities on skeletal X-ray 84.1 54.5 20.5 15.9 41.9 20 81.8 95 63 - 73 63 38 96 Findings on electrophoresis (%) Monocloncal heavy chain IgG IgA Monoclonal light chain 92.3 64.1 28.2 7.7 77.4 67.9 22.6 9.4 Deaths reported 7 14 Medication regimes First line Second line Melphalan / prednisolone Vincristine / adriamycin / dexamethasone Thalidomide Melphalan / prednisolone Cyclophophamide Melphalan Vincristine Prednisolone In summary, in line with findings from other countries, presentations of multiple myeloma are increasing in Thailand and there have been some changes in the clinical picture. We will continue to collect similar data in order to assess whether these trends continue.

PO-1034

PROSPECTIVE COMPARISON OF RESPONSE RATE AND LONG-TERM RESULTS OF VELCADE AND THALIDOMID BASED REGIMENS

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Introduction. The aim of this study was to compare prospectively groups of patients treated with Velcade(V) and Thalidomide (T) based regimens an impact of negative cytogenetic prognostic markers: deletion of 13q14, deletion of 17p13 (p53), amplification of CKS1B gene (1q21) and translocation t(4;14). ii) *Methods*. Two groups of multiple myeloma (MM) with comparable pre-treatment features were treated by T - based regimens (T group; No. of pts.24) and by V - based regimens (V group; No. of pts. 18). The T/V groups had following characteristics: median of follow-up 8.6/14.7 months; median age 65.0/62.5 years; stage IIIA 67%/50%; treatment lines already received for T group (1 line-75%; 2 -25%) and V group (1 line-61%, 2-22%, 3-17%). 94% in V group and 79% in T group of pts. received more than 4 cycles of V or T based regimens. Cytogenetic findings for T/V groups were as follows: deletion of 13q14 - 50%/62%; t (4; 14) - 53%/66%; deletion of 17p13 - 47%/41% and amplification of CKS1B gene was detected in 66%/63% pts. The response and other parameters such as time to progression (TTP); progression free survival (PFS) and duration of response (DOR) were assigned by IMWG criteria. *Results*. Overall response (OR) rate for T vs. V group was 83% vs. 39% including 0% vs. 0% CR, 29% vs. 11% VGPR and 54% vs. 28% PR. Median of TTP (the same for PFS) for T vs. V group was 6.9 (3.0-17.0) vs.11.0 (2.9-16.5) months. Median of DOR for T vs. V group was 5.0 (1.4-10.1) vs. 12.0 (6.3-14.9) months. It is too early evaluate OS. We have found no significant difference when compared incidence of each chromosomal aberration for TTP, DOR, PFS, OS and response rate except trend for shorter TTP for pts. Who had t (4; 14) in Thalidomide group (p=0,213) than in Velcade group (p=0,761). Conlusion: The better OR rate (83% vs. 39%) and VGPR rate after treatment in T group were not translated to better long-term results. Moreover at least trend to longer TTP (11.0 vs. 6.9 months) was seen in V group despite limited response rate in this cohort of pts. The results were not significantly influenced by cytogenetic findings. We are currently investigating whether or not this finding will be confirmed on large cohorts of patients.

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GROUP 11: Quality of lify, disease complications and treatmente toxicities

PO-1101

LIVING WITH MYELOMA: AN INTERPRETIVE PHENOMENOLOGICAL STUDY

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Introduction. Myeloma is a complex, debilitating cancer with no known cause. Despite recent advances in treatment, myeloma remains incurable. This interpretive phenomenological study was designed to explore the experience of living with myeloma, from the patient's perspective. The aim of this study was to elicit and learn from, the personal experiences of people diagnosed and treated for myeloma, to primarily inform nursing practice, and provide myeloma patients with the appropriate care and support. Interest in gaining insight into the issues that effect how patients live and cope with myeloma, arose from the first researcher's extensive experience caring for this patient group. *Methods*. A qualitative approach guided by the philosophy of Heidegger, was adopted for this study. Issues that affect patients (n=11), living with myeloma were explored through semi-structured interviews. Using van Manen's process of data analysis, hermeneutical engagement in the text was performed, and interpretation of commonalties as well as differences were articulated through themes. *Results*. Ten themes emerged, revealing the physical, psychological, and social impact of myeloma on an individual's well-being, living with an *unknown cancer*, altered body image, fatigue, and limited time with health professionals, stigma of cancer, fear of recurrence, physical space, loss, protecting others and luck/optimistic talk. The findings are presented in the four existentials of the lived world as proposed by van Manen these being, lived body, lived space, lived human relations and lived time. *Conclusions*. This is the first known qualitative study that explicates the unique descriptions of living with a diagnosis of myeloma. Although many of the findings are reflected in previous studies exploring the lived experience of different malignancies, the overarching theme of *living with an unknown cancer* is unique to this study. In pursuit of improved practice, it is imperative that nurses and other health care professionals understand the lived experience of their myeloma patients and the changing nature of their needs. Providing time for myeloma patients, in conjunction with sensitive listening skills, is a fundamental component required in determining individual concerns and promoting support tailored to patient need.

PO-1102

NURSING GUIDELINES FOR ENHANCED PATIENT CARE

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Introduction. Patients with multiple myeloma require education and support to receive and adhere to optimal therapy. The International Myeloma Foundation (IMF) recognizes that nurses play an essential role in managing patient care, and that there is a need for specific nursing guidelines, particularly for the use of novel anti-myeloma agents, such as proteasome inhibitors and immunomodulatory drugs. *Methods*. A permanent Nursing Leadership Board (NLB) was created in partnership between the IMF and 20 U.S. oncology nurses from leading cancer centers caring for patients with multiple myeloma. A pre-meeting needs assessment survey identified key NLB needs. A two day, on-site brainstorming meeting followed that included presentations, group discussions, feedback surveys, break-out groups, and consensus-building exercises. Post-meeting exchanges of information were conducted to create issue and position statements and establish consensus guidelines and recommendations that could be used by nurses in any type of medical facility. *Results*. The survey and meeting highlighted the need for specific nursing guidelines, strategies, and programs to educate and assist patients in managing key side effects of novel agents used to treat myeloma. Steroid toxicities, peripheral neuropathy, gastrointestinal side effects (nausea, vomiting, diarrhea, and constipation), thromboembolic events (deep vein thrombosis and pulmonary embolism), and myelosuppression (including thrombocytopenia, neutropenia, anemia, infection, and fever) were selected for the first consensus issue statements and recommendations. Guidelines were developed within breakout groups, followed by review by the whole NLB with IMF input. Guidelines include identification of toxicity by grade, patient education opportunities and information, and both pharmacologic and non-pharmacologic management strategies. Strategies include recommendations for managing the side effects in general, along with specific recommendations pertaining to the novel agents.

PO-1103

QUALITY OF LIFE IN MYELOMA PATIENTS UNDERGOING CONVENTIONAL INDUCTION THERAPY IS DEPENDENT ON QUALITY OF RESPONSE TO TREATMENT

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Background. Up to now, few data have been collected on the quality of life (QOL) of myeloma patients. Results of a recent study suggest that response to treatment is an important factor in this regard. Our aim was to further investigate the impact of response to treatment on the QOL of myeloma patients undergoing conventional chemotherapy. *Methods*. We prospectively collected QOL data using the EORTC QLQ-C30 in a study comparing continuous versus intermittent prednisone plus VMCP in 292 newly diagnosed myeloma patients. A QOL-questionnaire was distributed to each patient at each visit. Using the official EORTC scoring manual, we analyzed the relationship of response to global QOL, all functional scales and the symptom scale fatigue. *Results*. QOL data are available in 186 of 260 evaluable patients. Mean QOL scores during induction therapy were significantly associated with the quality of response achieved. This was still true for the following scales, if patients with progressive disease were excluded from the analysis: global QOL (p 0.02747), fatigue (p 0.00017), role functioning (p 0.04032, Figure), cognitive functioning (p 0.0224), and emotional functioning (p 0.01274). When QOL at the last visit of induction treatment was analyzed, global QOL (p 0.02273), physical functioning (p 0.01541) and fatigue (P 0.004616) were significantly associated with quality of response. In the case of global QOL and fatigue this was still true when patients with PD were excluded (p 0.04991 and p 0.009635, respectively). Mean physical functioning and cognitive functioning during induction were also inversely correlated with time to first response (p 0.04025 and p 0.04674, respectively). *Conclusion*. Quality of response during induction treatment significantly influences central parameters of QOL including cognitive function in myeloma patients undergoing conventional therapy. If patients with progressive disease are excluded, the magnitude of response is still associated with QOL. Even if survival cannot be prolonged by achieving a better response, myeloma patients might benefit from a better response by having a better quality of life.

PO-1104

PLASMA EXCHANGE IS AN IMPORTANT AND USEFUL ADJUVANT THERAPY IN CAST NEPHROPATHY

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Introduction. The role of plasma exchange (PLEX) in the treatment of cast nephropathy remains controversial. Three randomized clinical trials resulted in 3 different *Conclusions*. Serum free light chain (FLC) which is used as a marker of disease was not measured in any of the three trials. This study was conducted to determine if serum FLC can be used to guide PLEX in the treatment of cast nephropathy. *Methods*. Patients who underwent PLEX with a diagnosis of multiple myeloma from January 2003 to July of 2006 were recruited for the study. Laboratory data and renal biopsy were extracted from the electronic medical record. Renal response was defined as a 50% decrease in the serum creatinine from the peak level. *Results*. PLEX was performed on 40 patients during the study period. PLEX was performed either daily or every other day. Median number of PLEX performed was 6 (range 1-19). Cast nephropathy was identified in 18 of 28 renal biopsies. Other diagnoses included: amyloi-

dosis (1), light chain deposition disease (LCDD-3), LCDD with cast nephropathy (2), acute tubular necrosis (ATN-3) and diabetes nephropathy with ATN (1). Serum FLC was measured in 28 patients at baseline and after PLEX. No patient with biopsy proven cast nephropathy had a serum FLC less than 157 mg/dL. Fourteen patients with cast nephropathy also had serum FLC measured at baseline and after PLEX. In 9 patients, PLEX and chemotherapy resulted in > 50% decrease in FLC levels. In this group, 78% achieved renal response. In the 5 patients who had less than a 50% decline in FLC, none achieved a renal response. Renal response did not occurred in 6 patients despite > 50% reduction in serum FLC. These include 2 with LCDD, 1 with LCDD and ATN, 1 with diabetic nephropathy and ATN, 1 with cast nephropathy with an atypical interstitial nephritis and 1 cast nephropathy which was precipitated by intravenous contrast 1 month prior. *Discussion*. This small study revealed that serum FLC is a useful marker for screening and treatment of cast nephropathy. No patient with cast nephropathy had a serum FLC < 157 mg/dL. Thus the diagnosis of cast nephropathy should be questioned in patients who present with renal failure and serum FLC below this level. In those who have biopsy proven cast nephropathy, treatment with PLEX should be continued until at least a 50% reduction in serum FLC has been achieved. Ideally, a target of 150 mg/dL should be attempted since cast nephropathy is uncommon below this level. Renal biopsy was essential as reduction of serum FLC did not reverse renal failure when it was the result of ATN, LCDD and AL. Our findings showed that PLEX was an effective therapy when used in patient with biopsy proven cast nephropathy and when it was directed by serum FLC levels.

PO-1105

IMPACT OF R-HUEPO ADMINISTRATION ON IRON STATUS IN ANEMIC PATIENTS WITH MULTIPLE MYELOMA

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Introduction. Normal iron supply is important for erythropoiesis. Recombinant human erythropoietin (r-huEPO) stimulates erythropoiesis and increases iron demands of the erythroid marrow. Impaired iron supply may compromise response to r-huEPO therapy even in conditions with adequate iron stores, as it is the case in anemia of multiple myeloma (MM). It is therefore important to know the impact of r-huEPO administration on iron status, in order to explore the causes of r-huEPO unresponsiveness and optimise r-huEPO therapy. *Patients and methods.* Twenty-eight patients with a median age of 73.5 years (range 29-84) with symptomatic MM and disease related anemia were enrolled. All patients received r-huEPO at a dose of 30.000IU/wk for 6 consecutive weeks. The evaluated parameters of iron status were: serum ferritin, transferrin saturation (TSAT%), soluble transferrin receptor (sTfR), the ratio of sTfR to the logarithm of ferritin (sTfR-F index), percentage of hypochromic erythrocytes (HYPO%) and reticulocyte haemoglobin content (CHr). These parameters were evaluated in serial measurements at baseline and on weeks 1, 2 and 6. Response to r-huEPO (hemoglobin increase ≥2 g/dL) was evaluated after 6 weeks of therapy. *Results*. HYPO% and sTfR-F index significantly increased during all sequential measurements whereas TSAT% was significantly decreased from baseline to week 6 and from week 2 to week 6 (paired samples Wilcoxon test, $p<0.05$). HYPO% and sTfR-F index sequential changes between baseline and week 6 were the only positively correlated parameters (Spearman's test, $p<0.05$). Concerning response to r-huEPO eighteen patients responded (64%). With a median follow-up of 29 months (range 2-60), median overall survival was 36 months and did not differ statistically between responders and non-responders. Iron status markers either at baseline or during r-huEPO treatment did not influence overall survival. *Conclusions*. HYPO% and sTfR-F index significantly increased during all sequential measurements, from baseline to week 6, whereas TSAT% significantly decreased from baseline to week 6 and from week 2 to week 6. These results suggest that during r-huEPO treatment functional iron deficiency, which is a cause for r-huEPO unresponsiveness, is developed. In this case IV iron co-administration could optimise r-huEPO therapy.

PO-1106

RENAL RECOVERY IN 75% OF MYELOMA PATIENTS WITH CAST NEPHROPATHY FOLLOWING FLC REMOVAL BY HEMODIALYSIS

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Introduction. Irreversible acute renal failure (ARF) is associated with significantly reduced life expectancy in multiple myeloma patients (18 months versus 4 years). Cast nephropathy from excess serum free light chains (sFLCs) is the predominant cause of this dialysis-dependent ARF. Only 12-20% of these patients recover renal function. *Methods*. Extended hemodialysis using a high cut-off protein-permeable dialyser (Gambro HCO 1100) for rapidly lowering sFLCs was assessed for safety, efficacy and clinical outcomes in patients with multiple myeloma and dialysis-dependent ARF secondary to biopsy proven cast nephropathy. *Results*. Nine patients were studied: 7 with new onsets and 2 with refractory/relapsing disease (described in detail in a separate abstract). The chemotherapy employed was dexamethasone and thalidomide for new disease; Velcade, doxorubicin and dexamethasone for refractory/relapsing disease. Extended dialysis (up to 12 hrs/day) was very well tolerated. Consistent reductions in sFLCs concentrations were achieved during each dialysis session (45-81%). Seven patients, including the refractory/relapsing patients, achieved a sustained reduction in sFLCs of greater than 65% (range 65 -95%). These patients subsequently became dialysis-independent, following a mean treatment period of 21 days. The two patients had less sustained reductions in sFLCs concentrations and remained on dialysis (one did not respond to induction chemotherapy, the other had recurrent infections resulting in chemotherapy being stopped). *Conclusion*. extended daily hemodialysis with a light chain permeable dialyser rapidly reduced concentrations of sFLCs in patients who were responsive to chemotherapy. Dialysis independence occurred in patients who achieved a >60% sustained reduction in sFLCs (compared with approximately 30% achieved using plasma exchange). Renal recovery occurred in 78% of patients compared with 10-20% in published comparative series of patients treated conventionally. This could have a huge impact in the management and outcomes of patients with renal failure and multiple myeloma, if these results are replicated in more patients.

PO-1107

COMPARATIVE ANALYSIS OF THE EFFECT OF ADDITIONAL THERAPEUTIC MEASURES ON RENAL RECOVERY IN PATIENTS OF MULTIPLE MYELOMA ASSOCIATED CAST NEPHROPATHY PRESENTING WITH DIALYSIS REQUIRING ACUTE RENAL FAILURE

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Aim.To evaluate the effect of conventional haemodialysis using low-flux polysulfone dialyser with dialysis using high-flux polysulfone dialyser and conventional dialysis combined with plasma exchange,on renal recovery of myeloma cast nephropathy with acute renal failure. *Materials and Methods.* 12 newly diagnosed light-chain multiple myeloma patients with biopsy proven cast nephropathy who required dialysis for acute renal failure were randomly assigned to three groups each consisting of 4 patients.Gr.1 patients were put on alternate day haemodialysis with low-flux F6 dialyser.Gr.2 patients were put on alternate day haemodialysis with high-flux F80 dialyser.Gr.3 patients were put on alternate day plasma exchange combined with haemodialysis using low-flux F6 dialyser.All 3 groups had comparative demography and tumor burden. Patients were evaluated at 4[th],8[th] and 12[th] weeks.All of them received similar chemotherapy based on VAD regime. *Results*.Gr.2 patients showed 100%(4/4)renal recovery at 8th week.Gr.3 patients showed renal recovery 75%(3/4) at 8th week and 100%(4/4) at 12th week.Gr.1 patients had worst outcome with 25%(1/4) renal recovery at 12th week.Average dialysis requirement in Gr.1 patients was 33.75 compared to 13.5 in Gr.2 and 16.75 in Gr.3 patients.The cost for renal replacement therapy in Gr.2 patients was significantly lower compared to Gr.1 and Gr.3 patients. *Conclusions*.Dialysis using high-flux dialyser was most effective for renal recovery in myeloma cast nephropathy compared to conventional haemodialysis with low-flux dialyser alone or in combination with plasma exchange.The cost of therapy was also significantly lower.

