Results: Only N educated by SMM- and MM-MSC (both from patients at diagnosis, relapsed and refractory) significantly up-regulated Arg1, NOS2 and TNFα and exhibited suppressive effect with a reduction of T cell proliferation ($p<0.001$). By co-culturing educated-N with Human Brain Microvascular Endothelial Cells (HBMEC), we observed increased both tube length and number of branch points only in conditions where HBMEC were incubated with MM-MSC or MMref-MSC educated-N ($p<0.05$). Adding Bortezomib, Lenalidomide or Pomalidomide during co-culture of PBMC with MM-MSC, isolated N showed a significant reduction of pro-angiogenic activity but did not lose immunosuppressive ability. To examine if PC play a role in MSC "activation", before performing co-cultures with PBMC, we pre-treated HS-5 or HC-MSC with MM cell lines. PC pre-treatment drives healthy MSC to activate N in immunosuppressive and pro-angiogenic cells. Implanting of mixtures of fluorescently labeled MM cells and healthy- or MM-MSC into zebrafish, animals coinjected with PC and MM-MSC showed enhanced tumor colonization and growth compared with those injected with PC and healthy MSC.
Summary/Conclusions: Tumor microenvironmet transformation frommgUS to MM is associated with progressive activation of MSC that have a pro-tumoral activity. Indeed SMM- and MM-MSC polarize N in immunosuppressive and pro-angiogenic N (N2) in vitro. In addition, MM-MSC facilitate MM growth *in vivo* confirming their central role in tumor progression.

E1215

LONG TERM CR MULTIPLE MYELOMA PATIENTS STUDIED WITH NEXT GENERATION FLOW SHOW PREDOMINANTLY CURED *VSmg*US-LIKE MINIMAL RESIDUAL DISEASE PATTERNS: A STUDY OF THE GTMM-TUSCAN GROUP FOR MULTIPLE MYELOMA

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Background: CR is a prerequisite for long term responses, progression free survivals, and ultimately overall survivals and cure. In the era of novel agents, many MM patients can achieve stringent CR (sCR), *i.e.* disease disappearance at serological, immunoistochemical level plus negativity of free light chains (FLC). On the other hand most of these patients still will relapse and minimal residual disease (MRD) detection will play a crucial role in the very next future. Recently, two 8 colours tubes panel developed by the EuroFlow Consortium can detect MRD with an increased sensitivity and can be applied as standardized method to study multiple myeloma (MM) patients.
Aims: While many studies have looked at MRD status sequentially and soon after autologous or allogeneic stem cell transplantation with flow or molecular techniques, little is known about long term remission patients (>5-10 years) and in particular if more sensitive techniques such as NGF or NGS can still detect minimal disease in those patients. Aim of the study was to analyse patients with MM in >VGPR with next generation flow at>2 and>5 years of lasting remission.
Methods: Clinical assessment definition of CR status included serum and urine immunofixation, free light chain determination, imaging study with CT-PET, bone marrow aspirate, bone core biopsy. 56 MM patients (M/F= 30/26), were studied with NGF at two GTMM centers between February 2016 and February 2017. 28/56 (50%) patients were in sCR at the moment of the study at a median of 40 months after therapy (range 3-140). 28/56 (50%) patients were in VGPR at study analysis according to new IMWG response criteria. N= 12, 25 and 44 patients had a remission disease >5 years, >2 years, and <5 years, respectively. Two tube assay incorporated 8 antibodies each: CD38, CD56 β2-Microglobulin, CD19, κ Anti-Kappa Anti-Lambda CD45 CD138, and CD38, CD28, CD27, CD19, CD117, CD81, CD45 and CD138 (OneFlow™ *PCST* and PCD, BD Biosciences) and were utilized to detect MRD level with a lyse-wash-and-stain sample preparation protocol by flow cytometry (FACScanto II, BD, Biosciences). Accurate identification of BM plasma cells (PCs) and discrimination between phenotypically aberrant (aPC) and normal PC (nPC) were carried out after acquisition and analysis of >2 x 10^6 cells (Diva 8, BD Biosciences).
Results: MRD+ status was detected in 23/56 (41%) of the patients. 4/12 (2 33%) were MRD positive at >5 years remission (2 sCR, 2 VGPR) (median 96 months range 72 – 186 months); 20/44 (45%) were positive at<5 years of remission (3 CR; 17 VGPR)(median 9,5 range 3 – 46 months). 9/25 (36%) were MRD + after >2 years of remission (2 sCR, 7 VGPR) (median 46 months range 24 – 186 months). As expected being in sCR was correlated with a low MRD+ status 5/28 (18%) (2 patients after >5 years, 3 patients after <5 years). Interestingly looking at long lasting remission, *i.e.* >5 years, the 4/14 patients that resulted MRD+ displayed anmgUS like –plasmacell immunophenotype (prevalence of normal plasmacells *vs* aberrant monoclonal) with a PCn/PCtot ratio of 48%, 95%, 35%, 30%. CT/PET was positive in 22/56 patients. All patients in sCR were CT/PET negative.
Summary/Conclusions: In conclusion NGF showed that MM patients with long remission status can be considered disease free/cured with a high sensitivity method. MM patients that display anmgUS–like phenotype after achieving a CR can have long lasting remissions meaning disease control. Patients in sustained CR after 2 years can have high percentage of MRD negativity. Larger studies are warranted to identify patients who need treatment consolidation or continuous treatment based on MRD+ status *vs* others who could stay treatment free with social and economical benefits.

