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ABSTRACT BOOK

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groups compared with MGUS (Figure 1B). By a functional assay we have demonstrated that gene and protein overexpression drives to an increase of activity, comparing MGUS and MM at diagnosis versus MM at relapse (p-value ***<0.0001) (Figure 1C). We confirmed the correlation between higher mitochondrial burden and resistance to bortezomib in JJN3 and L363, and NCI-H929 and its resistant, NCI-H929 R20 (p-value*<0.05) (Figure 1D). *In vitro* drug assays showed a synergistic effect of tigecycline with bortezomib, suggesting that could be used as a potential therapy in combination for MM patients.
Summary/Conclusion: Mitochondrial machinery plays a critical role in the development, progression and resistance of MM patients. Mitochondrial protein components that generates the activity could be prospective targets for MM treatment. Tigecycline demonstrates synergistic effect with Bortezomib suggesting potential use as novel drug combination therapy in MM patients.

PS1277

ELOTUZUMAB PROMOTES SELF-ENGAGEMENT OF SLAMF7 BETWEEN NATURAL KILLER AND MULTIPLE MYELOMA CELLS TO ENHANCE CYTOTOXICITY

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Background: Elotuzumab is an immunoglobulin G1 monoclonal antibody targeting signaling lymphocytic activation molecule family member 7 (SLAMF7), which is highly expressed on multiple myeloma (MM) cells, natural killer (NK) cells, and, to varying degrees, other immune cells. Preclinical reports show that elotuzumab promotes potent NK cell–mediated antibody-dependent cellular cytotoxicity via Fc interaction with FcgRIIIA (CD16), resulting in killing of SLAMF7+ MM cells, which is further enhanced in combination with lenalidomide. Previous preclinical studies also suggest that elotuzumab can enhance NK cell activity via a costimulation mechanism independent of CD16 binding. In patients with relapsed/refractory MM, elotuzumab combined with lenalidomide and low-dose dexamethasone improves progression-free survival.
Aims: To characterize how elotuzumab affects interactions between NK and MM cells to enhance NK cell–mediated cytotoxicity.
Methods: We generated 2 MM cell lines (MM.1R and RPMI8226) modified to express low or high levels of SLAMF7, as well as the SKOV3 ovarian adenocarcinoma cell line transduced to express SLAMF7. CRISPR/Cas9 technology was used to generate a SLAMF7-deficient human NK-92 cell line. Variants of this SLAMF7-deficient line and an NK-92 line expressing high levels of SLAMF7 that expressed or lacked CD16 were also generated. Cytotoxicity was measured with CytoTox 96® (Promega) and xCELLigence® real-time cell analysis (ACEA Biosciences) platforms. A human SLAMF7 extracellular/T-cell receptor [TCR]-zeta intracellular fusion construct was transfected into a 3A9 mouse T-cell line and used as a reporter of SLAMF7 engagement by measuring interleukin-2 production using an enzyme-linked immunosorbent assay.
Results: Consistent with previous reports, addition of elotuzumab strongly enhanced cytotoxicity of CD16-expressing SLAMF7+ NK-92 cells against SLAMF7+ MM and SKOV3 cells. Elotuzumab also substantially boosted cytotoxicity by CD16-deficient SLAMF7+ parental NK-92 cells toward SLAMF7+ RPMI8226 and SKOV3 cells. Knockout of SLAMF7 on parental NK-92 cells, however, abrogated elotuzumab-mediated cytotoxicity toward SLAMF7+ SKOV3 targets. Additionally, by using a SLAMF7/TCR-zeta–expressing reporter cell line and plate-bound recombinant SLAMF7, we found that elotuzumab promoted reporter activity, suggesting it may facilitate or enhance SLAMF7–SLAMF7 interactions. Interestingly, other anti-SLAMF7 antibodies were ineffective in stimulating reporter activity.
Summary/Conclusion: We conclude that elotuzumab has an additional function as an NK cell–activating antibody. SLAMF7 naturally engages with itself in homotypic interactions. Elotuzumab uniquely promoted NK cell–mediated cytotoxicity in a CD16-independent manner, but only if both NK and target cells expressed SLAMF7. This suggests that elotuzumab can facilitate or enhance SLAMF7–SLAMF7 interactions between NK cells and MM targets. As elotuzumab is clinically used in combination with lenalidomide, additional studies are needed to understand the impact of combination treatment on elotuzumab-mediated SLAMF7–SLAMF7 interactions. Based on these preclinical observations, SLAMF7 expression on NK cells warrants further investigation as a potential biomarker for elotuzumab efficacy.