PO-1108

INFECTION IN MULTIPLE MYELOMA: PRELIMINARY RESULTS OF A MULTICENTER PROSPECTIVE STUDY

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Introduction. Infection is a frequent complication in patients with multiple myeloma (MM). The current treatment of MM involves different phases, including induction, autologous stem cell transplant (ASCT) and maintenance. These phases of treatment, added to the initial immunodeficiency, result in a cumulative immunosuppression that may lead to infections with different etiologies and risk factors in each treatment phase. The objective of this study was to describe the etiology and risks factors for infection, taking into account the phase of the treatment of MM. *Material and Methods*. A prospective multicenter study has been conducted in 6 South America hospitals since 2005. We have followed every episode of infection in MM patients, and collected data regarding etiology, treatment and outcome. These episodes were classified in tree groups: infection before ASCT (Group 1), infection during ASCT (Group 2), and infection during the maintenance treatment (Group 3). *Results*. Among 94 episodes of infection, 40 (43%) were in Group 1, 36 (38%) in Group 2, and 18 (19%) in Group 3. Most patients were in advanced stages of MM (67% in Durie Salmon III). Renal failure was more frequent among patients in Group 1 (35% vs. 8% in group 2 and 17% in group 3, p=0.01), whereas neutropenia was more frequent in Group 2 (58% vs. 10% group 1 and 22% group 3, p<0.001), and receipt of corticosteroids was more frequent in group 3 (61% vs. 32% in group 1 and 22% in group 2, p=0.02). The respiratory tract was the most frequent site of infection (36%), but bacteremia was more frequent during ASCT (47%). In 46% of episodes a microbiologic documentation was obtained. The mortality related to infection was 7% in Group 1, 6% in Group 2 and 11% in Group 3 (p=0.76) *Conclusion*. Risk factors and sites of infection differed according to the phase of treatment of MM. Infectious-related mortality was 7%. The recognition of different risk factors and etiologies in different phases of treatment may help to guide appropriate preventive and therapeutic measures.

PO-1109

HIGH RATE OF RENAL RECOVERY IN PATIENTS WITH CAST NEPHROPATHY TREATED BY REMOVAL OF FREE LIGHT CHAINS USING EXTENDED HEMODIALYSIS: A PHASE 1/2 CLINICAL TRIAL

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Introduction. Dialysis dependent renal failure in multiple myeloma (MM) patients is usually due to excess serum free light chains (sFLC) causing cast nephropathy. Only 12-20% of such patients recover renal function. We have previously demonstrated that extended dialysis using a high cut-off protein-permeable dialyser (Gambro HCO1100) rapidly lowers sFLC. Here we report the successful treatment of two patients with relapsed/refractory myeloma treated with PAD (bortezomib, adriamycin and dexamethasone) whilst receiving extended dialysis with the HCO1100. *Materials and Methods*. Patient 1 (64 yo male) was diagnosed with MM in 2006 and commenced cyclophosphamide/thalidomide/dexamethasone as primary treatment. He developed acute renal failure (GFR <15 mL/min requiring haemodialysis) whilst still on treatment. Renal biopsy confirmed cast nephropathy. He was commenced on pulsed dexamethasone and extended haemodialysis with the HCO1100. After 10 days there had been no response. He was then commenced on PAD chemotherapy. Before the completion of the first 21 day cycle, sFLC levels had dropped to and he was independent of dialysis (GFR). Patient 2 (66 yo male) received VAD chemotherapy and autologous transplant as primary therapy for his MM in 2004. In June 2006, he had evidence of disease progression, and commenced CTD. After responding, he commenced maintenance thalidomide. In December 2006, he presented with ARF requiring haemodialysis, with serum free lambda of 2530 mg/L). He was commenced on PAD and extended haemodialysis with the HCO1100. Before the end of the first 21 day cycle, sFLC levels had normalised (23 mg/L) and he was independent of haemodialysis. *Conclusions*. These two cases illustrate that 1) rapid reduction in free light chain production following PAD chemotherapy is achievable 2) rapid reduction in sFLC occurs when using extended dialysis with the HCO1100 membrane 3) the combination of PAD and the HCO 1100 results in rapid renal recovery The combination warrants further investigation in clinical study.

PO-1110

PENTASTARCH FOR ACUTE ATTACK OF SYSTEMIC CAPILLARY LEAK SYNDROME

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Systemic capillary leak syndrome (SCLS) is a rare condition characterized by unexplained episodic capillary hyperpermeability; edema, hypotension, hypoalbuminemia, hemoconcentration, renal shut-down and monoclonal gammopathy. Some patients were diagnosed multiple myeloma (MM) during follow-up but the pathogenesis of SCLS is still unclear and treatment is empirical. Death during acute hypotensive attack from cardiopulmonary collapse occurs in approximately one third of the cases. We experienced two cases of SCLS with severe acute attack and showed rapid improvement of hypotension by 10% pentastarch. Case 1: A 36-year-old woman visited with systolic blood pressure (SBP) 50 mmHg, dyspnea, oliguria, anasarca, Hb 19.2 g/dL, total protein/albumin 3.7/1.7 g/dL, BUN/creatinine 32.6/1.9 mg/dL and monoclonal IgG-kappa. There was no evidence of MM. SCLS was diagnosed and treatment was started with intravenous infusion of 9 L of normal saline (NS), dexamethasone and inotropics for 8 hours. Despite massive fluid therapy, SBP was not increased, and respiratory failure due to pulmonary edema was developed. After infusion of 10% pentastarch, SBP was gradually increased to normal within 12 hours. From the next day, diuresis phase started, other clinical and laboratory findings became normal range. Case 2: A 36-year-old woman was referred with anasarca, anuria, SBP 40 mmHg, Hb 21.1 g/dL, BUN/creatinine 22.6/2.8 mg/dL, total protein/albumin 4.4/2.5 g/dL and monoclonal IgG-kappa without evidences of MM. Two hours after using 0.5 L of 10% pentastarch and 2 L of NS, SBP and urine output increased to 127 mmHg and 50 ml/hour, respectively. Other clinical and laboratory findings restored and diuresis phase started within 1 day and there was no pulmonary edema. Acute management during hypotensive attack is very important to reduce mortality of SCLS. Because shift of fluid and protein by capillary hyperpermeability is the pathophysiology of this syndrome, massive crystalloid fluid infusion would not effective during hypotensive phase of SCLS and exacerbate edema. It was reported that egress from vascular compartment was limited to proteins under 200,000 dalton and we used pentastarch (240,000 dalton) and it showed dramatic responses. Pentastarch would be a very effective volume expander for the treatment of acute attack and might decrease the acute mortality of SCLS.

PO-1111

IMPACT OF THE RENAL FAILURE IN MULTIPLE MYELOMA

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Introduction. Cast nephropathy is a typical renal complication found in myeloma (MM) patients, and up to 50% of newly diagnosed patients have a decrease in creatine clearance. *Methods*. The study included 131 patients (75 men and 56 women, mean age 62.4 years) with MM followed up between October 1983 and June 2006. *Results*. 26.75% of patients presented (renal failure) RF in the onset of the disease. 73,56% of patients with RF are in stage III, while only 61.66% of the patients without RF are in stage III. 40% of patients with RF have IgA or light chains MM, while only 21.27% of patients with IgG presented RF (Mantel-Haenszel: Chi=3.36 and p=0.000). In 37.5% of patients with MM and RF was identified a precipitation factor of RF. 26,75% of patients with RF died within the first 2 months from the diagnosis. The 1 year survival of the patients with MM and persistent RF was 16.66%, compared to 66.66% for the patients whose urea and creatinine levels recovered, Fisher exact p=0.03. The same is also true for MM patients with persistent RF and patients with recovered RF. The urea and creatinine levels recovered in another 25%. The renal function recovery was positively influenced by the creatinine level of less than 3.5 mg% at presentation (66.66% in patients with reversible RF and only 16.66% in patients with persistent RF - Fisher exact p=0.04 - by the calcium level of less than

11 mg% (61.11% of patients with reversible RF had a calcium level of less than 11 mg%, versus 16.66% of patients with persistent RF (Fisher exact p=0.07). In 66.66% of MM patients with recovered renal function was identified a RF releasing factor, while this factor was present in only 27.77% of patients with persistent RF. Six patients with RF were dialysed; in two of them the renal function recovered, 3 remained in chronic dialysis program and one has died. *Conclusions*. RF is more frequent in patients with light chains and IgA MM than in IgG MM. The factors associated with renal function recovery were the degree of RF and presence of hypercalcemia

PO-1112

LEPTOMENINGEAL MYELOMATOSIS IN IGD-MULTIPLE MYELOMA. CAN IT BE PREDICTED?

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Introduction. Leptomeningeal myelomatosis (LMM), defined by the detection of myeloma cells (MC) in the cerebrospinal fluid (CSF) in the presence of suggestive symptoms, has an incidence of approximately 1% and a extremely poor prognosis. IgD-MM represents 1-2% of all MM patients. It has been suggested that meningeal seeding is a concomitant of aggressive MM rather than a sign of progression to more advanced disease. Some biological markers of aggressive MM have been described. Previous reports show a disproportionate number of IgD-MM in patients with LMM, which might indicate a propensity for this rare subtype to spread to the meninges. We present a patient with an IgD-MM and LMM. Patient: A 60-year-old woman presented with dyspnoea. CBC showed Hb 8.6 g/dL, platelets 25×10^9/L and WBC 12×10^9/L 6% plasmablastic MC ($CD38^+$, $CD56^-$, $CD19^-$). Pleural effusion had 79% of MC (CD56-). Bone marrow revealed 96% of MC with identical immunophenotype and a karyotype with diverse abnormalities including t(1;8). Serum M protein was 20.8 g/L (delta-lambda) and proteinuria 3.93 g/d (lambda). Creatinine, calcemia and LDH were normal while beta2-microglobulin was 7.4 mg/dL. She had multiple osteolytic lesions. A diagnosis of IgD-MM, IPI stage 3 was made and after 6 VAD courses she attained partial response. Six months after, she complained of progressive lower extremities weakness and right facial nerve palsy. Cranial MRI was normal. Spine MRI showed a soft enhancement of the cauda equina region. CSF had many atypical MC ($CD56^-$). Patient was diagnosed with LMM. Radiation therapy, IT methotrexate and AraC, and high-dose oral dexamethasone were instituted. However, MM progressed with a breast delta plasmacytoma, rising of serum M component and progressive renal failure. Patient died 2 months after LMM diagnosis. *Discussion*. Besides CD56 negativity, plasmablastic morphology, IPI stage 3 and circulating MC have been associated with LMM. These features were present in our patient at MM diagnosis. She had a translocation involving chromosome 1, frequently described in this situation. *Conclusion*. The potential higher risk for LMM in IgD-MM with the above mentioned features should alert clinicians to the possibility of this fatal complication and could indicate an earlier recognition or even prophylactic treatment.

PO-1113

ABDOMINAL EXTRAMEDULLARY PLASMACYTOMAS AS ASOLE MANIFESTATION IN RELAPSING MULTIPLE MYELOMA PATIENTS

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Introduction. Extramedullary Plasmacytomas (EMP) are rare in myeloma (MM) at diagnosis (3-5%). EMP arising in relapsing patients may be the only site of MM reappearance; differential diagnosis from other neoplasms presents difficulties. The incidence of EMP seems higher after auto/allo-transplantation. Prognosis is poor with standard /high-dose therapy and with Thalidomide/ Bortezomib.We present 3 cases developing abdominal EMP without systemic relapse. *Materials*. A 72 y male IgGk MM patient , stage IIIA in VGPR after 4 cycles of VAD, and while on dexamethasone maintenance, developed acute renal failure. Abdominal CT revealed a mass infiltrating both kidneys. Biopsy detected neoplastic cells with plasmablastic/anaplastic features, k-light chain positive, 70% $Ki67^+$; he expired in septic shock during treatment. In a 58y man with stage IIIA IgGλ MM, a VGPR was achieved after VAD and autoSCT. Three months later he developed hematuria and renal failure. CT showed a retro-peritoneal mass infiltrating the bladder, and laparoscopic biopsy revealed plasmablastic EMP; he died with disease progression after one cycle of bortezomib/dexamethasone. A 59 y man with IgA-λ MM in CR after VAD/ AutoSCT presented after 6 months with multiple skin infiltrates and elevated liver enzymes. Skin biopsy showed EMP while MRI of the liver showed two portal masses. A leg mass(10x10 cm) developed during treatment with DHEAP, and biopsy showed plasmablastic lymphoma. No response was obtained with bortezomib; PR was achieved with Melphalan (50 mg/m^{2}x 2) and radiation to the mass but he relapsed. He is alive under treatment with CHOP and Lenalidomide. *Discussion*. There are few reports of abdominal plasma cell neoplasms. In a review of abdominal plasmacytomas 4 /7 MM pts had involvement of perirenal space, 3/7of retroperitoneal and pelvic lymphnodes,3/7 of peritoneum, 2/7 of liver, 2/7 of subcutaneous tissues, and 1/7 of kidney In a retrospective study, of 432 pts with MM 19 (4.6%) developed EMP. There was no correlation with stage, LDH or Ig type. Response to chemotherapy was very poor. Zeiser *et al.*, reported that of 78 pts relapsing after BMT, 17% were EMP relapses. *Conclusion*. Auto-SCT is a well established treatment in MM; unfortunately relapses are the norm and EMPs seem to be noted more frequently of late.

PO-1114

BORTEZOMIB IN RENALLY IMPAIRED MULTIPLE MYELOMA PATIENTS

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Introduction. Approximately 30% of multiple myeloma (MM) patients present with renal impairment, which is associated with poor prognosis. Treatments for these patients should have a rapid mechanism of action and a safety profile not compromised by patients' renal impairment. This analysis of the phase 3 APEX study of bortezomib (VELCADE®) vs high-dose dexamethasone in relapsed MM patients assessed bortezomib efficacy and safety in patients with varying degrees of renal impairment. Additionally, bortezomib vs dexamethasone results were compared within renal subgroups. *Materials and Methods.* APEX eligibility criteria included creatinine clearance (CrCl) ≥20 mL/min. Response rate, time to progression (TTP), and overall survival (OS) were analyzed in subgroups of patients with CrCl <30, 30–50, 51–80, and >80 mL/min (severe, moderate, mild, no renal impairment, respectively). Intra- and inter-arm statistical comparisons of TTP and OS were conducted, and safety profile assessed, for subgroups with CrCl ″50 mL/min (moderate-to-severe) or >50 mL/min (mild/no impairment).

Table. Response rate, TTP, and OS in patients treated with bortezomib, by CrCl rate.

	Total population	*CrCl rate (mL/min)* <30	30–50	51–80	>80
N (total/response -evaluable)	330/313	17/15	45/43	141/137	127/118
Response rate (CR+PR),%	38	47	37	40	36
CR,%	6	0	9	8	4
Median time to first response, months	1.4	1.6	0.7	1.2	1.4
Median TTP, months	6.2	4.2	5.6	6.2	6.3
Median OS, months	29.8	22.0	22.8	30.0	NR

Response rate and TTP analyses based on initial APEX data, to enable comparison with dexamethasone arm, which was subsequently halted. OS analysis based on updated APEX data, with extended follow-up. NR = not yet reached.

Results. As shown (Table), response rates were comparable and time to response was similarly rapid across subgroups. TTP and OS were not statistically different between patients with moderate-to-severe and mild/no renal impairment, although there was a trend towards longer OS in the mild/no impairment subgroup (median TTP 4.9 vs 6.2 months, p=0.62; median OS 22.8 vs 30.0 months, p=0.07). Bortezomib showed greater efficacy vs dexamethasone in all subgroups, with higher response rates. In patients with moderate-to-severe and mild/no renal impairment, TTP was significantly longer (p"0.02) and there was a trend towards longer OS (p=0.09) with bortezomib. Incidences of adverse events (AEs), grade ≥3 AEs, serious AEs, and discontinuations and dose reductions due to AEs were similar in bortezomib patients with moderate-to-severe and mild/no renal impairment, whereas with dexamethasone, incidences of grade ≥3 AEs, serious AEs, and discontinuations and dose reductions due to AEs appeared higher in patients with moderate-to-severe vs mild/no renal impairment. *Conclusions*. Bortezomib is active and well tolerated in relapsed MM patients with varying degrees of renal impairment, including severe impairment; efficacy and safety are not substantially affected by moderate-to-severe vs mild/no renal impairment. Bortezomib has greater efficacy than dexamethasone regardless of degree of renal impairment.

PO-1115

ANTIBIOTIC PROPHYLAXIS CAN REDUCE THE INCIDENCE OF ONJ

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Introduction. ONJ is a serious complication of bisphosphonates administration, in particular zoledronate, in myeloma patients. Several risk factors may be involved and, among them, the bone infection subsequent to dental procedures, also of moderate complexity, can have a relevant pathogenetic role. In order to evaluate the possible protective effect of antibiotic prophylaxis before dental procedures, we conducted a retrospective study of clinical data concerning 101 zoledronate-treated myeloma patients from two different institutions. *Materials and Methods.* According to internal guidelines, the policy of prevention for infections caused by dental procedures for non-neutropenic patients was different among the two institutions. Institution A adopted an antibiotic prophylaxis (group A), which consisted in amoxicillin-clavulanate 2 gr/day p.o. or levofloxacin 500 mg/day p.o. from 1 day before to 3 days after any dental procedure. Patients from institution B did not receive any specific recommendation (group B). *Results*. Patients in the two groups did not differ for age, transplantation procedures, thalidomide and steroid treatment. The 47 group A patients received a median number of 15 (range 1-35) zoledronate infusion, with a median time of exposure of 30 months (range 1-64). The 54 group B patients received a median number of 12 (range 3-30) zoledronate infusion, with a median time of exposure of 12 months (range 3-60). The following dental procedures were performed: professional cleanings (14 in group A, 11 in group B), extractions (9 in group A, 13 in group B), implants (2 in group A), fillings (5 in group A, 4 in group B), prosthesis (17 in group A, 26 in group B). In group A there were no complications, while 8/54 group B patients (15%) developed ONJ (p=0.008). *Conclusions*. Group A patients seem to be protected by the antibiotic prophylaxis. These data suggest that antibiotic prophylaxis before any dental procedure in zoledronate-treated myeloma patients can have a protective role for ONJ development. A simple clinical intervention, like a course of low-cost antibiotics concomitant with a dental procedure, can be effective. Further, this observation can emphasize the role of oral microbial flora in the pathogenesis of ONJ, which, at present, is still unclear.