E1216

THE NOTCH PATHWAY IN THE INTERPLAY BETWEEN MYELOMA CELLS AND ENDOTHELIUM IN THE BONE MARROW NICHE

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Background: Angiogenesis is a hallmark of tumors, and it is a peculiar characteristic in bone marrow (BM) of multiple myeloma (MM) patients. MM is a still incurable disease that strongly depends on interactions with BM microenvironment. Endothelium of MM patients displays malignant behavior as compared to a healthy counterpart (1). MM displays a dysregulation of the Notch pathway due to Jagged ligands and Notch receptors overexpression. This condition brings to the generation of homotypic and heterotypic interaction loops that sustain MM cells. Moreover, Notch pathway represents a bridge in the dialogue with BM resident cells, including osteoclast and BM stromal cells (BMSCs), although its role in the crosstalk of MM and endothelium is still to be clarified.
Aims: The aim of this study is to investigate Notch role in MM crosstalk with endothelium exploiting 2D assays and 3D organoid systems to mimic tumor microenvironment (TME).
Methods: The Notch ligands, Jagged1 and 2, were silenced in the MM cell line RPMI8226 (RPMI8226$^{shJAG1/2}$) using an inducible lentiviral vector carrying two short hairpin RNAs targeting Jagged1 and 2. To mimic the endothelial compartment the human pulmonary arterial endothelial cells (HPAECs) were used and for the stromal compartment, the GFP$^+$HS5 cell line. Matrigel and wound healing assays were set up to investigate Notch role in modulating respectively the angiogenic potential of MM cells co-cultured with HPAECs and HPAEC motility in response to MM-derived soluble factors. To develop a TME-like system, a decellularized extracellular matrix (dECM) was used as a physiologic scaffold for organoid generation. dECM was produced by treating murine fibroblast NIH3T3 with ascorbic acid and was loaded with cells for organoids generation. We evaluated apoptosis of MM cells in single culture and co-culture with BMSCs or HPAECs by flow cytometry.
Results: Matrigel assay of HPAEC co-cultured with MM cells showed that direct contact increased angiogenic potential of HPAEC to form a grid of tubes; this effect is significantly reduced when HPAECs are co-cultured with RPMI8226$^{shJAG1/2}$ cells, indicating a key role of Notch signaling in endothelial stimulation. Wound healing assay demonstrated that Notch signaling affects HPAEC motility, since it is reduced when Jagged ligands are silenced. Concerning the 3D-organoid generation, our results indicate that the handcrafted dECM was a suitable scaffold. Moreover, apoptosis assays indicated that MM cells displayed an increased survival when cultured in the presence of BMSCs, that consistently with their recognized protective role; no significant difference in MM cell apoptosis was observed in the presence of endothelial cells. On the contrary, we have observed that endothelial cells were protected by MM cells suggesting that MM cells improve angiogenesis by preventing endothelial cells apoptosis.
Summary/Conclusions: These results indicate a novel role for Notch pathway in MM-EC crosstalk suggesting that the Notch pathway activation in MM cells can increase their proangiogenic potential. 3D-organoid mimics BM microenvironment and may be used as a novel tool to recapitulate the interactions of BM and tumor cells beyond the animal models.

References

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E1217

MIR-101-3P REGULATES BONE MARROW STROMA-INDUCED DRUG RESISTANCE IN MULTIPLE MYELOMA CELLS BY TARGETING SURVIVIN AND MODULATING CELL-CELL ADHESION

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Background: In multiple myeloma (MM), bone marrow stromal cells (BMSCs) protect MM cells against cell death by direct or indirect interaction. This phenomenon can partly explain *de novo* or acquired drug resistance in MM. Findings of relevant studies indicate activation of some oncogenic or survival pathways including PI3K/mTOR, Ras/MAPK, NFkB and Wnt. However; the potential regulatory mechanisms and druggable targets have not been clearly elucidated.