PS1278

CARFILZOMIB-INDUCED CARDIOTOXICITY: MOLECULAR MECHANISMS AND THE EMERGING ROLE OF METFORMIN AS A PROPHYLACTIC THERAPY

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Background: Carfilzomib (Cfz) is an irreversible proteasome inhibitor, which is used for the treatment of relapsed/refractory multiple myeloma (RRMM). In phase III trials, Cfz has been associated with higher cardiotoxicity and heart failure rates compared to the control treatment. Due to the severity of these adverse events and the lacking data regarding the induced cardiotoxicity, there is an imperative need for the elucidation and abrogation of the underlying mechanisms of Cfz-induced cardiotoxicity.
Aims: The aim of this study was to investigate the molecular mechanisms of Cfz-induced cardiotoxicity and to evaluate possible cardioprotective effects of concomitant medications based on our initial results.
Methods: Protocol 1: Male C57BL/6 mice, were randomized into: Control (/S 0.9%, n=7) and Cfz group (n=8). Based on the results showing below we also developed a second protocol using metformin (met); Protocol 2: Male C57BL/6 mice were randomized into: Control (/S 0.9%, n=8); Cfz (n=8) and Cfz+Met (n=10). Cfz (8 mg/kg ip) was administered every 48 hours in both protocols and Met (140 mg/kg po) every 24 hours for 6 days. Fastening glucose levels were monitored. At baseline and at the end of treatments mice underwent echocardiographic assessment. Animals were sacrificed and blood and myocardial tissue samples were obtained for the analysis of proteasome peptidases activity, protein phosphatase 2A (PP2A) activity and molecular signaling mechanisms. Protein kinase Akt, along with its downstream NO synthases; endothelial (eNOS) and inducible (iNOS), were identified as targets of possible endothelial dysfunction and inflammation. Moreover, the transcription factor FOXO1, downstream target of Akt and AMPKα, was identified in order to investigate possible changes in the expression of apoptotic factors. Finally, AMPKα was identified since – besides phosphorylating eNOS and FOXO1 – it functions as a regulator of autophagy.
Results: Administration of Cfz resulted in significant reduction of the chymotrypsin-like (CT-L) proteasome activity in myocardial tissue and peripheral blood mononuclear cells of Cfz-treated mice vs controls (p<0.01). Protocol 1: Reduction in fractional shortening (FS%) was observed in the Cfz group vs Control at Day 6 (39.87±0.47% vs 42.05±0.64% respectively, p<0.05). Cfz increased PP2A activity vs Control (p<0.05), without altering PP2A expression. A decrease in pAkt/tAkt (p<0.05), peNOS/teNOS (p<0.05), pAMPKα/tAMPKα (p<0.001) and an increase in the expression of iNOS (p<0.01) was observed in the Cfz group vs Control. Protocol 2: Met did not reduce fastening glucose levels at day 6 in Cfz+Met compared to Control and Cfz groups. Echocardiographic assessment at day 6 revealed that Met reversed Cfz-induced reduction in the FS% in Cfz+Met vs Cfz group (43.4±0.5% vs 41.5±0.4% respectively, p<0.05). AMPKα phosphorylation was significantly increased in the same group compared to Cfz group (p<0.01).
Summary/Conclusion: The present study demonstrates that Cfz induces cardiac dysfunction via increasing PP2A activity, leading to decreased phosphorylation of Akt, eNOS and AMPKα. The disturbance of Akt/AMPKα/eNOS axis and the increase of iNOS, suggests that Cfz might intervene with oxidative stress, apoptosis and myocardial energetic pathways. Thus, Cfz-induced increase in PP2A activity seems to be essential in the mechanism of cardiotoxicity. Met restored AMPKα phosphorylation and reversed Cfz-induced contractile dysfunction, emerging to be a potent pharmacological intervention for the management of Cfz-induced cardiotoxicity.