PO-1116

RISK FACTORS FOR BISPHOSPHONATE-RELATED OSTEONECROSIS OF JAW IN MULTIPLE MYELOMA PATIENTS

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Intravenous bisphosphonates (BPs) have been used for a long time in the treatment of bone disease secondary to multiple myeloma and solid tumors (especially breast cancer, prostate cancer and lung cancer), as well as osteoporosis, Paget disease and metabolic bone diseases. Their use has improved the quality of life of patients, reducing bone pain and preventing new skeletal events. The mechanisms of action of BPs have not been clarified: the principal action is devoted to osteoclastic activity inhibition and to induction of apoptosis. Moreover recent evidence has suggested an anti-angiogenic role, offering new opportunities for the treatment of different types of neoplasms. Pamidronate (P) and zoledronic acid (Z) are the most used BPs, and are generally well tolerated. Since 2003 several papers have reported the development of osteonecrosis of jaw (ONJ) as a possible long term complication of BPs treatment, in particular in those patients suffering from multiple myeloma. Possible risk factors for ONJ identified by investigators are: increased duration of exposure, type of BPs, advanced age, poor dental hygiene and prior history of dental procedures. Particularly, in the literature, the risk for ONJ seems higher in patients assuming Z than in patients treated with P alone or with P followed by Z. The duration (median from 22 to 39 months) of treatment and the cumulative dose are also important risk factors for ONJ development. The prevalence has not yet been clearly determined, ranging from 6% to 10% in cancer patients, according to different authors. Typical presentation is an exposed bone, sometimes in association with infection of the lesion. Most myeloma patients showed ONJ after tooth extraction. Recent clinical staging has been reported for ONJ, helping direct appropriate local treatment (Ruggiero *et al.*, 2006 OOOE). In our experience, we retrospectively analysed 105 multiple myeloma (MM) patients treated with BPs for a long term, to evaluate incidence, possible risk factors, clinical features and outcome of ONJ. *Patients and Methods*. One hundred-eighteen patients, treated with BPs therapy, were evaluated for osteolytic lesions in two Italian haematological departments. Only MM patients (n = 105) were considered for the analysis. All patients were treated with monthly intravenous BPs cycles (P 90 mg and Z 4 mg). The ONJ diagnosis was based on: subjective symptoms, such as pain, and oral cavity inspection that showed soft-tissue swelling and exposed bone, X-Ray, computed tomography and histopathology examination. Treatment included conservative approaches and surgical procedures. Conservative management consisted of empiric antibiotic therapy with amoxicillin and claritromicin in patients with negative cultures, local irrigations (topical clorexidine 0.12%), hyperbaric therapy and systemic ozone therapy. Surgical management consisted of minor debridement to remove necrotic tissue, marginal mandibular resections and partial maxillectomy Data were analyzed for descriptive statistics, and then were studied by univariate analysis including Fisher's exact test and Pearson's test for cross tabulation of qualitative variables and general linear model for quantitative data. Time to event analysis was carried out by Kaplan-Meier method followed by logrank test, plus smoothing techniques by means of Epanechnikov kernel, to evaluate the cumulative hazard over time. *Results*. Among 105 MM patients, 44 were males (41.9%) and 61 females (58.1%); median age was 69 years (range 34-89). ONJ was uniformly distributed among gender (Fisher's exact test: p=0.113), and was not associated with age (Wald's test: p=0.254). Only 7 patients (0.7%) received P alone, whereas 36 (34.3%) were treated with Z, and 62 (59.0%) received P and then Z . For P, median treatment duration was 18 months (range: 1 to 25) in patients receiving P and 23 months (range: 1 to 81) in those receiving both drugs. For Z, median treatment duration was 20 months (range: 1 to 38) in patients treated with Z alone, and 24 months (range: 1 to 35) in those receiving both drugs. The cumulative doses for P and for Z , respectively, were not significantly different comparing the single and the double-drug arm (median test: p=0.499 and p=0.530, respectively). ONJ prevalence was 0% (0 cases) for P alone, 8.3% (3 cases) for Z alone and 22.5% (14 cases) for P+Z treatment, without significant differences among groups (Fisher's exact test: p=0.102). Measured incidence rate of ONJ, starting from diagnosis, was 0.0014 new cases per patient month (CI95%: 0.0004 to 0.0045) in subjects treated with Z alone, and 0.0029 new cases per patient month (CI95%: 0.0017 to 0.0048) in subjects receiving first P, and then Z. Incidence rates were not significantly different, but the estimate of adjusted odds ratio of ONJ between groups was 2.523, indicating a significantly higher risk in patients treated with both drugs in sequence (Pearson's). At development of ONJ, 60 patients (57%) were treated with thalidomide and 6 of them (10%) developed ONJ, with a significant association between the events (Fisher's exact test: p=0.026), but with no association between thalidomide and the type of BP treatment (Fisher's exact test: p=0.209). Mandible was involved in ten patients and maxilla in seven. The typical symptoms and signs were pain and exposed bone. Eleven patients (10.5%) had a history of previous tooth extraction. Sixteen cases (15.0%) had necrosis of the bone, five (4.8%) exposition of the bone and five (4.8%) a fistula in another cavity. Among microbiologic cultures only Actinomyces was found in one case. Management of ONJ consisted in: systemic antibiotic therapy (12 patients), partial osteotomy (7 patients), hyperbaric oxy-

gen therapy (5 patients), local irrigation (4 patients) and local radiotherapy (1 patient). Only one patient did not need treatment.One patient had a spontaneous complete resolution, 10 presented a partial response with minimal debridmet and local antibiotic therapy, six had no improvement. All patients interrupted BPs therapy after the occurrence of ONJ. *Discussion*. In our study we considered 105 cases with MM and our analysis was focused on dosage and duration of treatment. In this series 17.8% of patients developed a proven ONJ. Sequential treatment with pamidronate and zoledronic acid presents an important odds of ONJ development. Concurrent therapy with thalidomide represents a significant and independent additional risk factor for ONJ. Concerning the management of ONJ, in our experience major debridement surgery did not give good results, while conservative therapy gave better long term ones. All patients with ONJ interrupted BPs therapy . At present, following the suggested guide lines (Ruggiero *et al.*, 2006 J Clin Oncol Prac), no new cases of ONJ have been recorded in our centers.

PO-1117

FREQUENT MYELOMA INVOLVEMENT FOUND AT HISTOLOGIC EVALUATION IN PATIENTS FORMERLY DIAGNOSED AS HAVING OSTEONECROSIS OF THE JAWS

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Osteonecrosis of the jaws (ONJ) is a rare complication that has been described in patients who had previously undergone radiotherapy for head and neck cancer or similar disorders. Recently, a growing epidemic of ONJ has been reported and this has been attributed to the widespread use of bisphosphonates for treatment and prevention of bone involvement in multiple myeloma (MM) or solid tumors. In the present study we have retrospectively reviewed all MM patients treated at our Institution who have subsequently presented a clinical picture - namely pain, mucosal swelling, exposed bone and purulent discharge- suggestive for ONJ. From May 2003 to present, 15 patients (6M, 9F) were analyzed, their median age was 65, all patients but three had received autologous stem cell transplantation, either single (n=2) or double (n=9); four patients had relapsed after first-line treatment and were receiving thalidomide-dexamethasone as salvage therapy. All the patients had been treated with bisphosphonates, either pamidronate alone (n=2) or pamidronate followed by zoledronic acid (n=2) or zoledronic acid alone (n=11); median number of bisphosphonate infusions was 22 (range 10-45). Diagnostic work-up included only panoramic and tomographic imaging in 2 patients, while in 13 patients histological examination of surgically removed bone was performed. Surprisingly, in 5 patients (38%) a consistent infiltrate of monoclonal plasmacells was detected, thus suggesting involvement by underlying disease. Complete repair or significant healing of the lesions occurred in 10 patients after antimicrobial therapy, either alone (n=3) or combined with surgery (n=5) or with thalidomide-dexamethasone salvage therapy (3 patients with MM at histological examination). Four patients did not show a sustained improvement; specifically, two patients with MM at histological examination underwent major surgery prior to salvage treatment thus resulting in persistent disability. According to our results it can be concluded that tumor involvement is not uncommon in MM patients diagnosed as having ONJ. For this reason, at variance to what is recommended in published guidelines, in MM patients with ONJ, biopsy of the lesion should be performed, though with caution, in order to rule out tumor involvement and to set up the most appropriate treatment.

PO-1118

OSTEONECROSIS OF THE JAW IN MULTIPLE MYELOMA PATIENTS.

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Background. Osteonecrosis of the jaw (ONJ) is a complication observed in patients with a history of aminobisphosphonate use. *Methods*. In order to define ONJ and gain insights into its pathophysiology, clinical, radiographic, biochemical, and microarray profiling studies were conducted in 11 patients with multiple myeloma (MM) with ONJ. *Results*. Eleven patients between the ages of 57 and 81 yrs were treated with either pamidronate (n=3), zoledronate (n=4), or both agents sequentially (n=4) for a mean of 38.7 months (range: 9-81 months). Patients presented with both maxillary and mandibular lesions. Radiographic studies demonstrated radiolucency and sclerosis on plain films. An increased glucose metabolism and mineralization at sites of ONJ, evidenced by the maximum standardized uptake value (SUVmax) on fluorodeoxyglucose (FDG) and sodium fluoride-positron emission tomography (NaF-PET) scans, respectively was noted. The target-to-background ratio of SUVmax was significantly greater for NaF-PET scans than FDG-PET, suggesting that NaF-PET may have greater sensitivity to confirm a diagnosis of ONJ. Transcriptional profiling of peripheral blood mononuclear cells (PBMCs) using the Affymetrix U133Plus 2.0 gene chip in all 11 MM patients was compared with 10 age matched MM controls and 5 normal donors. Serum and urine bone turnover markers were similarly tested. Genes and proteins involved in osteoblast and osteoclast signaling cascades were significantly downregulated in patients with ONJ. *Conclusions*. ONJ is a complication of long-term aminobisphosphonate use. 18F-NaF PET scan has greater sensitivity than FDG-PET to confirm ONJ. Gene and protein studies are consistent with altered bone remodeling, evidenced by suppression of both bone resorption and formation.

PO-1119

BRAZILIAN RECOMMENDATIONS: BISPHOSPHONATES AND ONJ

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Introduction. Maintaining dental health is becoming increasingly important in patients with cancer who may receive long-term therapy with multiple regimens. For example, osteonecrosis of the jaw (ONJ), a pathology characterized by exposed bone in the oral cavity with variable healing, is an uncommon event that has been reported in patients with cancer receiving complex treatment regimens. Recently, ONJ has been reported in cancer patients whose treatment regimens include bisphosphonates, potent inhibitors of osteoclast-mediated bone resorption that have become integral components of therapy for patients with malignant bone disease. *Materials and Methods*. Literature searches were performed to assess the current awareness of the etiology, clinical characteristics, and risk factors of ONJ. In addition, a local multidisciplinary panel of Brazilian experts in the field collaborated to develop guidelines for reducing the risk and minimizing the morbidity of ONJ in patients undergoing bisphosphonate therapy. *Results*. Osteonecrosis of the jaw has been reported in patients undergoing therapy with intravenous bisphosphonates and, less commonly, oral bisphosphonates. Although the pathogenesis of ONJ, which is likely to involve multiple factors, is not well understood, local and systemic risk factors have been recognized. Local risk factors include dental extraction, trauma from prosthesis (eg, bridges and dentures), and poor oral hygiene. Systemic risk factors include use of chemotherapeutic agents or intravenous bisphosphonates, immunosuppression, and malignant bone disease. Osteonecrosis of the jaw has been reported more frequently in patients with multiple myeloma than in patients with solid tumors. Based on these risk factors, we recommend that patients with cancer undergoing bisphosphonate therapy receive routine dental examinations with preventive dentistry before initiating therapy, avoid invasive procedures during therapy, and maintain good oral hygiene throughout the continuum of care. The potential risks and benefits of continuing bisphosphonate therapy in patients with dental pathologies should be discussed openly with the patient on a case-by-case basis. *Conclusions*. Identified risk factors may provide insight into the pathophysiology and prevention of ONJ. Research efforts to provide more definitive guidance for the management of ONJ are ongoing.

PO-1120

KYPHOPLASTY FOR SPINAL FRACTURES IN MULTIPLE MYELOMA

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Introduction. Approximately 70% of multiple myeloma patients suffer from painful spinal compression fractures. Introduced in 1992, balloon kyphoplasty has shown significant benefits in pain relief and height

restoration of vertebral fractures. This abstract evaluates the safety and efficacy of kyphoplasty in 41 consecutive patients with Multiple Myeloma treated at the H. Lee Moffitt Cancer Center. *Materials and Methods*. A total of 62 kyphoplasty procedures were performed in a 24 month period. The use of medical records including all pre and post op films (MRI, CT, plain films, and bone scans), ambulatory care notes, and operative notes were reviewed on all 41 patients. *Results*. There were 23 females (56%) and 18 males (44%). The age range was 39 to 87 years old, with a median age was 59. Ten (24%) of these patients underwent radiation therapy to the vertebral involvement site. Of the 62 procedures, 23 (37%) were thoracic kyphoplasties, while 22 patients (35%) were lumbar kyphoplasties, 15 patients (24%) underwent a combined thoracic and lumbar kyphoplasty, and 2 patients (3%) underwent sacroplasties. Eight (13%) of the 62 cases were complicated by methylmethacrylate extravasation. Eight-five percent of the cases were without sequelae. There was one case of pneumothorax that resolved with chest tube placement. In regards to pain relief, 9 patients (22%) received partial pain relief, 30 patients (73%) received substantial relief and 2 patients (5%) received no pain relief. It was also found that of the 41 patients, 14 (34%) developed new fracture sites over an average time frame of 4 months. *Conclusions*. The kyphoplasty procedure is efficacious in the treatment of vertebral compression fractures related to multiple myeloma. It has shown to produce significant pain relief in the majority of the patients with minimal complications.

PO-1121

VTE MANAGEMENT RECOMMENDATIONS FOR LEN/DEX IN MM

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Introduction. In January 2007, an international group of multiple myeloma (MM) specialists reached a consensus on practical recommendations regarding the management of lenalidomide treatment (in combination with dexamethasone) in patients with relapsed/refractory MM, who have received at least one prior therapy. Here, we focus on recommendations for prevention and management of venous thromboembolism (VTE) in these patients. *Methods*. A moderated round table discussion. *Results*. Treatment with lenalidomide/dexamethasone (Len/Dex) in patients with relapsed/refractory MM has been reported to be associated with a rate of VTE of 12%. Therefore, it is important to assess the VTE risk and consider the use of prophylaxis. There was consensus that the following factors increase the risk for VTE in this setting: high-tumour mass, concomitant chemotherapy, doxorubicin, high-dose dexamethasone, erythropoietin use, ongoing infection/inflammation, older age, thrombophilia, previous VTE, or pre-existing coagulation disorders. Neither baseline coagulation studies nor screening for VTE in asymptomatic patients are recommended. Sonography for diagnosis of VTE is recommended in symptomatic patients. We recommend prophylactic anticoagulation if any of the above risk factors is present at treatment with Len/Dex and no prophylaxis in patients without risk factors. The risk of VTE is particularly high in the first 4-6 months of therapy. At present there is no evidence of the best prophylaxis: daily aspirin, low-molecular-weight heparin (LMWH), or therapeutic doses of warfarin, are the options. The panel suggests the use of low-dose aspirin (ASA) (81-100 mg) or prophylactic dose of LMWH. Low-dose warfarin is not recommended, therapeutic-dose warfarin seems to be associated with an increased risk of severe haemorrhage. All patients need to receive clear instructions on how to proceed in case clinical symptoms of VTE occur. When VTE has occurred, the patient can be continued on treatment with Len/Dex or retreated after stabilization depending on the severity of the VTE. For therapeutic anticoagulation, patients previously on ASA should be switched to LMWH; and patients already on prophylactic doses of LMWH should receive therapeutic doses. *Conclusion*. In relapsed/refractory patients who receive lenalidomide, VTE prophylaxis with ASA or LMWH is suggested if at least one of the above listed risk factors is present.

PO-1122

MANAGEMENT OF NON-HAEMATOLOGICAL TOXICITY OF LEN/DEX

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Introduction. In January 2007, an international group of multiple myeloma (MM) specialists reached a consensus on practical recommendations regarding the management of lenalidomide treatment (in combination with dexamethasone) in patients with relapsed/refractory MM, who have received at least one prior therapy. This communication focuses on recommendations for management of non-haematological adverse events (NHAE) in these patients. VTE management recommendations are presented separately. *Methods*. A moderated round table discussion. *Results*. Treatment with lenalidomide/dexamethasone (Len/Dex) is well tolerated in patients with relapsed/refractory MM. Nevertheless, some NHAE may occur. The most common NHAE of all grades in two randomised studies were fatigue (38%; versus 37% with dexamethasone alone), constipation (39% vs. 19%), diarrhoea (29% vs. 25%), nausea (22% vs. 19%), muscle cramps (30% vs. 21%), rash (16% vs. 8%); and paraesthesia (12% vs. 13%). The most common NHAE leading to dose reduction/interruption were fatigue (4%) and pneumonia (2%). A randomised study comparing lenalidomide combined with high-dose or low-dose dexamethasone demonstrated considerably less toxicity with low-dose dexamethasone; however, efficacy data from this study are not yet available. Atrial fibrillation (AF) appears to be more common in patients treated with Len/Dex (grade 3/4 NCI: 3% vs. 1%), especially if high-dose dexamethasone is used or if patients had previous AF, and regular monitoring is recommended. In contrast to monotherapy with lenalidomide alone, rash is less frequent with Len/Dex. In case of grade ≥2 rash, we recommend treating the patient with antihistamines. If persistent, continuous low-dose prednisone (10-20 mg/d) should be added. Rash is mostly self-limiting with a duration of several weeks but in some cases dose reduction or discontinuation of lenalidomide is necessary. In case of fatigue, other causes such as anaemia, infection, depression or hypothyroidism should be ruled out. Also, patients benefit from counselling. Dose reduction may be considered for severe fatigue. Dexamethasone therapy may predispose patients to infection; therefore we recommend the use of routine antibiotic prophylaxis for all patients upon initiation of Len/Dex treatment. In addition, vaccinations (influenza, pneumococci, meningococci and haemophilus) should be considered. *Conclusion*. A concise strategy for NHAE management of Len/Dex is presented, which will aid safe and efficient administration.

