was significantly poorer as compared to the prevoius one in all the cases (3.36 x 106CD34+ cells/kg *vs* 8.3 x 106 CD34+ cells/kg, p<0.001). PBSC yield was not influenced by number of transplants, response to therapy and treatment with Interferon. Twenty-two patients were submitted to ASCT; hematopoietic recovery was 11 days for PMN > 500/mmc and 13 days for platelets > 20000/mmc. Grade III-IV non hematological toxicity of the whole transplant program was observed in 3/22 (13%) cases. Median progression-free survival was 27 months. *Conclusions*. Although these data should be confirmed in a larger prospective study, a second PBSC mobilization followed by ASCT seems to be is feasible in patients with MM who have previously received a transplant procedure.

PO-107

DISTINCT GENE AND MIRNA EXPRESSION PROFILES IN SEQUENTIAL MYELOMA SAMPLES AT DIAGNOSIS AND RELAPSE

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Introduction. Multiple myeloma (MM) is characterized by a highly heterogeneous clinical course, which may be only partially explained by the presence of major genetic alterations. High-resolution technologies are improving the knowledge of the molecular mechanisms underlying MM disease evolution. To provide useful information concerning the molecular alterations associated with tumor progression in MM, we generated global gene and miRNA expression profiles in sequential samples of MM patients, evaluated at diagnosis and relapse. *Methods.* Global gene expression profiles of highly purified plasma cells (PCs) from 17 symptomatic MM and 2 primary PC leukemia (PCL) patients were generated at diagnosis and relapse after first line therapy, by means of Affymetrix Gene 1.0 ST array. Twelve (11 MM, 1 PCL) paired samples for which material was available were also profiled on Affymetrix miRNA 3.0 array. Unsupervised and paired supervised analyses were carried out by dChip and SAM software, respectively. Functional annotation studies were performed using DAVID 6.7 and miRBase tools. Main genomic aberrations were investigated by FISH analysis. *Results.* Concerning major genomic aberrations, acquisition of del(13) and del(17) were respectively observed in one and two relapsed MMs of 15 analyzed cases; 1q gain aberration was acquired in 2 MMs and 1 PCL, among 11 tested patients. In particular, 1q gain and del(17) were both acquired in one relapsed MM. Main IgH chromosomal translocations found at onset were confirmed at relapse. No IgH translocation was acquired in any patient at relapse. Paired supervised analysis identified 171 differentially expressed genes, all but one up-regulated in baseline MM/PCL compared to relapsed samples. In particular, a stronger overexpression pattern at diagnosis was observed in seven patients: the two PCL cases carrying the t(11;14) and t(14;16) respectively, the two MAF-translocated MMs and 3 MMs without major IgH translocations, two of which acquired del(17) at relapse. Deregulated transcripts were mainly specific of genes involved in RNA processing, including spliceosome components, and comprising also proteasome genes. Furthermore, we evidenced the differential expression of ATG5 and M6PR, implicated in drug response mechanisms involving autophagy process, and CRBN, whose downregulation has been linked to drug resistance in MM. Furthermore, five miRNAs were found positively modulated in relapse compared to diagnosis phase; among them, miR-25, a negative regulator of TP53, was found to be associated with high-risk MM and miR-4428 is a novel candidate miRNA in human B cell malignancies. *Conclusions.* Microarray technologies may be useful in identifying genes and miRNAs specifically deregulated in MM progression. These data may contribute to our understanding of mechanisms involved in drug resistance and clonal evolution in MM, and to improve the risk-stratification of patients.

PO-108

NOVEL THERAPEUTIC TARGETS IN MULTIPLE MYELOMA: PROTEIN KINASE CSNK1A DEPENDENT SIGNALING NETWORK

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Introduction. Multiple myeloma (MM) is the second most frequent hematologic malignancy, accounting for about 10% of all blood tumors. Dwelling of MM in the bone marrow microenvironment favours uncontrolled growth, genetic clonal selection and inherent drug resistance. MM cells and stromal cells also produce osteoclast-promoting cytokines as well as osteoblast inhibitory factors, such as potent antagonist of the Wnt pathway, which, in turn, is essential for osteoblastogenesis. Clinically, MM patients face the repeated relapses as well as the devastating bone disease. Novel therapeutic targets are thus urgently needed. Casein Kinase 1α (CK1α) is a serine/threonine kinase essential for the function of signaling pathways that are involved in MM pathobiology: the canonical Wingless (Wnt)/β-catenin cascade; the Hedgehog (Hh) pathway; the NF-κB pathway; p53-driven response, here by stimulating the binding to MDM2 and p53 inhibition. However, despite these notions could suggest a role of CK1α in growth, survival and proliferation of malignant plasma cells, and tumor-microenvironment interactions/osteoblastogeneis, its function in MM and related bone disease has, to the best of our knowledge, never been investigated. *Methods.* CK1α expression and activity has been analyzed in MM cells and controls; the consequences on MM cell survival and proliferation and osteoblastogenesis of CK1α inhibition with small ATP-competitive compounds or by RNA interference has been investigated. CK1α-dependent signaling events have been analyzed by different methods. *Results.* We analyzed a number of data sets in available repositories and found that CK1α is significantly over expressed across the evolution from normal to highly malignant plasma cells in most of the human datasets as well as in a mouse myeloma model. CSNK1α mRNA and CK1α protein were found highly expressed expression in purified CD138+ malignant plasma cells from MM patients. Treating MM cells with the CK1α inhibitor D4476 or RNA interference caused a variable amount of apoptosis and growth arrest, even in co-cultures with protective BM stromal cells, which were instread spared by the cytotoxicity. Surprisingly, the combined treatment with bortezomib caused a much stronger cytotoxic effect on MM cells than the single treatments. Analysis of signaling networks revealed a strong inhibition of β-catenin phosphorylation and Akt activation as well profound perturbations of the ER stress-unfolded protein response pathways, especially of the IRE1α and PERK-dependent branches. *Conclusions.* The data we have collected so far allow concluding that CK1α could be a growth-propelling kinase in MM. The identification of the exact mechanism of action and of the pathways targeted by CK1α in MM plasma cells is the subject of future research.

