of MM patients have lost their immune competence and become anergic to ZA-stimulation. The aim of this work was to identify strategies to overcome myeloma-induced BM γδ T-cell immune dysfunction and restore their anti-myeloma activity. *Methods.* γδ T cell immunophenotype: four color flow cytometry was used to determine the subset distribution of γδ T cells, the expression of the inhibitory receptors (PD-1 and BTLA) and of its ligands (PD-L1 and HVEM) in the tumor microenvironment. γδ T cell activation: MM BMMC were cultured for 7 days in presence of IL-2 and ZA. On day 7, percentages and total counts of viable γδ T cells were calculated with the trypan blue staining and flow cytometry. BM γδ T cell proliferation and CD107 expression was also evaluated in the presence of anti-PD1 blocking mAb. Myeloid-derived suppressor cells (MDSC) and regulatory T cells (Tregs) inhibition: to test the role of MDSC and Tregs in the anergy of MM BM γδ T cell, BMMC were cultured for 7 days with IL2 and ZA in presence or absence of functional inhibitors of these suppressor cells (sildenafil and 1-methyl-tryptophan for MDSC;OX40L and anti-TGFβ for Tregs). *Results.* Our results point to a functional exhaustion of BM γδ T cells driven by the local microenvironment as the main cause of immunoparesis. Tumor cells and bystander cells in the BM microenvironment of MM patients have an extremely accelerated Mevalonate pathway activity leading to high concentrations of extra-cellular IPP in the tumor site. We investigated the expression of programmed death-1 (PD-1) receptor, a negative regulator of T cell activation and proliferation, on BM and PB γδ T cells from MM patients and healthy donors. Interestingly, MM BM γδ T cells showed the highest PD-1 expression, mainly in the subset with the highest proliferative capacity (central memory). Moreover, our data indicate that the late removal of Tregs and MDSC, negative regulators locally recruited by myeloma cells, is not sufficient to reinstate the immune competence of BM γδ T cell. Besides, our results reveal that BM γδ T cells dysfunction is an early and long-lasting event during the disease evolution. *Conclusions.* Our results evidence that neutralization of the PD-1/PDL-1 axis partially reinstates BM γδ T cell reactivity and improves their cytotoxic ability in MM patients.

## C024

### THE ROLE OF NOTCH PATHWAY IN MULTIPLE MYELOMA PROGRESSION TOWARD IL-6 INDEPENDENCE

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*Introduction.* Multiple myeloma (MM) is a heamotologic malignancy characterized by proliferation of neoplastic plasma cells in the bone marrow (BM). Initially, myeloma cells strictly depend on BM, which supports tumor progression through adhesion molecules and soluble mediators as interleukin-6 (IL-6). Later, at the stage of plasma cell leukemia, MM cells acquire mutations resulting in proliferation indipendent from environmental factors such as IL-6. Recently, Notch signaling has been shown to be upregulated during MM progression and to positively regulate cell proliferation, drug resistance and BM infiltration. The aim of this study is to evaluate if Notch signalling plays a role in the acquirement of IL-6 independence. *Methods.* The human MM cell lines CMA03, INA-6 and XG1 were maintained in RPMI-1640 medium supplemented with IL-6 (respectivetly at 10, 2.5 and 1ng/ml). CMA03/06, OPM2 and U266 were maintened in the same condition withouth IL-6. The murine fibroblasts NIH3T3 were used as BMSC mimic and maintained in DMEM. Viable cells were counted by trypan blue exclusion assay. Notch inhibition was obtained by using the γ-secretase inhibitor DAPT at 50mM, soluble Jagged1 was used at 5mg/ml. qRT-PCR reactions were performed by Maxima™ SYBR GreenqPCR Master Mix. Silencing of Jagged1 and 2 was obtained by transient expression of specific siRNAs (Select RNAiTM siRNA system, Invitrogen). *Results.* The global expression analysis of the MM model of IL-6 independence acquisistion represented by CMA03 and CMA03/06 cell line (Verdelli *et al.* Genes Chromosomes Cancer, 2014), indicated that Notch pathway activation may contribute to the IL-6 independence in MM by inducing proliferative signals. Accordingly, we showed here that the activation of Notch signaling, induced by stimulation with soluble Jagged1 ligand, partially rescued IL-6 dependency in XG1 cells. Otherwise, Notch signaling inhibition obtained with DAPT in three different IL-6-indipendent MM cell lines (CMA03/06, OPM2 and U266) resulted in a significant decrease of cell growth which could be reverted by IL-6. This confirms that Notch and IL-6 are complementary in activating MM cell proliferation. Of note, Notch withdrawal induced by Jagged1/2 silencing, decreased IL-6 expression in OPM2 and U266 cell lines. This suggests that Notch-directed IL-6 regulation might have a biological significance in those MM cell lines which express high IL-6 levels. More frequently, BM stromal cells represent the main source of IL-6 for those MM cells which do not display an autonomous production. Results from co-culture systems indicate that surface Jagged expressed on MM cell lines induced Notch-directed IL-6 production in stromal cells. This effect was reverted by silencing Jagged1/2 in MM cells. *Conclusions.* These results suggest that Notch pathway activation may contribute to the transition from IL-6-dependent to IL-6-independent cell growth, and that its inhibition may result in decreased cell proliferation.