PO-1123

RECOMMENDED MANAGEMENT OF CYTOPENIA FOR LEN/DEX IN MM

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Introduction. In January 2007, an international group of multiple myeloma (MM) specialists reached a consensus on practical recommendations regarding management of lenalidomide treatment (in combination with dexamethasone) in patients with relapsed/refractory MM, who received ≥1 prior therapy. This communication focuses on recommendations regarding the management of cytopenias in these patients. *Methods*.

Moderated round table discussion. *Results*. In published studies, 11.6% of patients treated with lenalidomide/dexamethasone (Len/Dex) had NCI grade 3 neutropenia and 3.3% grade 4 neutropenia (Chen *et al.*, Blood 2006; 108:3556). This was the most frequent reason for discontinuation of therapy and dose reduction. The rate of grade 4 febrile neutropenia was <1%. Thrombocytopenia occurred in 11.1%. Risk factors for cytopenia during Len/Dex include low counts at baseline, previous chemotherapy and response to treatment. Patients with renal insufficiency were reported to suffer from more severe thrombocytopenia, but age has not been reported to be a risk factor. Recommendations regarding monitoring and management of cytopenia during treatment with Len/Dex for relapsed/refractory MM have been developed. In case of a normal baseline full blood count (FBC), biweekly monitoring is recommended. If baseline FBC is abnormal because of MM infiltration, treatment should still be pursued with full dose and at least weekly monitoring. Standard dose-reduction strategies should be followed for all other causes of abnormal baseline and follow-up FBC. As a general rule, G-CSF can be used in neutropenic patients. In case of neutrophils <1,000/μL, G-CSF is recommended to prevent dose reduction and febrile neutropenia aiming at >500/μL neutrophils. If neutrophils fall <500/μL, Len should be interrupted and restarted (same dose for first fall and no other toxicity; lower dose for subsequent falls) once neutrophils >500/μL. Similarly, if platelets fall <50,000/μL, anticoagulation should be stopped and in case of thrombocytopenia <30,000/μL, Len should be interrupted and restarted at a lower dose once platelets >30,000/μL. Also, antibiotic prophylaxis with cotrimoxazole should be applied if patients receive Len with high dose Dex. Patients should receive clear instructions to seek medical care within 3 hours if febrile while neutropenic. *Conclusion*. A stringent strategy for the management of cytopenia due to treatment with Len/Dex is recommended.

PO-1124

DOWNREGULATION OF PU-1 BY IMIDS INDUCES NEUTROPENIA

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Introduction. Previous clinical trials have shown that IMiDs induce high response rates in patients with relapsed or refractory multiple myeloma, and induce erythropoietic and cytogenetic remission in patients with MDS. The most common dose limiting adverse effect of IMiDs is severe neutropenia. Our investigations indicate that IMiDs have no direct toxic effects on hematopoietic progenitors, but rather promote a shift in hematopoietic progenitor lineage commitment toward myeloid precursors (Koh *et al.*, Blood 2005, Anderson at al, Blood 2006). It was the aim of this study to characterize the biologic mechanism(s) underlying neutropenia IMiD treatment. Methods and *Results*. In colony forming assays using human CD34+ hematopoietic precursors treated with CC-5013 or CC-4047, we observed a 8.3 fold increase in granulocyte progenitor formation (CFU-G) at the expense of CFU-GM, BFU-E and CFU-E in comparison to vehicle controls. After 4 days *in vitro* treatment with CC-4047, expression of the transcription factor PU.1 protein showed complete down-regulation. PU.1 gene knock-down models show increased granulopoiesis with impaired neutrophil maturation, resulting in a failure to attain neutrophil functional competence. To analyze medullary changes in granulopoiesis in human subjects treated with CC-5013, we evaluated sequential bone marrows from 6 patients comparing bone marrow features prior to treatment, during treatment, and at the time of grade 4 neutropenia. Marrows at the time of neutropenia showed an accumulation of myeloid precursors (myelocytes, metamyelocytes) supporting impaired myeloid maturation. In 5 out of 6 patients the myeloid:erythroid (M:E) ratio increased with a M:E median of 1.95 pretreatment and 9.2 at the time of the neutrophil nadir. At the same time, bone marrow cellularity was unchanged. *Conclusions*. Our data show that IMiDs downregulate PU.1, a key transcription factor involved in granulocyte differentiation. The loss of PU.1 results in medullary accumulation of immature myeloid precursors, with corresponding neutropenia in the peripheral blood. Our findings show that IMIDs do not induce neutropenia by marrow suppression but rather by transient block of maturation.

PO-1125

ACYCLOVIR 400 MG ONCE DAILY IS SUFFICIENT FOR VARICELLA-ZOSTER PROFYLAXIS DURING BORTEZOMIB TREATMENT

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Introduction. Varicella-zoster virus (VZV) reactivation is common in patients with multiple myeloma treated with bortezomib. None of our 47 patients receiving bortezomib for relapsed multiple myeloma who received prophylactic different dose of acyclovir during the bortezomib treatment had VZV reactivation, whereas in ten patients who were not taking acyclovir herpes zoster occurred in three patients (33%). Thus, our results show that prophylaxis with acyclovir is an effective strategy to prevent VZV reactivation during therapy with bortezomib. *Methods*. Between December 2004 and December 2006, we treated 57 relapsed MM patients with bortezomib. The patients were treated with standard dosage schedule (intravenous infusions of bortezomib 1.3 mg per square meter of body area on days 1, 4, 8, and 11 of a 21-day cycle). Data from clinical trials show that the incidence of herpes zoster during bortezomib therapy is about 13%. *Results*. Our first ten patients treated with bortezomib did not receive any varicella-zoster virus (VZV) prophylaxis and herpes zoster developed in three of these patients (33%). Cutaneous herpes zoster developed on the trunk in two patient and on the face in one patient. Clinical manifestation of herpes zoster was rather typical. The therapy with bortezomib was subsequently interrupted and all three patients received treatment with acyclovir intravenously. One of these three patients is still suffering from severe postherpetic pain. Based on this experience, we started to use prophylaxis with acyclovir 400 mg per os 3 times daily during bortezomib therapy. We did not note any VZV reactivations in the 32 consecutive patient receiving VZV prophylaxis including no VZV reactivation in five patients who already had VZV reactivation before the bortezomib treatment. Than we reduced the prophylactic dose of acyclovir to 400 mg per os 1 times daily in the next 15 consecutive patient. We did not note any VZV reactivations in this 15 patients including 3 patients who already had had VZV reactivation before the bortezomib treatment. *Conclusions*. In conclusion, VZV reactivation is quite common and serious consequence of bortezomib therapy in MM patients and often leads to interruption of the treatment. According to our experience, prophylaxis with acyclovir is very effective and should be considered for all patients with MM treated by bortezomib. The minimal sufficient dose of acyclovir for the prophylaxis of VZV reactivation remains to be established but in our hands the dose of 400 mg of acyclovir once daily was effective in 100% of cases. Supported with research program MSMT of Czech republic Nr. LC 06027

PO-1126

HERPES ZOSTER WITH USE OF BORTEZOMIB IN MULTIPLE MYELOMA

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Background. Bortezomib, a proteasome inhibitor, has been used for patients with refractory and relapsed multiple myeloma. However, the adverse effects associated with use of bortezomib such as neuropathy has been emerged as a problem to overcome. Herpes zoster was recently reported as a possible adverse events associated with bortezomib in the APEX trial. Herpes zoster is the consequence of reactivation of latent Varicella-zoster virus (VZV) from dorsal root ganglion. A recent *in vitro* study suggests cellular localization of varicella zoster virus ORF29p may be affected by proteasome inhibition leading to nuclear accumulation of VZV. Although some concomitantly administered drugs such as dexamethasone might contribute to the occurrence of herpes zoster, the bortezomib-induced proteasome inhibition itself may result in reactivation of VZV. *Materials*. We analyzed the incidence of herpes zoster in patients treated with bortezomib-containing regimen. 61 refractory and relapsed multiple myeloma patients were analyzed. All the patients were heavily pre-treated with more than at least two kinds of therapeutics. *Results*. All the patients received bortezomib (1.3 mg/m^2) on days 1, 4 , 8, and 11 of a 3-week cycle and they received dexamethasone simultaneously. 18 patients showed herpes zoster during or after bortezomib based

treatment (18/61, 29.5%), and this was higher than the reported incidence of western countries. Among 18 patients, only one patient had a previous history of herpes zoster while he was treated with previous cheomotherapy regimens containing high- dose dexamethasone. Thus, 17 patients never had herpes zoster before they were exposed to bortezomib even though they were heavily treated with other agents including dexamethasone. 12 patients showed herpes zoster during treatment while 4 patients showed herpes zoster after the completion of the planned 8th cycle of bortezomib and dexamethasone treatment. There was no significant relationship between peripheral blood counts and VZV reactivation. *Conclusions*. Bortezomib was associated with a higher incidence of herpes zoster in patients with heavily pre-treated multiple myeloma. Thus, the use of prophylactic acyclovir should be considered, and the further study should be warranted to understand the underlying mechanism for this bortezomib-associated herpes zoster.

PO-1127

THAL MONOTHERAPY INDIVIDUAL PATIENT ANALYSIS: TOXICITY

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Introduction. Thalidomide is an effective agent in relapsed/refractory Multiple Myeloma (MM). However, it is associated with considerable toxicity, which may be dose- and/or duration-related. The objective of this analysis was to address the question of dose-toxicity relationship utilising individual patient data from published studies. *Methods*. Authors of published studies evaluating thalidomide monotherapy in patients with relapsed/refractory MM were asked to send in individual patient data including parameters on dose of thalidomide, response and toxicity. *Results*. For analysis of toxicity, data from 216 patients are evaluable to date. 21% of patients discontinued therapy because of toxicity. Overall, somnolence and neurotoxicity were the most frequent adverse events: somnolence occurred in 69% (95% CI 63-75) of patients, but only 2% (95% CI 1-5) experienced somnolence III°. Peripheral neuropathy (PNP) occurred in 68% (95% CI 63-76) with 9% PNP III° (95% CI 6-14). Neutropenia occurred in 36% (95% CI 30-43) with 15% ≥III° (95% CI 11-21). Also of note, the rate of cardiac adverse events was 5% (95% CI 36-10) with 3% ≥III° (95% CI (2-7). The rate of venous thromboembolic events (VTE) was 6% (95% CI 3-10) with 2% ≥III° (95% CI 1-5). For somnolence, PNP and neutropenia, there was a clear correlation between severity of adverse events and cumulative dose of thalidomide after 3 months (p<0.01) as well as daily dose at 3 months (p<0.01). Of patients taking 200 mg/d or more 86% had PNP ≥I°, 27% had somnolence ≥II° and 38% neutropenia ≥II°. In contrast, of patients taking < 200 mg/d only 60% had PNP ≥I° (p<0.01), 10% (p<0.01) had somnolence ≥II° and 16% neutropenia ≥II° (p<0.01). Interestingly, a correlation between severity of toxicity and duration of treatment could only be found for PNP (p<0.01). *Conclusions*. This first analysis of our large data base of patients with thalidomide monotherapy confirms a dose dependent severity of adverse events. However, although we found a 21% discontinuation rate because of toxicity, higher grade toxicities were rare. Interestingly, duration of treatment was clearly correlated with the development of PNP.

PO-1128

THE COST OF TREATING MULTIPLE MYELOMA IN SOUTH-WEST SWEDEN

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Introduction. Approximately 560 patients are diagnosed with Multiple Myeloma (MM) in Sweden each year. Few studies have estimated the cost of treating these patients. In this study the resource utilization and costs associated with the treatment of MM in Sweden were estimated. *Materials and Methods*. A retrospective chart review was performed on patients who initiated their first line chemotherapy in 2001 at predetermined hospitals in South-Western Sweden. Data was collected until patients' death or December 31 2005. Direct hospital-based resources and their corresponding costs (Euro 2006) were calculated for drugs (chemotherapy, bisphosphonates, interferon, analgetics, blood cell enhancement), autologous stem cell transplantation (ASCT), in- and outpatient visits, laboratory tests, radiotherapy, and therapy induced or comorbidity related events. *Results*. 94 patients were included in the study, of which 22% were still alive at study completion. Median age at diagnosis was 76 years (range 34-98). A total of 3,023 patient months were recorded, on average 32.5 months per patient. First, second, and third line treatment lasted 24.1, 5.8 and 2.6 months, respectively, and included 2.9, 2.6, and 3.1 chemotherapy drugs per patient. Of the 80 patients who received chemotherapy in the first line, 72 were prescribed melphalan, and 55 patients received a combination of melphalan and prednisone, which is recommended in Swedish treatment guidelines. The average cost per patient was Euro 46,277, or Euro 1,424 per patient-month. Therapy induced and comorbidity events, ASCT and inpatient care constituted together 70% of the total costs. The cost for chemotherapy drugs increased from Euro 29 in first line treatment to Euro584 in third line, but still only amounted to 6% of the total costs. All cost items, apart from surgery, inpatient stays and lab tests, increased successively with line of treatment. *Conclusions*. The cost of treating MM in Sweden is more than Euro 45,000 per patient, of which 70% is accounted for by therapy induced or comorbidity related events, ASCT and inpatient care. Hence, effective new technologies that can reduce consumption of these resources may generate economically important cost offsets.

Poster Session III

PO-1201

GENOMIC MUTATIONS IN THE APRIL/TACI/TRAF PATHWAY IN WALDENSTROM'S MACROGLOBULINEMIA (WM)

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Introduction. IgA and IgG hypogammaglobulinemia are a common finding in patients with WM with 74% and 63% of patients in our studies exhibiting IgA and IgG levels respectively below the lower limit of normal. An extension of this study looking at the impact of therapy on IgA and IgG levels concluded that disease reduction or even attainment of a CR failed to improved IgA and IgG levels in statistically significant manner. These results provided the impetus to look for a genetic link between WM and hypogammaglobulinemia and we subsequently uncovered novel germ line mutations in the Blys/April receptor Taci (TNFRSF13B) in 6 of the 29 (21%) patients studied and these correlated with IgG and IgA hypogammaglobulinemia (p<0.0001 for both). To expand on these findings we have now sequenced the entire length of Taci including introns and the 2kb 5' and 3' flanking regions as well as the upstream and down stream genes April, Blys, Traf2, and Traf5. *Materials and Methods*. DNA from the BCWM1 cell line and CD19[+] selected bone marrow cells from 22 patients with WM were used for genomic sequencing studies. *Results*. No notable genomic variants were observed in Blys and Traf5 in this population. Novel variants were discovered in the first exon of April in 4/17 (23%) of the samples studied and in 2/22 (9%) of the Traf2 samples in exons 5-6. A region of increased homozygosity was also noted 1.5kb upstream of Taci. *Conclusions*. These studies clearly demonstrate an unusually high frequency of mutations and abnormalities in the April-Taci-Traf2 pathway. Taci mutations have already been associated with hypogammaglobulinemia and common variable immunodeficiency (CVID) while CVID itself has been associated with increased rates of lymphoma. While the current numbers are too small for rigorous statistical analysis with clinical data, further studies are underway at our institution to expand these results through further sequencing of additional WM patient samples, healthy donor samples, and samples from extended families of patients with WM.

PO-1202

DELETIONS OF CHROMOSOMES 6Q AND 13Q, AND TRISOMY 4 ARE THE MOST COMMON CYTOGENETIC ABNORMALITIES IN WALDENSTRÖM MACROGLOBULINEMIA. PRELIMINARY RESULTS OF A MULTICENTRIC STUDY

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The genetic bases of Waldenström Macroglobulinemia (WM) are poorly understood. We studied by conventional cytogenetic (CC) and Fluorescence *in situ* hybridization (FISH) analysis a cohort of 83 untreated WM patients, enrolled in a prospective randomized trial from the French Cooperative Group on Chronic Lymphocytic Leukemia and Waldenström Macroglobulinemia (FCG-CLL/WM). The sex ratio was 57M/26F, the mean age at diagnosis was 67 years [40-85]. The mean percentage of lymphoplasmacytic cells was 50% [8-90]. CC was systematically performed on bone marrow or peripheral blood, and FISH analysis carried out using 7 probes CEP4, CEP12, 13q14, 11q22 (ATM), 17p13 (TP53), IGH Abbott, 6q21 Q-Biogene, on metaphases and interphase nuclei. Out of 67/83 successful karyotypes, 45% showed abnormalities. There were 7 (11%) 6q deletions, 5 (8%) trisomy 4/partial trisomy 4, 3 (5%) trisomy 18, 2 (3%) trisomy 12. Using FISH deletions of 6q21 were observed in 14/50 cases (28%), 13q14 in 8/54 cases (15%), TP53 5/54 cases (9%), ATM 3/53 cases (6%). Trisomy 4 was present in 7/53 cases (13%), and trisomy 12 in 3/55 cases (5%). No rearrangement of IGH was observed in the 17 analyzed cases. The 6q deletion is the most frequent reported cytogenetic abnormality in WM. We find 28% of 6q deletion, a low percentage compared to the literature [39-54%]. This could be explained either by the difference in the probe used or we did not select for lymphoplasmacytic cells before cytogenetic analyses. Interestingly we confirmed our recent observation that trisomy 4 is recurrent in WM (15% if partial trisomy 4 is included). Furthermore we observed in this large series a frequent 13q14 deletion (15%). In conclusion, cytogenetic abnormalities in WM differ from those commonly reported in other B-cell neoplasms and confirm the originality of this disease. Indeed 6q deletion is frequent compared to CLL or marginal zone lymphoma (MZL) and 13q14 deletion is rare compared to CLL. In our series trisomy 12 is rare compared to atypical-CLL and MZL. We didn't observed cytogenetic involvement of the IGH locus, which is frequent in multiple myeloma or lymphoplasmocytic lymphoma. Finally trisomy 4 is present in WM but not reported in other B-cell malignancies. Searches for correlations with clinical and other biological parameters are ongoing.