PO-109

NOTCH TARGETING PREVENTS MULTIPLE MYELOMA ASSOCIATED OSTEOCLASTOGENESIS

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Introduction. Multiple myeloma is an incurable hematological tumor stemming from malignant plasma cells. MM cells accumulate in the bone marrow (BM) and establish complex interactions with normal BM stroma, which promotes tumor survival, bone disease and drug resistance. Recent evidence indicate that Notch pathway is deregulated in MM and plays a role in the pathogenesis of this tumor by modulating tumor cell biology and the crosstalk between MM cells and the BM niche. Activation of the Notch signaling is mainly due to the the aberrant expression of two of the Notch ligands, Jag1 and Jag2, that causes an increase in the ability of MM cells to trigger Notch activation in neighboring tumor cells and cells of the BM niche. In this work, we

investigated the contribution of Jag1/2 to the osteoclastogenic potential of MM. *Methods*. The γ-secretase inhibitor DAPT was used at a final concentration of 50μM. Osteoclasts (OCL) differentiation of Raw264.7 cells was induced by treatment with 50ng/ml mRANKL or co-culturing with MM cells or their conditioned medium. After 5-7days cells were stained using the TRAP Kit and counted. For bone resorption assay, Raw264.7 cells were cultured on Osteo Assay Surface plates under differentiation conditions for 7-10 days. Images of the resorbed areas were captured and the % of resorbed area was measured using the Wimasis image analysis software. Select RNAiTM siRNA system (Invitrogen) was used according to the manufacturer instructions for the selective inhibition of Jag1 and Jag2. Transfection was performed by electroporation using two plasmids coding the intracellular Notch1 and Notch2.Total RNA was isolated using TRI-Reagent. cDNA was prepared through MMLV-RT, then quantitative PCR was performed by Maxima SYBR Green qPCR Master Mix. ELISA Assay was performed using biotin-conjugated goat anti-human RANKL (Merck-Millipore) and Streptavidin-HRP-labeled secondary antibody. Flow citometry was performed using an anti-human RANKL antibody (Abcam) and a Alexa488-conjugated secondary antibody (Life Technologies). Jag1 recombinant peptide was used at 0.5μg/ml. anti-RANKL neutralizing antibody was used at 0.1μg/ml. *Results*. Our results indicate that Notch2 signaling is essential for OCL differentiation and activity. Furthermore, this work supports the hypothesis that Jag ligands expressed by MM cells promote OCLs differentiation in two different ways: through the release of RANKL or by direct contact with OCL precursors. Jag ligands are able to mediate the interactions of MM cells with the BM niche which further promotes MM cell osteoclastogenic ability. Indeed, Jag1/2 silencing inhibits MM cell ability to interact with BM stromal cells and to induce osteoclastogenesis. *Conclusions*. Our work demonstrates that Notch signaling drives MM-induced osteoclastogenesis and osteolysis and suggest that Jag1/2 might present new promising therapeutic targets to reduce MM-associated bone disease.