PO-1203

RE-EVALUATION OF THE T(9;14)(P13;Q32)

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Introduction. The t(9;14)(p13;q32) which deregulates PAX5 as a consequence of its juxtaposition to the IGH locus was originally described in patients with lymphoplasmacytic lymphoma (LPL). Subsequent studies have however failed to confirm this finding. Indeed IGH translocations *per se* appear to be very rare events in LPL/WM. The t(9;14) has however been described in some patients with splenic marginal zone lymphoma, diffuse large B-cell lymphoma and post transplant lymphoproliferative disorders. In order to further clarify this we have evaluated a large series of B-cell lymphomas for the presence of the t(9;14). *Materials and Methods.* 296 cases of B-cell lymphoma were evaluated by interphase FISH for the t(9;14). Cases of follicular lymphoma and mantle cell lymphoma were specifically excluded as they contain disease defining IGH translocations. Samples were initially evaluated for IGH rearrangements using a dual colour IGH break-apart probe set (Vysis) and cases with a split signal (indicative of an IGH rearrangement) were further evaluated for rearrangements of PAX5 using another dual colour break-apart probe set (Dako). *Results*. 96 cases of diffuse large B cell lymphoma and 49 cases of extranodal marginal zone lymphoma were evaluated but none harboured the t(9;14). A further 141 cases of CD5- lymphoproliferative disorders (comprising primarily patients with WM, splenic marginal zone lymphoma and non-IgM LPL) were also evaluated and a single patient was found to have both an IGH and PAX5 rearrangements indicative of an underlying t(9;14). This patient had peripheral blood lymphocytosis, splenomegaly and bone marrow infiltration but no serum paraprotein and was considered to have splenic marginal zone lymphoma. *Conclusions*. We would conclude that the t(9;14) is a very rare abnormality in B cell lymphoma but it may be seen in a small minority of patients with splenic marginal zone lymphoma.

PO-1204

PHARMACOGENOMIC IN WALDENSTROM'S MACROGLOBULINEMIA (WM): HCNT1 EXPRESSION AS A POSSIBLE PREDICTIVE BIOMARKER OF CLINICAL RESPONSE AT 2-CDA

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Background. WD is a lymphoproliferative disorder with a median survival of 5 years. Symptomatic cases are treated with alkylating agents, nucleoside analogues and the monoclonal antibody rituximab. Despite the advances in WD therapy, some pts do not respond favorably or suffer severe adverse drug effects. Pharmacogenomic studies have shown that expression of specific transporters and receptors molecules contribute to variable drug activity. *Aim*. To identify genetic factors able to influence the clinical response to 2 CDA, using a pharmacogenomic approach. *Methods*. Using an ABI PRISM 7000 Real Time platform we amplified seven genes, encoding for equilibrative and concentrative nucleoside transporter (hENT1, hCNT1), deoxycytidine and deoxyguanosine kinase (dCK, dGK), 5'-nucleotidase (5'NT), ribonucleotide reductase catalytic and regulatory

(RR1, RR2) subunits, in the blood marrow of 21 WD pts before treatment. Pts were treated with 4 courses of 2-CDA (0.1 mg/kg sc for 5 days) in combination with Rituximab at standard schedule. Relative quantitation was performed using the Delta CT calculation: the value of gene expression was normalised to the calibrator (healthy tissue cells). *Results*. Clinical responses were evaluated according to Response Criteria (3rd International Workshops on WM) 2 months after the end of chemotherapy; in the pts who achieved a MR (5) and SD (1) the levels of hCNT1 were found to be 15 times lower (median 6,88E-03, range 1,4E-02 - 7E-05) than in the pts who achieved a PR (10) and a CR (1) (median 1,03E-01, range 4,06E-01-2,46E-02 p=0.014).The remaining four, showing very low values of expression (median 4,24E-03, range 1,68E-02- 00E0 p=0.045), failed the treatment: 3 of them for 2-CDA-related toxicity and one for PD. No correlation was found for the other genes. *Conclusions*. hCNT1 seems to be a gene involved in 2-CDA activity and its expression seems to correlate with clinical response. The lower hCNT1 expression detected in pts who didn't achieve CR or PR suggests a possible relationship between reduced hCNT1 levels and a diminished clinical activity of 2-CDA. Thus it might be important to explore the possibility of standardizing an absolute quantitative method in order to identify a threshold value which could be predictive of drug resistance.

PO-1205

TRANSGENIC MOUSE MODELS OF MACROGLOBULINEMIA WALDENSTROM

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Aims. Accurate mouse models of human plasma cell neoplasms including macroglobulinemia Waldenstom (MW) are needed to study genetic and epigenetic changes during oncogenesis and to test new intervention strategies for improved clinical outcome. Considering that the presently available pre-clinical models of MW are limited to xenografting human MW cells into SCID mice or human fetal bone engrafted in SCID mice, we sought to design new mouse strains that are prone to spontaneously arising IgM$^+$ plasma cell neoplasms that reside in the bone marrow. *Materials and Methods*. Plasma cell neoplasms arise in mice that carry a widely expressed human IL-6 transgene (H2-Ld-IL-6 developed by T. Kishimoto, Osaka University); a Bcl2 transgene (EìSV-Bcl-2-22 developed by A. Harris and J. Adams, WEHI, Melbourne); a His6-tagged mouse Myc gene inserted in three different locations of the mouse Ig heavy-chain locus (iMyc transgenes); or one of the iMyc genes plus a second, cooperating transgene: IL-6, Bcl-2, or the Bcl-XL transgenes developed by B. Van Ness and T. Behrens, University of Minnesota, respectively. Plasma cell tumors of this sort undergo immunoglobulin isotype switching and express, therefore, IgG or IgA in the great majority of cases. We hypothesized the tumorigenesis might be arrested at the MW-typical IgM$^+$ stage if the tumors arose on a genetic background in which isotype switching has been abrogated. *Results*. We crossed IL-6, iMyc, and Bcl-XL transgenic mice with mice deficient in isotype switching because they carry two null alleles of the gene encoding AID (activation induced cytidine deaminase; T. Honjo, Kyoto University). Unlike their AID-proficient counterparts that developed IgG$^+$ or IgA$^+$ plasma cell tumors, the AID-deficient offspring developed IgM$^+$ tumors that typically exhibited lymphoplasmacytoid features with a variable potential to undergo terminal plasmacytic differentiation. Bone marrow infiltration by IgM$^+$ tumor cells, splenomegaly and, in advanced cases, leukemic dissemination of tumor cells were routinely observed. *Conclusions*. Our findings define the first step toward a new mouse model of human MW. Additional studies of the present mouse strains are warranted to determine the tumor precursor (post-GC/memory B cell?) and the role of numerous biological factors known or suspected to be involved in the natural history of MW (BLyS, del 6q21-22, bone marrow environment).

PO-1206

AN ANIMAL MODEL FOR WALDENSTROM'S MACROGLOBULINEMIA

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Introduction. Waldenstrom's macroglobulinemia (WM) is a B-cell lymphoproliferative disorder characterized by predilection for bone marrow (BM) involvement and secretion of IgM paraprotein. The purpose of this study is to establish an animal model mimicking closely the disease. *Materials and Methods.* Compact cores of human cancellous bone obtained from adults undergoing total hip arthroplasty were implanted in the hindlimb muscles of NOD-SCID mice and were allowed to mature for eight to twelve weeks. Freshly obtained WM cells were transplanted employing three methods: a) i.m. injection of Ficoll-separated BM aspirate from WM patients (3×10^6 cells) close to the bone implant; b) i.v. injection of similarly obtained cells into the tail vein (1×10^6cells) and c) implantation of freshly harvested un-manipulated BM core biopsy from WM patients to the contralateral hindlimb of animals carrying bone fragment from non WM individuals. Mice were followed for up to 6 months. Tumor progression was determined by monitoring human immunoglobulin M (IgM) levels in murine plasma and by histopathologic evaluation, including immunohistology for expression of human CD20 and IgM. *Results*. All animals regardless of their treatment (a: i.m. injections, n=10; b: i.v. injections, n=6; c: bone biopsy, n=10) showed elevated levels of human IgM one month after the introduction of WM cells. However, only a small minority (10%) of mice injected i.m. (group a) maintained elevated IgM beyond 5 months. In all i.v. injected mice IgM was gradually diminishing over time. On the other hand, over a half (60%) of mice implanted with the bone marrow core biopsies showed a steadily increasing level of IgM, indicative of the development of the disease. Positive cells for both CD20 and IgM were found in the BM core biopsies from the WM patients and the human bone graft opposite to the implanted site suggesting of metastasis. Murine tissue histopathologic evaluation is ongoing. *Conclusions*. The implantation of whole BM core biopsies from WM patients enables the creation of a successful WM model, as it preserves the essential interaction of WM cells with their microenvironment. The colonization by WM cells of the uninvolved human bone graft may allow the study of factors related to its aggressiveness and adhesion to stroma.

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PO-1207

EXPRESSION OF THE KAPPA MYELOMA ANTIGEN ON THE CELL SURFACE OF BONE MARROW ASPIRATES FROM WALDENSTROM'S MACROGLOBULINEMIA PATIENTS

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In this study we assessed bone marrow isolates from Waldenstrom's macroglobulinemia (WM) patients for the cell surface expression of kappa myeloma antigen (KMA), which has previously been described in multiple myeloma. KMA is a membrane associated kappa light chain present on malignant plasma cells isolated from kappa type multiple myeloma patients and multiple myeloma (MMkappa) cell lines. A murine monoclonal antibody (mKap) that recognizes a conformation-dependent epitope on KMA has been developed in our laboratory and a chimeric version of the antibody was shown to mediate significant ADCC of MMkappa cells using human PBMCs as effectors.[1] A chimeric version of this antibody is due to enter a Phase I/II clinical trial for MMkappa patients in Australia in late 2007. Although the immunnophenotypic profile of the malignant clone in WM and MM is different both are lymphoproliferative disorders in which the neoplastic cells express monoclonal immunoglobulin light chain.[2] Demonstration of KMA on the surface of malignant cells from WM patients would provide a rationale for treatment with the chimeric antibody. Archived bone marrow aspirates from eleven confirmed WM patients were studied by flow cytometry for the presence of surface (s)KMA positive cells. Seven of the patient samples were WMkappa, and three of these contained a subpopulation of sIgM$^+$ sKMA$^+$ cells. The proportion of sIgM$^+$ cells that were sKMA$^+$ ranged from 10 to 15 percent. Interestingly, 2 out of the 3 patient samples also contained a subpopulation of sKMA$^+$ cells that were sIgM negative. None of the four WMlambda patient samples analysed contained sKMA$^+$ cells. These preliminary results demonstrate that KMA is expressed in some WMkappa patients. We therefore propose that the chimeric version of mKap should be considered for assessment as a potential therapeutic in WMkappa. Further characterisation of the phenotype of the WM KMA$^+$ subpopulation is ongoing in our laboratory. *Acknowledgements.* Biospecimens were provided by the Peter MacCallum Cancer Centre Tissue Bank, a member of the ABN-Oncology group,

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PO-1208

IMMUNOPENOTYPIC AND MOLECULAR PROFILE OF WALDENSTROM'S MACROGLOBULINEMIA (WM) AND SMALL LYMPHOCYTIC LYMPHOMA (SLL) PTS: REPORT OF A MULTICENTER STUDY

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Background. WM represents a B-cell lymphoproliferative disorder primarily characterized by bone marrow infiltration by lymphoplasmacytic lymphoma. Immunophenotypic study is of great value in the differential diagnosis of this uncommon disease and molecular studies are going to better investigate this entity. The typical immunophenotype for lymphoplasmacytic cells should include the expression of strong surface Ig and cytoplasmatic IgM and is CD19$^+$, CD20$^+$, CD22$^+$, CD79a$^+$, FMC7$^+$, CD5$^-$, CD10$^-$, CD23$^-$, CD43±. Molecular studies have shown that WM cells usually have somatic VH mutated genes. SLL is the tissue counterpart of CLL; infiltrating cell morphology and immunophenotype are the same of B-CLL. *Aim and Methods*. To evaluate the immunophenotypic (including ZAP70 and CD38) and molecular profile (IgH rearrangement by PCR) on marrow and peripheral blood of 43 newly diagnosed/pretreated pts affected by WM (28) or SLL (15) requiring a treatment enrolled in a multicenter trial evaluating a combination therapy with Rituximab and 2CdA. *Results*. Considering WM pts, we found that only 50% of them presented a typical immunophenotype profile. On the contrary, in 82% of the pts with a diagnosis of SLL the immunophenotype was according the histopatologic diagnosis. Simultaneous expression of CD5$^+$, CD23$^+$, CD43$^+$ was found only in the 7% of WM pts versus 87% of SLL pts. Considering ZAP70, CD38 and IgH rearrangement, 61% of WM pts resulted ZAP70 positive with a concordance between ZAP70$^+$ and CD38$^+$ of 62% and 46% of pts showed a monoclonal IgH rearrangement suggestive for pre germinal centre status origin. On the contrary, 86% of evaluable SLL pts were positive for ZAP70 expression, with a concordance between ZAP70$^+$ and CD38$^+$ of 55% and 73% of them presented a IgH rearrangement. When both marrow and peripheral blood were evaluated for immunophenotypic study, the concordance between bone marrow and peripheral blood immunophenotype was 55% in WM versus 100% in SLL pts. *Conclusions*. In our experience, the typical immunophenotypic panel confirmed only half of histopatologic diagnosis of WM compared to 82% of SLL diagnosis. Molecular study of IgH rearrangement does not seem to confirm the exclusive post germinal status origin for WM cells.

PO-1209

CXCR4 AND VLA-4 INTERACTION PROMOTES ADHESION OF WALDENSTROM'S MACROGLOBULINEMIA CELLS

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Background. Waldenstrom Macroglobulinemia (WM) is characterized by widespread involvement of the bone marrow (BM) and lymphadenopathy in 20% of the patients. Adhesion of malignant cells to the bone marrow microenvironment induces proliferation and resistance to therapy. Chemokines and adhesion molecules regulate the interaction of WM cells with their microenvironment. We hypothesized that the SDF-1/CXCR4 axis plays an important role on the regulation of adhesion in WM. *Methods*. The level of CXCR4 and adhesion molecules (VLA-4 and LFA-1) was determined using flow cytometry and RT-PCR in patient samples and WM cell lines (BCWM.1 and WM-WSU). Adhesion was determined using an adhesion assay coated with the VLA-4 ligand fibronectin (EMD Biosciences, San Diego, CA) in the presence or absence of SDF-1 a 10-100 nM, R&D, MN). Co-immunoprecipitation was performed with CXCR4 and VLA-4 antibodies (BD pharmingen, San Diego, CA). The CXCR4 inhibitor AMD3100 (10-100 mM, Sigma, MO), the Gi protein inhibitor pertussis toxin PTX (10-200 ng/mL, Sigma, MO), and anti-VLA4 antibody (Calbiochem, CA) were used to inhibit adhesion in WM cells and downstream signaling pathways. These studies were confirmed using CXCR4 knockdown with lentivirus infection (RNA Consortium, MA). Immunoblotting for proteins downstream of CXCR4 was performed. *Results*. WM tumor cells from patients and cell line expressed high surface expression of CXCR4 (mean 70%) and VLA-4 (mean 95%). Adhesion of WM cells to fibronectin was significantly increased compared to BSA control, and SDF-1 induced a significant increase in adhesion of WM cells to fibronectin, up to 85% increase compared to control. AMD3100 20 mM inhibited adhesion by 50% compared to SDF-1 stimulated control. Similar results were obtained with PTX 200 ng/mL and anti-VLA-4 antibody (10 ng/mL). These results were confirmed using CXCR4 knockdown in WM cells. To identify the mechanism of regulation of adhesion by CXCR4, we investigated the interaction of CXCR4 and VLA-4 receptors and demonstrated that CXCR4 and VLA-4 co-immunoprecipitated in response to SDF-1 stimulation indicating a direct interaction of these two receptors. *Conclusion*. These studies demonstrate that the CXCR4/SDF-1 axis promotes adhesion of WM tumor cells to the BM microenvironment through its interaction with the adhesion molecule VLA-4.

PO-1210

THE CXCR4/SDF-1 AXIS REGULATES MIGRATION IN WALDENSTROM'S MACROGLOBULINEMIA

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Background. Waldenstrom Macroglobulinemia (WM) is characterized by the widespread involvement of the bone marrow (BM) at diagnosis, implying a continuous (re) circulation of the WM cells in the peripheral blood and (re) entrance into the BM. The process of homing and migration is regulated by cytokines and chemokines. We sought to investigate the role of chemokine receptors, and in specific the SDF-1/CXCR4 axis on migration in WM cells. *Methods*. Flow cytometry for CXC and CC chemokine receptors (CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CCR2, CCR4, CCR5, CCR6 and CCR7) on patient samples and WM cell lines (BCWM.1 and WM-WSU) was performed. Migration towards serial concentrations of SDF-1 was determined using the transwell migration assay (Costar, NY). The CXCR4 inhibitor AMD3100 (10-100 mM, Sigma, MO), Gi protein inhibitor pertussis toxin PTX (10-200 ng/mL, Sigma, MO) were used to inhibit CXCR4 signaling. These studies were confirmed using CXCR4 knockdown with lentivirus infection (RNA Consortium, MA). Immunoblotting for proteins downstream of CXCR4 was performed. *Results*. The following chemokine receptors were expressed on patient CD19$^+$WM cells and WM cell lines: CXCR1 (mean 60%), CXCR2 (mean 47%), CXCR4 (mean 47%), CXCR5 (mean 69%), CCR4 (mean 54%) and CCR6 (mean 61%). We next determined the effect of SDF-1 on migration and signaling pathways in WM. SDF-1 (10-100nM) induced migration in a bell-shaped curve with 30nM inducing maximum migration (110% compared to control). SDF-1 30nM induced a rapid activation of signaling pathways downstream of CXCR4 including pERK1/2, pAKT, and pPKC at 1 min, with maximum activation at 5 min. The CXCR4 inhibitor AMD3100 inhibited migration of BCWM.1 in the presence of 30nM SDF-1, with AMD3100 10mM inhibiting migration at 59% of control. Similar results were observed on patients' CD19$^+$WM cells, with inhibition of migration of patients' cells at 50% compared to control. Those results were confirmed using lentivirus knockdown of CXCR4 receptor and with the use of PTX, with 30-50% inhibition of migration of WM cells compared to control. AMD3100-inhibition of migration was through inhibition of pERK1/2, pAKT and pPKC. *Conclusion*. CXCR4/SDF-1 regulates migration in WM indicating a potential role in homing.

PO-1211

COX-2 EXPRESSION IN WALDENSTROM MACROGLOBULINEMIA

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Introduction. Cyclooxygenase-2 (COX-2) is the key enzyme involved in prostaglandin synthesis and it is expressed in many solid malignancies including colon, breast and lung cancers. We and others have previously demonstrated that COX-2 is extensively expressed in multiple myeloma and as such it may have both therapeutic and prognostic value. There is however very little data regarding COX-2 expression in Waldenstrom macroglobulinemia (WM) and other lymphoproliferative disorders. *Materials and Methods.* In this study COX-2 expression was assessed by standard streptavidin-biotin peroxidase immunohistochemistry utilising a COX-2 monoclonal antibody (Clone SP21; Labvision, Fremont, USA). A known COX-2 positive colon cancer was used as a positive control but positive staining observed in endothelial cells, histiocytes and megakaryocytes served as internal controls in most cases. *Results*. 121 patients were included in this analysis. 23 were considered to have WM by consensus panel criteria while the remainder had diffuse large B-cell lymphoma (DLBL, n=19), follicular lymphoma (FL, n=20), Hodgkin lymphoma (HL, n=18), Burkitt lymphoma (BL, n=10), mantle cell lymphoma (MCL, n=10), CLL (n=12) and extranodal marginal zone lymphoma (ENMZL, n=9). COX-2 expression was demonstrable in all cases of WM but appeared to be confined to the plasma cell component. COX-2 expression was also noted in the associated mast cells. COX-2 expression was also demonstrable in the majority of patients with HL (11/18, 61%). In these cases expression was documented in >50% of the RS cells. COX-2 expression was seen in a minority of patients with other lymphomas - DLBL 2/19 (11%), FL 1/20 (5%), BL 1/10 (10%), MCL 1/10 (10%), CLL 2/12 (17%) and ENMZL 0/9. *Conclusions*. COX-2 is extensively expressed in WM but it appears to be confined to the plasma cell component of the disease. The significance of this is unclear at present but COX-2 inhibition may be of value in the treatment of WM. COX-2 is expressed in a significant proportion of patients with HL. This is intriguing and supports the emerging hypothesis that RS cells are derived from B-cells that have undergone aberrant / abortive plasma cell differentiation. COX-2 is also expressed in a minority of patients with other lymphomas.

PO-1212

SERUM SOLUBLE SYNDECAN-1 IS INCREASED IN WM/LPL AND SMZL

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Introduction. Syndecan-1 expression is restricted to the B-cell lineage including precursor B-cells, normal and malignant plasma cells. Multiple myeloma (MM) plasma cells express syndecan-1 but It is currently controversial whether syndecan-1 is expressed on malignant cells of B-cell chronic lymphocytic leukemia (B-CLL). Syndecan-1 can be shed from the surface of cells and circulate as a soluble factor (s-synd-1). Elevated serum s-synd-1 levels have been reported to be an independent adverse prognostic marker in MM. Its levels and significance has not been reported yet in Waldenstrom's macroglobulinemia (WM), lymphoplasmacytic lymphoma (LPL) and splenic marginal zone lymphoma (SMZL), a disease that, in the presence of a serum moclonal IgM component, is not easily differentiable from WM. *Aims.* To determine s-synd-1 in WM/LPL and SMZL patients at diagnosis. *Patients and Methods*. 35 patients with WM, 19 with LPL (not secreting IgM), 19 with SMZL and 26 healthy individuals (HI) were studied. S-synd-1 levels were determined by ELISA (Diaclone Research, France), in sera drawn before any treatment and kept frozen. The median follow-up of alive WM patients was 78 months (8-198), 40 months (4-157) for LPL patients and 35 months (15-233) for SMZL ones. *Results*. The median s-synd-1 levels were 39 ng/mL (9-86), 89 ng/mL (25-175), 95 ng/mL (27-400), 105 ng/mL (23-295) and 82 ng/mL (36-360) in the serum of HI, IgM-MGUS, WM, LPL and SMZL patients respectively. Differences between HI and IgM-MGUS, WM, LPL and SMZL were all statistically significant ($p<0.001$, $p=0.004$, $p<0.001$and $p<0.001$ respectively). No correlation between s-synd-1 and disease parameters such as IgM level, hemoglobin, leukemic picture, presence of splenomegaly or lymphadenopathy, increased LDH, bone marrow infiltration's extend or with survival, was found. *Conclusions*. The elevated serum s-synd-1 levels found at diagnosis reveal a role for this molecule in these diseases. Due to their usually very indolent course, a larger series and an even longer follow-up as well as serial measurements, are needed to reveal possible correlations with disease characteristics and survival, unless other protective mechanisms, absent in MM, retain in these diseases the possibility to counteract the adverse impact of elevated s-synd-1. Researches are needed.

PO-1213

DIFFERING SERUM CYTOKINE LEVELS IN LPL AND WM

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Aims. to investigate the serum concentrations of selected proinflammatory (IL-6, IL-1, TNF), proangiogenic (VEGF), growth inhibitory (TGF-beta-1) or stimulating (Blys) cytokines in the serum of WM and LPL (non IgM-secretory) patients at diagnosis. *Patients and Methods*. 35 WM and 18 LPL patients were studied. 69% of WM and 56% of LPL patients were males. The median age of WM patients was 64y (40-80) while it was 56y (42-80) in LPL. Hemoglobin level was <10 g/dL in 26% vs 5%, platelet counts were $<150\times10^9$/L in 11% vs 17%, lymphocytosis was present in 9% vs 28%, spleen was enlarged in 6% vs 12%, lymphadenopathy was present in 29% vs 67%, bone marrow was infiltrated in 100% vs 78% of patients with WM and LPL respectively. Serum cytokine levels were determined by ELISA in frozen sera drawn at diagnosis. *Results*. In WM and LPL patients, serum IL-6 ranged from undetectable to 5.8 pg/mL (median 1.2 pg/mL) and from undetectable to 4.5 pg/mL (median 0.45 pg/mL) respectively, serum IL-1 ranged from undetectable to 500 pg/mL (median 6.3 pg/mL) and from undetectable to 13 pg/mL (median 5 pg/mL) respectively, TNF was undetectable in both groups, serum VEGF ranged from 50 to 5000 pg/mL (median 450 pg/mL) and from 50 to 940 pg/mL (median 300 pg/mL) respectively, serum TGF-beta-1 ranged from 6300 to 615000 pg/mL (median 48000 pg/mL) and 7500 to 162000 pg/mL (median 55350 pg/mL) respectively, serum Blys ranged from 88 to 13200 pg/mL (median 258 pg/mL) and from 148 to 1700 pg/mL (median 600 pg/mL) respectively. Differences were statistically significant for serum VEGF and IL-6 that were higher in WM than in LPL patients ($p=0.014$ and 0.042 respectively); serum VEGF levels were found increased in both groups as compared to healthy individuals (HI) while serum IL-6 levels were found increased in WM as compared to HI but not in LPL. Serum Blys levels were higher in LPL patients as compared to both WM patients and HI ($p=0.001$ and 0.018 respectively). *Conclusions*. The differences observed in cytokine levels between WM and LPL may reflect some different biologic mechanisms of these similar if not identical disorders.

PO-1214

DISTINGUISHING IGM MYELOMA FROM WM

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Introduction. IgM paraproteins usually occur in the context of WM and other lymphoproliferative disorders and are only very rarely encountered in patients with myeloma. IgM myeloma is a very rare and poorly defined entity. Historical studies have suggested that it is characterized by clinical and laboratory features intermediate between those of WM and MM. Patients with IgM paraproteins and plasmacytic bone marrow infiltration therefore present a considerable diagnostic challenge. IgG and IgA myeloma is however readily distinguished from WM on the basis of immunophenotype and genotype as the neoplastic plasma cells are characterized by aberrant expression of CD19, CD45 and CD56, IGH translocations and deletions of chromosome 13. The latter are of course rarely if ever encountered in WM. We have therefore evaluated the immunophenotype and genotype of 10 patients considered to

have IgM myeloma. *Material and Methods*. Plasma cells were phenotyped by flow cytometry to determine the expression of CD45, CD38, CD19, CD56 and CD138. Immunohistochemistry was used to assess expression of CD20, PAX5, MUM1/IRF4, Cyclin D1, IgM, IgA, IgG, kappa, lambda. Interphase FISH was used to examine cases for IGH rearrangements and deletion of chromosome 13. *Results*. The median paraprotein concentration in this cohort was 19 g/L. The bone marrow infiltrate consisted of a monomorphic plasma cell population which was characterized by expression of CD138, CD38, MUM1/IRF4 and cytoplasmic IgM in all cases. There was no evidence of a B-cell population by morphology, flow cytometry or immunohistochemistry although one case was CD20[+]. Aberrant expression of CD19, CD56 and / or CD45 (typical of myeloma plasma cells) was demonstrable in 7/9 cases. FISH studies demonstrated evidence of IGH rearrangements in all cases examined (n=7). These comprised the t(11;14) in 4 cases and the t(4;14) in one patient. The translocation partner was not identified in the remaining patients. 3/7 patients had 13q deletions. Cyclin D1 was expressed in 6/8 cases. *Conclusions*. We would conclude IgM myeloma is characterized by phenotypic and genotypic features typically seen in standard class-switched myeloma. By utilizing plasma cell phenotyping and interphase FISH it is possible to make a clear distinction between IgM myeloma and WM.

PO-1215

REGRESSION OF LYMPHOPLASMACYTIC LYMPHOMA AFTER TREATMENT OF CHRONIC HEPATITIS C

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Introduction. An association between hepatitis C virus (HCV) infection and B-cell lymphoma, including lymphoplasmacytic lymphoma, has been suggested and debated. If HCV infection is linked to the cause of lymphoplasmacytic lymphoma, anti-viral treatment may be effective to lymphoma cells. *Materials and Methods.* We report a case of 76-year-old female with lympoplasmacytic lymphoma. Prior to the diagnosis of lymphoma, elevated plasma transaminase level was observed for more than six months. Serum anti-HCV antibodies and HCV-RNA (genotype Ib) detected by reverse transcriptase-polymerase chain reaction assay were present. IgM-kappa monoclonal gammopathy was confirmed by immunoelectrophoresis assay, but mixed (type II) cryoglobulinemia was not complicated. She was treated by several types of chemotherapy including CHOP regimen, however, complete remission was not obtained. The patient was then started on 3MU of interferon-alpha (IFN) three times a week. *Results*. Aftter 6 months of treatment, cervical lymphadenopathy was regressed. Viral load of HCV-RNA decreased after IFN treatment. *Conclusion*. HCV infecton may be linked to the pathogenesis of lymphoma in this case. HCV infection was frequently obsereved in the cases of lymphoplasmacytic lymphoma (or immunocytoma) in Italy, however, a lack of association with HCV infection and Waldenstroem's macrogloblinemia was recently reported from the United States. Further studies are needed to clarify the cause of these geographical variations.

PO-1216

WALDENSTROM'S MACROGLOBULINEMIA OF THE STOMACH

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Background. Gastrointestinal involvement in Waldenstrom's maroglobulinemia (WM) is exceedingly rare. Small-intestinal involvement in WM has been reported in less than 20 cases worldwide and the cases that are strictly limited to the stomach are less than five. *Aim*. The aim of this study is to report a case of gastric Waldenstrom's macroglobulinemia that presented with upper gastrointestinal bleeding. *Materials and Methods*(Case Report). A 58-year-old man was admitted to the hospital with mild epigastric pain and melena for the past 3 weeks prior to admission. Physical examination was unremarkable with the exception of mild splenomegaly. *Results*. A CBC disclosed a Hgb of 8.3 g/dL and the blood smear revealed the presence of plasmacytoid lymphocytes and rouleaux formation. Total protein was 6.1 g/dL with an albumin of 2.2 and globulins of 3.9 g/dL. Serum IgM was 2.87 g/dL (normal range 0.062 to 0.27 g/dL). Immunoelectrophoresis showed the presence of an IgM (kappa) monoclonal gammopathy. A Sia test was positive and serum viscosity was 1.6. Abdominal CT revealed mild splenomegaly and *thickening* of the gastric wall. A UGI endoscopy showed diffuse nodularity and ulcerations of the mucosa involving the fundus and the body of the stomach while the antrum was completely spared. The stomach was poorly distensible but there was no active bleeding or oozing at the time of the endoscopy. Histologic examination of several gastric biopsies disclosed an intense and diffuse infiltration of the lamina propria with plasmacytoid lymphocytes. Immunoperoxidase staining showed aggregates of IgM-positive cells. Giemsa stain was negative for Helicobacter pylori of biopsies obtained from both antrum and fundus of the stomach. *Conclusion*. Although gastrointestinal involvement in Waldenstrom's macroglobulinemia is rare, the entity should be included in the differential diagnosis of all nodular gastric lesions and appropriate special studies be pursued.

PO-1217

WALDENSTROM'S MACROGLOBULINEMIA (WM) IN JAPAN. RESULTS OF A RETROSPECTIVE SURVEY

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Introduction. Wm is less frequent than B-CLL and myeloma in the USA, with a 3.4 (males) and 1.7(female) age-adjusted incidence per million person-year at risk. Familial clustering and 6q21-22 deletion are found in up to 20% and 70% respectively. In Japan, Wm was described including some family cases but no survey has been done. The exact incidence, assumed to be very low like for B-CLL and characteristics remain to be reassessed. *Methods*. We sent to the 22 K-HOT institutions (Kuyshu Island, Japan) a retrospective questionnaire to record all Wm cases diagnosed from 1995 to 2004. *Results*. Fifty-five cases were diagnosed over 10 years by 11 institutions. We confirmed 48 Wm cases (2003 consensus panel) and excluded 4 marginal zone lymphoma and 4 IgM myeloma. Median age was 68(41-88) year-old and male:female ratio 33:15. Clinical presentation included B symptom (12.5%), lymphadenopathy; (23.0%), hepatomegaly (6.0%), splenomegaly (12.5%), hyperviscosity (14.5%), peripheral neuropathy (2%) and amyloidosis (2.0%). The median level of the IgM kappa (76%) or lambda (24%) was 26,45 g/L (8,8-94,4). The median neutrophils, haemoglobin and platelets were 3.07(1.02-6.99), 10.5(5.9-15.3) and 203(28-417) respectively with the presence of atypical lymphocytes in 21%. Conventional cytogenetics was abnormal in 6/30 cases revealing del(6)(q) (n=2), del(Y) (n=1), t(11;16)(q13;p11) (n=1) and t(1;12)(q21;q13) (n=1). No apparent 1st degree family history was recorded. For 44 patients, the median follow up is 27.6 months (0-174). Following a 1st line treatment given in 31 patients [fludarabine (9), alkylating (14), anthracycline (associated with rituximab in 3 cases) (7) based chemotherapy and steroids(1)] after a median of 17 weeks (1-267), the median event free survival was 9.6 months (0-112). Overall, 7 patients died, from treatment toxicity (n=1), disease progression (n=5) and secondary myeloid leukemia (n=1). *Discussion*. Wm appears infrequent in Japan. However, not every Hospital of Kyushu Island (25 millions people) participates to the study so the incidence could be at least of 0.2 per million person-year in this part of Japan. Characteristics were not peculiar, with no apparent family clustering, the occurrence of 6q deletion (underestimated by the absence of FISH) and 2 new translocations.

PO-1218

RESVERATROL EXERTS ANTIPROLIFERATIVE EFFECT AND INDUCES APOPTOSIS IN WALDENSTRÖM'S MACROGLOBULINEMIA

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Background. Resveratrol (3,4',5-trihydroxystilbene) is a polyphenolic natural product, synthesized by a wide variety of plant species including grapes. It has gained considerable attention because of its anti-cancer properties, as demonstrated in solid and haematological malignancies, including multiple myeloma. We therefore examined Resveratrol

for its anti-tumor activity in Waldenström's Macroglobulinemia (WM). *Methods*. We examined the effect of increasing concentrations of resveratrol (5-80 mM) on WM cell lines (BCWM.1), IgM secreting low-grade lymphoma cell lines (WM-WSU, MEC-1, RL), primary CD19+ WM cells and bone marrow stromal cells (BMSCs) isolated from bone marrow of patients with WM, after appropriate informed consent. [3H]-thymidine uptake and calcein-AM assay were used to evaluate the effect of resveratrol on proliferation and cytotoxicity respectively. Apoptosis and cell cycle analysis were investigated at 24h by flow cytometry using Annexin V-propidium iodide (PI) staining and PI-staining respectively. Apoptotic and cell signaling pathways targeted by resveratrol were investigated by Western Blot at 24 h and 6 h respectively. Since BMCSc confer growth and resistance to conventional treatments, we also tested the effect of resveratrol on WM cells co-cultured with BMSCs. *Results*. Resveratrol induced significant cytotoxicity and inhibition of DNA synthesis at 24 and 48 h on BCWM.1 with an IC50 of 10-20 mM. Similar data was obtained with primary CD19+ WM cells. In contrast, resveratrol did not trigger significant reduction of proliferation of peripheral blood mononuclear cells isolated from healthy donors. Importantly, it induced apoptosis in BCWM.1 and primary CD19+ WM cells, as demonstrated by flow cytometry. Dose-dependent apoptosis at 24h with induction of caspases 3, 8, 9 and PARP cleavage was also observed, including reduction of Mcl-1 and increase of p53. In parallel, resveratrol caused accumulation of BCWM.1 in sub-G1 phase. To better elucidate the mechanism of action of resveratrol in WM, we next examined downstream molecules and observed that resveratrol inhibited Akt phosphorylation in BCWM.1 cells in a dose-dependent manner. Phosphorylation of GSK3a/b, downstream target protein of Akt, was also markedly inhibited, as well pERK and pAKT. Adherence of BCWM.1 cells to BMSCs triggered increased [3H] thymidine uptake, and resveratrol inhibited this up-regulation in a dose-dependent manner. *Conclusion*. These *in vitro* data demonstrated that resveratrol has significant antitumor activity in WM, providing the framework for clinical trials in WM patients.