PO-110

RELATIONSHIP BETWEEN CIRCULATING PLASMA CELLS AND CYTOGENETIC RISK IN PATIENTS WITH MULTIPLE MYELOMA

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Multiple Myeloma (MM) is a clonal B-cell disorder characterized by accumulation of malignant plasma cells (PC) in the bone marrow (BM). Circulating plasma cells (PCs) can be detected in the peripheral blood of a significant proportion of patients with MM and their presence is a well-known prognostic factor. Indeed, the appearance of circulating PCs in the blood could indicate relative indipendence from adhesion to the microenvironment and, therefore, signifies more aggressive disease. In this study, we examined the relationship between the number of PCs and citogenetic risk in patients with newly diagnosed MM. We analyzed peripheral blood from patients with Monoclonal Gammopathy of Undetermined Significance (MGUS; n=15), Smoldering Myeloma (SM; n=28), Solitary Plasmacytomas (SP; n=3) and active MM (n=105). These patients were followed by the UOC Ematologia at the "Mazzoni" Hospital from January 2006 to December 2013, with a median follow-up of 25 months. We analyzed clinical, laboratory and cytogenetic data of patients with active MM. However, cytogenetic analysis was not evaluable for 15 patients. The number of PCs was detected by flow cytometry using a simple two-colour approach. Cells were stained with fluorescence-labeled CD38 and CD45 antibodies and 50,000 events were acquired and analyzed for each patient. PCs were identified by gating on CD38bright+/CD45- cells. Using a receiver operating characteristics (ROC) analysis, we assessed that ≥41circulating PCs is the optimal cut-off for defining poor prognosis. The 8-years probability of Overall Survival (OS) and Progression-Free Survival (PFS) in patients with <41 and ≥41circulating PCs, was 32% *vs* 8% (p=0.017) and 29% *vs* 0% (p=0.0008), respectively. Patients with high-risk cytogenetics (n=24) had poor prognosis, independently of circulating PCs (PC<41 *vs* PC≥41: OS=0% *vs* OS=16%, p=n.s.; PFS=0% *vs* 17%, p=n.s.). Patients with standard-risk cytogenetics (n=66) showed a better prognosis associated to a lower number of circulating PCs (PC<41 *vs* PC≥41: OS=36% *vs* 10%, p=0.026; PFS=37% *vs* 0%, p=0.0001). These data were confirmed by multivariate analysis (Cox model) for the subgroup with standard-risk cytogenetics, in which the presence of ≥41 circulating PCs, older age, DS stage >I and lack of maintenance therapy, adversely affected OS and PFS. All patients with SP showed no circulating PCs. In all cases of MGUS or SM, circulating PCs, when detected, were <20. In summary, our results suggest that the quantification of circulating PCs by flow cytometry could provide useful prognostic information for newly diagnosed MM patients with standard-risk cytogenetics.

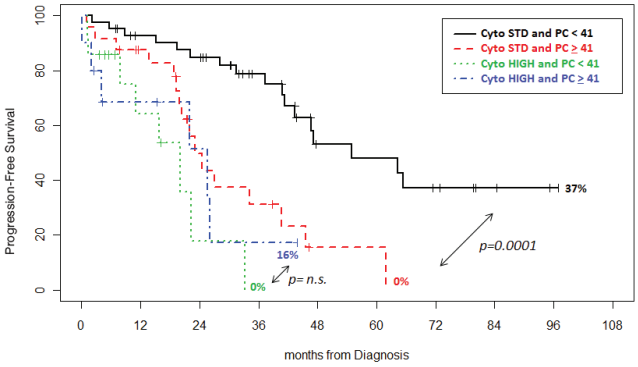

Figure 1.

PO-111

IN VITRO IMPAIRMENT OF EZH2 IN HUMAN MULTIPLE MYELOMA CELL LINES CAUSES A DECREASE IN CELL PROLIFERATION

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Introduction. EZH2 (Enhancer of Zeste 2) is the SET containing catalytic subunit of the Polycomb Repressive Complex 2 (PRC2), which is comprised of SUZ12, EED, RbAp48 and JARID2 among other interacting proteins that function to repress transcription through H3K27 trimethylation. EZH2 is over-expressed in solid tumors and is associated with poor prognosis and metastatic progression. EZH2 knockdown, blocks proliferation, cell invasion, tumor growth and metastasis, whereas its over-expression produces an oncogenic phenotype. In prostate cancer, the oncogenic function of EZH2 requires the activity of MMSET (Multiple myeloma Set Domain) protein (Asangani IA. *et al.*, Molecular Cell 2013). Our previous studies indicated that EZH2 is up-regulated in tumor progression in multiple myeloma (MM). To investigate the role of EZH2 in MM, we performed knock-down experiments in human myeloma cell lines (HMCLs). In particular, we focused on two HMCLs characterized by the t(4;14) translocation associated with MMSET over-expression. *Methods*. HEK 293T cells have been transfected with a lentiviral vector pGIPZ EZH2 sh and with an empty pGIPZ vector as control (Thermo Scientific Open Biosystems expression Arrest GIPZ Lentiviral shRNA mir). The supernatants from GFP positive cells were used to infect HMCLs KMS11 and KMS34. GFP positive cells were collected and processed for quantitative real time PCR (Q-RT-PCR) (TaqMan miRNA assays, Applied Biosystems), and immunoblotting (WB). *Results*. To investigate the role of EZH2 in MM, we impaired its expression in KMS11 and KMS34 HMCLs both characterized by the t(4;14) translocation. The impairment of EZH2 expression, confirmed by Q-RT-PCR and WB analyses of GFP positive cells, was associated with a 40% reduction in cell proliferation. The analysis of MMSET protein amount in KMS11 HMCL GFP positive cells showed a 60% reduction if compared with GFP positive control cells. *Conclusions*. Our preliminary data suggest that impairment of EZH2 is able to reduce cell proliferation in *in vitro* myeloma cell lines. In addition, EZH2 knock-down induces a significant reduction of MMSET protein level. This is in agreement with the model described in prostate cancer and are currently under investigation in our laboratory.