PO-1219

SIMVASTATIN, AN HMG-COA INHIBITOR, INDUCES *IN VITRO* ANTITUMOR ACTIVITY IN WALDENSTROM'S MACROGLOBULINEMIA

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Background. Waldenstrom's Macroglobulinemia (WM) is an incurable lymphoplasmacytic lymphoma with limited options of therapy. The presence of hypocholesterolemia has been observed among patients and recently investigated by us (Patterson *et al.*, ASH 2006). Our recent data showed an inverse relationship between the IgM serum level and LDL cholesterol serum level on 110 patients (p=0.0004). Interestingly, the patients who received statins had the lowest IgM value (p=0.004). We therefore sought to demonstrate *in vitro* efficiency of statins (Simvastatin, Lovastatin and Pravastatin) on WM tumor cells growth, survival and signaling. *Methods*. WM cell lines (BCWM.1 and WSU-WM) and IgM secreting low-grade lymphoma cell lines (MEC-1, RL) were used. Primary CD19+ malignant cells were obtained from WM patients after informed consent. Cytotoxicity was measured using the MTT survival assay, growth inhibition using thymidine uptake assay and apoptosis was assessed by flow cytometry using apo2.7. Since bone marrow stromal cells (BMSC) confer growth and resistance to conventional treatments, we also tested the effect of statins on WM cells co-cultured with BMSC. Immunoblotting for signaling pathways was performed at different doses (2.5-50 uM) of therapy at 24h. Statins induced-cytotoxicity was also addressed in combination with known agents in WM disease such as fludarabine, bortezomib, dexamethasone. *Results*. Simvastatin induced significant inhibition of proliferation (IC_{50} 2.5-25 uM) and showed cytotoxic effect (IC_{50} 5-50 uM) in WM and IgM secreting cell lines at 72h. Lovastatin gave similar results whereas pravastatin showed no efficacy. Similar effects of simvastatin were demonstrated in primary CD19+ WM cells at 5 days. Those effects were reversed by the addition of mevalonate and geranylgeranylpyrophosphate (GGP), but not of squalene or farnesylpyrophosphate (FPP), suggesting an important role of geranylgeranylated proteins in survival of WM tumor cells. Simvastatin was also capable of decreasing IgM secretion by BCWM.1 cells in a dose dependant fashion (2.5-25 uM) at 24 hrs. Interestingly, this effect was entirely abrogated with addition of mevalonate and equally partially reversed by GGP, squalene and FTP, suggesting an important role of the 3 pathways for IgM production by WM tumor cells. In addition, simvastatin overcame tumor cell growth induced by co-culture of WM cells with BMSC, that confers resistance to conventional agents. Simvastatin induced apoptosis at 24h through induction of caspases -3, -8, -9 and PARP cleavage. Simvastatin also inhibited PI3K/Akt and MAPK pathways through inhibition of Akt and ERK phosphorylation. Finally, simvastatin induced-cytotoxicity was enhanced by bortezomib or fludarabine. *Conclusion*. Our study therefore shows that simvastatin has significant antitumor activity in WM *in vitro*, providing the framework for clinical trials to improve patient outcome in WM.

PO-1220

PROTEIN KINASE INHIBITOR, ENZASTAURIN, INDUCES *IN VITRO* AND *IN VIVO* ANTITUMOR ACTIVITY IN WALDENSTROM S MACROGLOBULINEMIA

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Background. Waldenstrom s Macroglobulinemia is an incurable lymphoplasmacytic lymphoma with limited options of therapy. We have previously demonstrated upregulation of PKCb in WM using protein array techniques, and confirmed increased expression in WM using immunohistochemistry. PKCb regulate cell survival and growth, as well as migration and homing in many B-cell malignancies. We therefore hypothesized that inhibition of PKCb will induce cytotoxicity in WM. *Methods*. In this study, we examined the effect of serial dilutions of the PKCb inhibitor enzastaurin (2.5 uM to 20 uM) on WM cell lines (BCWM.1 and WM-WSU), IgM secreting low-grade lymphoma cell lines (MEC-1, RL), as well as primary CD19+ WM cells and WM cells adherent to bone marrow stromal cells (BMSCs), which induce resistance to conventional therapy. Cytotoxicity was measured by MTT assay and inhibition of cell proliferation was determined by thymidine uptake assay. Apoptosis was measured by flow cytometry using Annexin V and DAPI staining at 48 h. Cell DNA content analysis was performed using DAPI staining on fresh cells. Cell signaling pathways targeted by enzastaurin were determined using immunoblotting at 6 h (2.5 to 10 uM) and at 7.5uM (10 min to 12 h). The effect of enzastaurin *in vivo* was determined using a subcutaneous WM model in SCID mice. Enzastaurin was given by oral gavage (80 mg/kg twice daily). *Results*. Enzastaurin demonstrated time and dose-dependent inhibition of PKCb in WM cells. It induced a significant decrease of proliferation at 24 and 48 h in all cell lines tested with an IC50 of 2.5 to 10 uM, even in the presence of DOPPA, a specific PKCb stimulator. Similar effects were demonstrated in primary CD19+ WM cells, with no cytotoxicity on peripheral blood mononuclear cells indicating selective toxicity on malignant cells. Enzastaurin induced dose-dependent apoptosis at 24 and 48 h with induction of caspases 3, 8, 9 and PARP cleavage. Analysis of cell DNA content confirmed apoptosis at low doses of enzastaurin (5 uM). To further determine the mechanism of action of enzastaurin in WM, we examined downstream molecules. It significantly inhibited Akt phosphorylation and Akt kinase activity, as determined by inhibition of phosphorylation of GSKa/b fusion protein. In addition, enzastaurin inhibited p-MARCK, and p-S6R. Enzastaurin overcame resistance induced by co-culture of WM cells with bone marrow stromal cells. Furthermore, enzastaurin (2.5 to 5uM) in combination with bortezomib (2.5 to 10 nM), another active agent in WM, demonstrated strong synergistic activity using the Calcusyn software for synergy. Finally, *in vivo* animal studies demonstrated significant inhibition of WM tumor growth in the enzastaurin treated mice (n=11), compared to control mice (n=8) (p=0.028). *Conclusion*. Enzastaurin has significant antitumor activity in WM *in vitro* and *in vivo*, providing the framework for clinical trials evaluating enzastaurin as a new therapeutic agent in patients with WM.

PO-1221

PERIFOSINE, AN ORAL BIOACTIVE NOVEL AKT INHIBITOR, INDUCES *IN VITRO* AND *IN VIVO* ANTITUMOR ACTIVITY IN WALDENSTROM MACROGLOBULINEMIA

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Background. Waldenstrom s Macroglobulinemia (WM) is a low-grade

lymphoplasmacytic lymphoma with limited options of therapy. The PI3k/Akt pathway is a critical regulator of cell survival. Our previous studies using proteomic analysis have demonstrated upregulation of members of the PI3k/Akt pathway in WM. We examined whether the new Akt inhibitor perifosine (NSC 639966; Keryx, NY) induces cytotoxicity in WM. *Methods*. WM cell lines (BCWM1 and WSU-WM) and IgM secreting low-grade lymphoma cell lines (MEC1, RL) were used. Primary CD19[+] malignant cells were obtained from patients after informed consent. Inhibition of proliferation was measured using the MTT assay; DNA synthesis was measured using the thymidine uptake assay and apoptosis using Apo2.7 flow cytometry. Bone marrow stromal cells (BMSC) confer growth and resistance to conventional treatments. We therefore, tested the effect of perifosine on WM cells co-cultured with BMSC. Immunoblotting for signaling pathways was performed at different time (6 hrs to 16 hrs) and doses of therapy. *In vivo* activity of perifosine was assessed using a SCID-irradiated model with subcutaneous tumors in which perifosine was administered by oral gavage daily (35 mg/kg/day). A two-sided t-test was used to determine statistical differences. *Results*. Perifosine inhibited phosphorylation of Akt in a dose- and time- dependent fashion, as well as downstream GSK3a/b and ribosomal phospho-S6. Perifosine inhibited Akt activity as confirmed by Akt kinase assay. Perifosine induced significant cytotoxicity and inhibition of DNA synthesis with an IC50 of 5-20uM in all cell lines tested. Similar effects were observed in primary CD19[+] patient WM cells. Perifosine induced apoptosis in WM cells as demonstrated by flow cytometry. The mechanism of apoptosis induced by perifosine was through activation of SAPK/JNK pathway, followed by caspase-8, -9 and PARP cleavage. The JNK inhibitor SP600125 abrogated perifosine-induced apoptosis. The growth inhibitory effects of perifosine were significant even in the presence of BMSC, IL-6 and IGF-1, which induce resistance to conventional therapies. Importantly, perifosine did not induce cytotoxicity in healthy donor peripheral blood mononuclear cells or in hematopietic stem cells in a methylcellulose colony forming cell assay, indicating lack of toxicity on normal cells. Interestingly, MAPK members such as MEK/ERK were activated by perifosine. The MEK inhibitor U0126 significantly enhanced perifosine-induced cytotoxicity in WM cells, indicating that this combination may be synergistic *in vivo*. Finally, perifosine induced significant reduction in WM tumor growth *in vivo*, as compared to control cohort treated with vehicle only at week 6 (p=0.05). *Conclusion*. Perifosine has significant antitumor activity in WM both *in vitro* and *in vivo*. These results provide the framework for clinical evaluation of perifosine in WM.

PO-1222

THE COMBINATION OF PERIFOSINE WITH BORTEZOMIB AND RITUXIMAB PROVIDES SYNERGISTIC ANTI-TUMOR ACTIVITY IN WALDENSTROM'S MACROGLOBULINEMIA

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Background. There is a need to study combination of agents to enhance the activity of current therapeutic regimens in WM. Rituximab (R) and bortezomib (B) have demonstrated clinical activity in WM. Perifosine (P) is an oral AKT inhibitor that has showed *in vitro* and *in vivo* activity in WM. We sought to determine the effect of the combination of perifosine with bortezomib and rituximab. *Methods*. The WM cell lines (BCWM.1, WM-WSU) and IgM secreting cell lines (RL, MEC-1) were used as well as primary CD19[+] patient samples. Cytotoxicity was measured using the MTT survival assay, growth inhibition using thymidine uptake, apoptosis and cell cycle analysis using flow cytometry. Cytotoxicity of rituximab in combination with other agents was tested using ADCC assay. *Results*. Low doses of perifosine (5 mM, 48h) induce 30% cytotoxicity that was synergistically enhanced to 50% and 65% in combination with B (5 and 10 nM) respectively. B+P strongly induced apoptosis, cytotoxicity and growth inhibition in WM cell lines, IgM secreting cell lines as well as in primary CD19[+] patient WM cells and in the presence of BMSC, which induce resistance to conventional therapies. Importantly, B+P did not induce cytotoxicity in healthy donor peripheral blood mononuclear cells or in hematopoeitic stem cells in a methylcellulose colony-forming assay, indicating lack of toxicity on normal cells. The combination of B+P significantly enhanced Rituximab-induced ADCC specific lysis to 65% (40:1 ratio) (p<0.001), while single agent R as well as P+R or B+R induced less than 40% specific lysis. Interestingly, B+P induced-apoptosis was enhanced with R in presence of IL-2 treated mononuclear cells or when conjugated to an anti human IgG1 Fc antibody within the first 24 hours of treatment. B+P induced apoptosis through induction of caspase-8, -9, then -3 and PARP cleavage, as well as through decreased expression of BCL-XL and MCL-1. The combination of B+P+R induced a significant increase of caspase-9 cleavage. Perifosine induced time- and dose- dependent inhibition of Akt phosphorylation and downstream phosphorylation of GSK3a/b and S6R using immunobloting and Akt kinase assay. R strongly inhibited the Akt pathway alone and in combination with B+P. *Conclusion*. The combination of B+P+R provides strong synergistic activity by targeting different signaling pathways activated in WM. These results provide the framework for clinical evaluation of the combination B+P+R in WM.

PO-1223

PHASE II TRIAL OF PERIFOSINE (KRX-0401) IN RELAPSED AND/OR REFRACTORY WALDENSTROM'S MACROGLOBULINEMIA: PRELIMINARY RESULTS

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Introduction. We previously demonstrated that Akt is upregulated in samples of patients with WM and that the Akt inhibitor perifosine induces growth inhibition *in vitro* and *in vivo* in xenograft models in WM. This phase II study aimed to determine safety and activity of perifosine in patients with relapsed/refractory WM. *Methods*. Patients who had at least one previous therapy for WM and who had relapsed or refractory disease were eligible. Other eligibility criteria included symptomatic disease, > 2 weeks from last therapeutic agent used, >10% involvement with lymphoplasmacytic cells in the bone marrow, IgM paraprotein > 2x upper limit of normal, absolute neutrophil count >1000/mm^2 and platelet count >75,000/mm^2. NCI CTCAE v3.0 was used for toxicity assessment. Response was assessed by criteria established at the second consensus panel for WM. Serum free light chain assay was also performed to determine its role in assessing response in comparison to IgM protein measurement. All patients received 150 mg perifosine daily for 28 days per cycle for 6 cycles; response was assessed after 2 cycles. *Results*. To date, 13 patients have been enrolled. Of those, 9 were males. The median age was 64 years (range, 52-80 years). The median IgM at baseline was 2980 (range, 1110-7670); β2 microglobulin (β2M) was 2.5 (range, 1.7-4.7), with elevated B2M in 6 patients. The median follow up was 3 months (range, 1-5 months). Of the 7 patients evaluable, 1 had a minimal response (25% reduction) and 6 had stable disease. The median IgM reduction was 14% in the 7 evaluable patients (0-25%). One patient whose IgM rose in the first month had a 50% reduction from the peak of IgM level at 3 months, indicating a delayed response. Patients tolerated perifosine well without significant toxicities: grade 3 nausea/vomiting in 1 patient resolved with dose reduction to 100 mg; grade 3 anemia in one patient was related to disease progression; and a single patient had grade 3 hemmorhoidal bleeding unrelated to therapy. *Conclusions*. Oral Perifosine is well tolerated and demonstrates encouraging activity in patients with relapsed WM. Updated data will be presented.

PO-1224

CLADRIBINE (2-CDA) AND RITUXIMAB COMBINATION TREATMENT FOR PATIENTS WITH WALDENSTROM'S MACROGLOBULINEMIA (WM) OR SMALL LYMPHOCYTIC LYMPHOMA (SLL): CLINICAL PRELIMINARY REPORTS OF A MULTICENTER STUDY

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Background. There is evidence that combinations of nucleoside analogues and rituximab are effective for treatment of WM. *Aim*. To test the efficacy of 2-CDA in combination with Rituximab in the treatment of newly diagnosed/pretreated WM or SLL pts requiring systemic treatment. *Methods*. The combination therapy consisted of Rituximab at standard schedule (375 mg/mq) on day 1 followed by 2-CDA 0.1 mg/kg (sc injection) for 5 consecutive days. Each cycle was administered monthly for 4 times. From December 2003, 43 pts (28 with WM and 15 with

SLL) have been enrolled in this multicenter trial. Pts characteristics include: sex (M/F) 27/16, median age 63 (range 27-79 yrs), 27 newly diagnosed (16 WM, 11 SLL). Relatively to WM pts, median IgM level at the time of treatment was 2567mg/dL; anemia (10pts), neurologic symptoms (6pts) and symptomatic crioglobulinemia (4pts) represented the main reasons to start the treatment. *Results*. With the exception of three pts (all WM pts) who dropped out from the study due to cardiac toxicity occurring after the first Rituximab infusion, the therapy was well tolerated even if flu-like syndrome was more frequent (30%) and severe (25%) in the WM pts than in those affected by SLL (17% and 8% respectively). Treatment delays occurred only in five patients because of haematologic toxicity (two pts due to G3 neutropenia) or extraematologic complications. No major infections were observed. With a median follow-up of 12 months, 34 pts (21 WM and 13 SLL pts) are evaluable for response. Pts with WM achieved CR/PR in 11 cases (30% of them presenting a molecular clearance in blood marrow), MR in 6 cases, SD in 1 case and PD/NR in 3 cases. Nine pts with SLL obtained CR, 3 pts PR and the remaining a PD. *Conclusions*. On the basis on the preliminary results above described, the combination of 2-CDA and Rituximab seems to be safe and active in WM and SLL pts requiring a treatment. The ongoing pharmacogenomic analysis associated with the study, might contribute to allow a prediction of the clinical response to such combination treatment.

PO-1225

FLUDARABINE, CYCLOPHOSPHAMIDE AND RITUXIMAB AN EFFECTIVE REGIMEN CHARACTERIZED BY HIGH INCIDENCE OF DELAYED RESPONSES IN WALDENSTROM'S MACROGLOBULINEMIA

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Background. Rituximab is an active and well tolerated agent in the treatment of Waldenstrom's Macroglobulinemia (WM) whether used in untreated or in refractory/relapsed patients. The treatment with anti-CD20 monoclonal antibody is associated with the risk of transient exacerbation of clinical effects of the disease related to the *flare* of IgM paraprotein after its administration. High response rates, ranging from 55% to 89%, have been reported with the Fludarabine plus Cyclophosphamide combination even in pretreated patients. Fludarabine and Cyclophosphamide are synergistic with Rituximab *in vitro* in lymphoma cell lines, and the administration of the three drugs is associated with higher response rates in chronic lymphocytic leukemia and other lymphoprolipherative disorders. Based on these data we conducted this study to define the efficacy and tolerability of Fludarabine, Cyclophosphamide and Rituximab (FCR) in symptomatic patients with WM. *Patients characteristics and Methods*. Fifteen patients received the FCR regimen. The median age was 57 years (range 31-76), sex ratio M/F 13/2, and the median time from diagnosis of WM to chemo-immunotherapy treatment of 46 months (range 1-156). Three patients received FCR as first line treatment while 12 patients had been previously treated, with a median of 2 lines of therapy (range 1-6), five of them having received previous treatment with anti CD20 and anti CD22. At the time of FCR treatment 50% of patients had refractory disease. Splenomegaly and lymphadenopathy were present in 20% and 33% of patients respectively. In 50% of cases hemoglobin was <10 g/dL, serum albumin <3,5 g/dL in 36%, and serum β2 microglobulin >3 mg/dL in 50%. Median serum monoclonal protein was 2.52 g/dL (range 6.47-0.8) and median IgM level was 3615 mg/dL (range 277-10900), being > 4000 mg/dL in the 53% of patients. FCR regimen was administered every 4 weeks and consisted of: Rituximab 375 mg/sqm iv day 1, Fludarabine 25 mg/sqm iv day 1-3, Cyclophosphamide 250 mg/sqm iv day 1-3. Anti-infective prophylaxis was based on cotrimoxazole 800 mg, 3 times per week and acyclovir 800 mg per day. Response criteria were defined according to the Second International Workshop on WM, updated in 2006. Responses were evaluated one month after the end of FCR last course. To assess delayed responses further bone marrow evaluations were performed in case of progressive reduction of monoclonal protein. *Results*. Fourteen patients are evaluable for response as in one patient treatment is still ongoing. Seventy-five FCR courses have been administered, with a median of 6 (range 3-6). Overall 86% of responses (12 pts) were observed, being all categorized as partial remissions. Of note, 7 of the 12 responding patients showed a complete resolution of symptoms, adenopathy/organomegaly on computed tomography and malignant cells were not detectable at bone marrow immunophenotype and hystologic evaluation, but a persistence of a positive serum immunofixation was detected with a median serum monoclonal protein of 0.3 g/dL. Of the 2 non responding patients, one showed a stable disease after 6 courses of treatment while in the other a progressive disease was documented during treatment. Toxicity: Mild or moderate effects related to Rituximab infusion such as fever, hypotension and cutaneous rash were seen in 4 (28%) patients and were limited to the first and second infusion. None of the patients developed IgM *flare*. Hematologic toxicity was the main source of adverse effects, grade III-IV neutropenia was observed in 66% of courses administered. Three responding patients showed a grade III prolonged neutropenia after the 6th course lasting 2, 3 and 4 months, respectively. Two episodes of FUO were recorded, and one clinically documented pneumonitis. With a median follow-up of 8 months (range 1-36) of the 12 responding patients 11 are alive and progression free, one patient died two months after the end of treatment while in PR for disseminated Aspegillosis. Two patients received an autologous peripheral blood stem cell transplant. As regards delayed responses, one patient in PR converted to CR after 4 months from the end of treatment and underwent autologous peripheral blood stem cell transplant while in CR. In 6 cases a progressive reduction of monoclonal component has been detected and is still documented even in the patient with 27 months of follow-up. *Conclusion*. FCR regimen is active and well tolerated in symptomatic WM even in previously heavily pre-treated patients. The high incidence and long lasting episodes of neutropenia did not translate in major infectious episodes. Progressive delayed responses have been observed even in patients with longer follow-up.

PO-1226

FLUDARABINE PLUS CYCLOPHOSPHAMIDE AND RITUXIMAB (RFC) IN WALDENSTROM'S MACROGLOBULINEMIA (WM): RESULTS IN 25 PATIENTS

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Treatment of Waldenstrom's Macroglobulinemia relies on alkylator agents, nucleoside analogs and/or monoclonal antibody based therapies. We showed previously that combination of fludarabine and cyclophosphamide yields a 78% response rate (RR). We performed a retrospective study in 25 WM patients (pts) treated with RFC regimen in 7 French centers. The median age was 61 years (range: 40-77 years), the median IgM level measured by electrophoresis was 27.3 g/L (range: 8,7-55,5 g/L), the median haemoglobin level was 9,3 g/dL (range: 6-13,5 g/dL), the median platelet count was 144×10^9/L (range :$30\text{-}500\times10^9$/L), the median β2 microglobulin level was 3,4 mg/L (range: 1,8-8.3 mg/L). In all, 23/25 pts had previously been treated with a median of 2 lines of therapy (range: 1-6), including 3 autologous stem cell transplantation (ASCT). 17 patients had relapsed disease and 9 patients had refractory disease. RFC regimen was given every 4 weeks and consisted in: Rituximab 375 mg/m^2 IV Day 1, Fludarabine 40 mg/m^2 per os Day 1 to Day 3, Cyclophosphamide 250 mg/m^2 per os Day 1 to Day 3. Response was assessed 3 months after the last RFC cycle according to response criteria agreed by the 3rd International Workshop on WM (Kimby, E, 2006). 25 pts received the first cycle of RFC, and 22 received two or more cycles (median of 4 cycles, range 2-6). Main toxicity was hematological, including 12 grade III-IV neutropenia, 6 grade III-IV thrombocytopenia and 2 grade III-IV anemia. Response was evaluated in 20 pts (2 ongoing and 3 early discontinuation treatments), including 16 partial responses (PR), 2 minor responses and 2 stable diseases. Of note 7/20 very good PR were observed (> 90% decrease in M-protein). Overall RR was 90%, with a median duration of 8 months. With a median follow-up of 14 months (range: 3-36 months), 25 pts are alive. 2 ASCT and 3 Allogeneic SCT were performed. 3 pts relapsed (8, 10 and 13 months). RFC association in heavily treated patients with poor prognostic factors gives a very high response rate with acceptable toxicity and could be offered to relapsed/refractory patients.

PO-1227

2-CDA-CYCLOPHOSPHAMIDE ± RITUXIMAB FOR SYMPTOMATIC WM

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Introduction. Long term outcomes of patients with symptomatic Waldenstrom's macroglobulinemia (WM) treated with nucleoside analog based therapy have not been defined clearly. *Methods*. We retrospectively updated our experience with 47 previously untreated patients with symptomatic WM, treated between 7/96-5/02 with 2 consecutive 6-week courses of 2-chlorodeoxyadenosine (2-CdA) 1.5 mg//m^2 sc tid x 7d + cyclophosphamide (Cy) 40 mg/m^2 po bid x 7d (29 patients) alone or with rituximab (Rit) 375 mg/m^2 iv q wk x 4 wk (18 patients). Responding patients were followed without further treatment until relapse, defined by a 25% increase in M-protein from nadir. *Results*. With 2-CdA-Cy, overall response rate (ORR) was 83%, including complete response (CR) in 4% (1 patient). With 2-CdA-Cy-Rit, ORR was comparable at 94% (p=0.24), including CR in 17% (3 patients) (p=0.11). Median times to remission were similar at 2.3 (2-CdA-Cy) and 2.4 months (2-CdA-Cy-Rit), yet there was a trend towards longer duration of first remission (DOR) for patients treated with 2-CdA-Cy-Rit (58.6mos vs. 25.6mos) (p=0.20). Since many patients with WM are asymptomatic and do not require treatment at first sign of relapse, we also evaluated time to re-treatment (TTR). Median TTR (56.3 months) was appreciably longer than DOR for patients treated with 2-CdA-Cy, and has not been reached in those treated with 2-CdA-Cy-Rit (p=0.02). Among 12 patients who required re-treatment after 2-CdA-Cy, median duration of second remission was 41.6 months. The regimen administered to 7 of these patients was 2-CdA-Cy-Rit, which may explain the longer DOR noted at re-treatment. With a median follow-up of 68.5 months, only 1 patient initially treated with 2-CdA-Cy-Rit has required re-treatment. Median overall survival has not been reached for either regimen (p=0.21). Three of 3 deaths on 2-CdA-Cy-Rit, and 3/14 on 2-CdA-Cy, have been unrelated to WM. *Conclusion*. After only 2 courses of treatment with 2-CdA-Cy combinations, we have observed high response rates with little toxicity, long remission durations, longer time to need for re-treatment, and prolonged survival. When re-treatment was necessary, second remissions were long, further supporting the use of limited 2-CdA-Cy based regimens as the treatment of choice for previously untreated patients with symptomatic WM.

PO-1228

AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) FOR PATIENTS WITH WALDENSTROM'S MACROGLOBULINAEMIA. AN ANALYSIS OF 201 CASES FROM THE EUROPEAN BONE MARROW TRANSPLANT REGISTRY (EBMT)

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Lymphoma WP of the EBMT

Introduction. Waldenstrom's macroglobulinaemia (WM) is a relatively rare lymphoma which primarily affects elderly patients. Standard doses of alkylating agents, purine analogues and anti-CD20 monoclonal antibody effect response rates of up to 60%. However, complete response is infrequent and there is no cure. Due to the mostly indolent nature of the disease and the older age of patients larger series on the role of high dose therapy and ASCT have not been published. *Patients and Methods*. We report a retrospective multicentre study of 201 WM patients (132M/69F), who underwent an ASCT between January/1992 and December/2005. The median age at transplant was 53 years (22-73) and the median time from diagnosis to transplant was 18 months (3-239). The patients had already received a median number of 2 (1-10) lines of therapy before ASCT. At transplantation, 40 patients (20%) were in CR1, 24 (12%) in ≥CR2, 83 (41%) in PR1, 27 (13%) in ≥PR2, 27(14%) had relapsed or refractory disease. Conditioning regimens used were BEAM in 44% of the cases, cyclofosphamide or melphalan/TBI in 28%, high-dose melphalan in 14% and other protocols in 14%. In 88% of the patients peripheral blood was used as the source of stem cells. Results All patients but three successfully engrafted. With a median follow-up of 26 months (5-163), 112 (56%) patients are alive and free of disease, 73 (36%) patients have relapsed after a median of 14 months (1-110) post ASCT. Fifty-two patients died, 36 (18%) from disease progression and 16 (8%) from regimen toxicity. Non-relapse mortality was 6% at 1 year. The actuarial OS was 86% at 1 year, 75% at 3 years, and 61% at 5 years. The probability of relapse was 20% at 1 year, 38% at 3 years and 55% at 5 years with an estimated PFS of 74%, 54% and 33% at 1, 3, and 5 years, respectively. *Conclusion*. This study suggests that ASCT is a safe procedure and that can achieve prolonged remissions in this population of heavily pre-treated patients.

PO-1229

HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) IN WALDENSTROM MACROGLOBULINEMIA (WM), UPDATE OF THE FRENCH EXPERIENCE IN 54 CASES

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Introduction. HSCT has been developed in few Wm cases and is nowadays challenged by other innovative approaches. However, high dose therapy followed by autologous HSCT (HD-auto) produces high response rate and some long term responses while allogeneic HSCT performed after either myeloablative (MA-allo) or reduced intensity conditioning (RIC-allo) regimens may be cure of Wm (Dreger 1998, Tournilhac 2003, Maloney 2006). *Methods*. We updated and extended our retrospective experience on 32 HD-auto, 11 MA-allo and 11-RIC-allo performed from 1990 to 2006 in 51 patients from 18 institutions. A MA-allo and a RIC-allo were performed in 1 and 2 cases respectively following relapse after a 1st HD-auto. *Results*. Data are presented in the Table and Figure.

Table.

	HD-auto	*MA-allo*	*RIC-allo*
Nb	32	11	11
Age at HSCT (y)	56 (34-68)	46 (24-57)	56 (36-64)
Interval diagnostic - HSCT (m)	38 (7-286)	50 (9-77)	74 (17-149)
Status at T : ≥ 3 lines	50%	64%	81%
chemoresistant (Stable or PD)	25%	36%	55%
Conditioning regimens	BEAM (13); other (3) TBI/melphalan (9), TBI/endoxan (7); (3)	TBI/endoxan (9), TBI/melphalan (2)	TBI/Fludarabine (10) other (1)
HLA mached allogenic donor	/	Sibling (9), Unrelated (2)	Sibling (8), Unrelated (2), Cord blood (1)
Median follow up (m)	45 (3-121)	68 (3-132)	22 (2-60)
Relapse	18 (56%)	4 (36%)	0
Death	14 (44%)	5 (45%)	3 (27%)
WM progression;Toxicity	10 ; 4	1 ; 4	0 ; 3
Median event free survival (m)	32 (2-119)	36 (3-132)	Not reached
Overall survival (1; 3; 5 y)	87%; 77%; 58%	64%; 54%; 54%	82%; 68%; 68%
Transplant related mortality	12.5% (only 2[nd] cancer)	36%	27% (one 2nd cancer)

Acute GVHD developed following 9 MA-allo [Grade I-II (n=8), Grade III-IV (n=1)] and 8 RIC-allo [Grade I-II (n=7), Grade III-IV (n=1)]. Chronic GVHD developed following 7 MA-allo [limited (n=5), extensive (n=2)] and 5 RIC-allo [limited (n=2), extensive (n=3)]. Transplant related mortality included GVHD, sepsis or 2[nd] cancer. *Conclusion*. We confirm that autologous HSCT achieves some long term responses even in heavily pretreated patients. Allogeneic HSCT induces very long term disease control and may cure WM. Specially, the RIC-allo gives impressive results on disease control in a set of older patients, mostly heavily pretreated with refractory disease, nevertheless the toxic mortality and the incidence of chronic GVH disease and its impact on quality of life has to be mentioned.

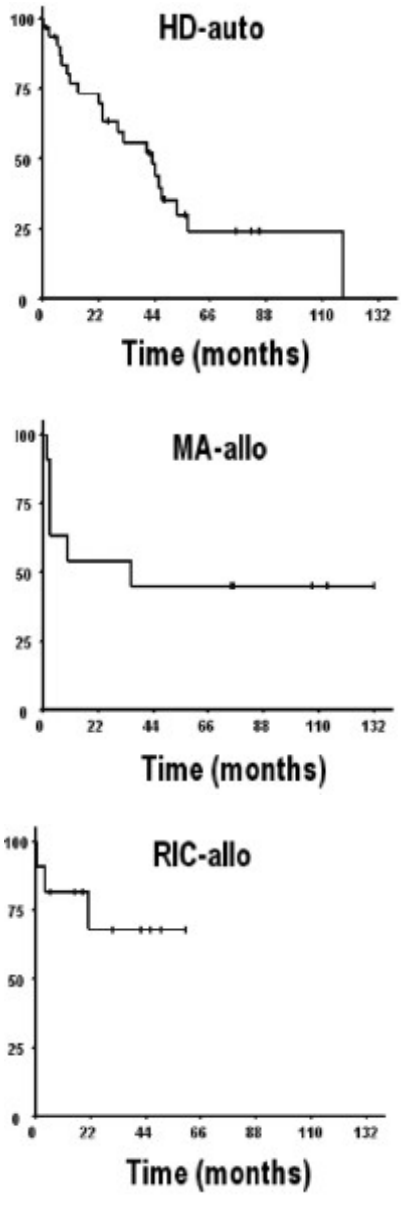

Figure. Event Free Survival (%).

PO-1230

SERUM FREE LIGHT CHAIN IS A MARKER OF TUMOR BURDEN AND OF PROGNOSTIC IMPACT IN WALDENSTROM'S MACROGLOBULINEMIA

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Background. We sought to determine the value of serum free light chain (sFLC) in WM. *Methods*. We analyzed 170 samples, of which 101 were WM and 69 IgM-MGUS. sFLC levels were performed using Freelite reagents on a Dade-Behring Nephelometer. *Results*. The mean (±se) sFLC was significantly higher in WM as compared to IgM-MGUS, with 109.8 mg/L (±16) in WM and 25.8 mg/L (±2) in IgM-MGUS, $p<0.0001$. In addition, sFLC correlated with the serum IgM level (r=0.27; $p=0.008$) and serum viscosity (r=0.35, $p=0.008$), but not with the bone marrow involvement. Mean (±se) sFLC measurement also separated WM patients with asymptomatic versus symptomatic disease ($p<0.001$). Elevated sFLC correlated with poor prognostic markers in WM, such as high serum B2M, anemia, thrombocytopenia and leucopenia. We next defined a threshold of sFLC at 50 mg/L based on its ability to correctly classify MGUS and WM with over 90% specificity. This cut off was able to correctly classify patients with low β2M (≤3 mg/L) and high B2M (>3 mg/L), ($p<0.001$). A prognostic model were then developed based on B2M, anemia (hemoglobin <10 g/dL) and thrombocytopenia (platelet count $<120\times10^9$/L). A point was given for elevated B2M of >3 mg/L, anemia, or thrombocytopenia, with score of 3 indicating elevated B2M in combination with anemia and thrombocytopenia. Elevated sFLC (≥50 mg/L) was able to correctly classify patients with a score of 2 points or more versus 1 point in this model ($p<0.005$). Although the follow-up is short in our sequential study of sFLC measurement, it appears that a decrease in half the initial sFLC values on 2 different measurements correlated with sustained response for patients undergoing therapy. Sequential sFLC measurements also correlated with the adverse prognosis impact on the progression free survival. Patients with increase values on at least 2 sFLC measurements had shorter progression free survival. *Conclusion*. sFLC clearly differentiated patients with WM and IgM MGUS. In patients with WM, sFLC significantly correlated with poor prognostic markers. Future studies are needed to validate the role of sFLC as a prognostic marker of survival in WM.

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