PS1279

JAGGED1/2 INHIBITION PROMOTES TUMOR CELLS RESPONSE TO BORTEZOMIB IN A ZEBRAFISH MODEL OF MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) is the second most frequently diagnosed hematological malignancy and today is still incurable, mainly due to the development of drug resistance that causes relapse and contributes to the fatal outcome of this disease. MM cells accumulate in the bone marrow (BM) and establish complex interactions with the surrounding normal cells, forcing them to assume a pro-tumor behavior. In this process, a key role is played by the two Notch ligands Jagged1 and 2, whose dysregulated expression causes an aberrant activation of the Notch pathway both in MM cells and in the BM niche cells.
Aims: We aimed to validate the effect of Jagged1/2 silencing on MM cells resistance to the standard-of-care drug Bortezomib by: i) *in vitro*, on co-culture of MM cell lines and BM stromal cells (BNSC); ii) *ex vivo*, on co-culture of primary cells from MM patients and BMSC; iii) *in vivo*, using a zebrafish xenograft model of MM that allows a rapid and reliable screening of MM cells response to chemotherapics.
Methods: Jagged1/2 expression was inhibited transiently in MM cell lines using specific siRNAs and constitutively in primary MM cells using a lentiviral vector that encodes specific shRNAs. Cells were cultured alone or co-cultured with BMSC and treated with 8nM Bortezomib. Apoptosis was evaluated by Annexin V staining and flow cytometry. For *in vivo* experiments cells were stained with the CM-Dil vital dye, resuspended in PBS + 3% polyvinyl pyrrolidone and injected in the yolk area of 2dpf (days post fertilization) zebrafish embryos. Injected embryos were treated with 10μM Bortezomib or DMSO for 48h. MM cells growth in zebrafish was evaluated by fluorescence microscopy and tumor area were calculated using ImageJ and normalized on tumor volume at the time of injection.
Results: Jagged1/2 blockade reduces MM cells ability to induce Notch activation in BMSC, causing a decrease in their capacity to sustain MM resistance to Bortezomib. Results obtained *in vitro* on MM cells lines were further validated on co-culture of primary CD138+ cells and BMSC from newly diagnosed MM patients. The analysis performed on xenotransplanted embryos showed that the treatment with 10μM Bortezomib caused a decrease of about the 50% in tumor growth in comparison to DMSO-treated controls, with no effect on embryos viability. Jagged1/2 knockdown alone has a comparable effect to Bortezomib, while the combination of Bortezomib and Jagged1/2 inhibition results in a stronger decrease in tumor growth of about the 75% in comparison to the vehicle-controls.
Summary/Conclusion: Our findings demonstrate that Jagged1/2 inhibition represents a suitable strategy to promote MM response to the standard of care drug Bortezomib, contrasting BM-induced drug resistance.

PS1280

TGFB INHIBITION IN COMBINATION WITH CHEMOTHERAPY REPAIRS EXISTING LYTIC BONE LESIONS IN A NOVEL PLATEAU PHASE MODEL OF MULTIPLE MYELOMA

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Background: Multiple myeloma (MM) causes a destructive bone disease in >85% of patients and current therapies do little to repair existing bone damage. We previously identified that combined bone anabolic and anti-resorptive therapy repairs osteolytic lesions in mice with high tumour load. In patients, if bone repair agents were given, they would be administered in combination with chemotherapy.
Aims: This study aimed to determine if bone recovers after chemotherapy and if this is enhanced by bone anabolic therapy.
Methods: Human U266-GFP-luc MM cells were i.v. injected into NSG mice (n=5-7/group). After tumour and lytic bone lesion development, mice were administered first-line chemotherapeutics (bortezomib±lenalidomide)±a bone anabolic (SD208; transforming growth factor β receptor 1 inhibitor) or vehicles for 2 weeks. Tumour and bone lesions were monitored *in vivo* by bioluminescence imaging (BLI), serum paraprotein ELISAs and μCT. Flow cytometry, histomorphometry, μCT, TRAP and P1NP ELISAs and QPCR were performed for endpoint analyses.
Results: Chemotherapy significantly reduced total body tumour burden and paraprotein, and increased survival. Combined chemotherapy was more effective than either given alone, reducing tumour to levels undetectable by BLI and paraprotein. However, flow cytometry revealed low tumour levels of 100MM cells/10^6 bone marrow cells. Lytic bone lesions developed ~8 weeks after tumour inoculation. Vehicle treated mice exhibited progressive bone lesion development and virtually no trabecular bone at endpoint. Lesions in mice administered bortezomib±lenalidomide were unchanged after 1 week but began to repair after 2 weeks, with significantly reduced TRAP+ osteoclasts and increased osteoblasts, indicating recovery of bone. Mice treated with chemotherapy + anabolic SD-208 exhibited enhanced repair of bone lesions, with partial repair of perforating cortical lesions on all tibial surfaces within 1 week and complete repair of lesions within 2 weeks. SD-208 also significantly increased trabecular bone volume after 2 weeks.
Summary/Conclusion: This study identified SD-208 enhances MM bone lesion repair when combined with first-line chemotherapeutics. Future studies combining SD-208 and chemotherapy with anti-resorptive therapy will identify optimum treatment regimens for translation of bone anabolic therapy into MM clinical trials.

PS1281

MULTIPLE MYELOMA: SINGLE PLARFORM ABSOLUTE COUNT OF CIRCULATING PLASMA CELLS AT DIAGNOSIS CORRELATE WITH POOR PROGNOSIS PARAMETERS

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Background: Risk stratification of newly diagnosed multiple myeloma (NDMM) patients is based on clinical and laboratory parameters. In previous studies, circulating plasma cells (CPC) showed a significant correlation with more aggressive disease: CPC were detected by flow cytometry after separation by ficoll gradient, which can reduce the recovery of plasma cells, or by acquiring a defined total number of events, and the absolute CPC number was obtained using an automated hematology analyzer.
Aims: This is the first study to perform a single platform absolute count of CPC. We compared CPC with patients' baseline characteristics.
Methods: We collected 413 peripheral blood (PB) samples of NDMM patients enrolled in the UNITO-MM-01/FORTE. For the single platform tube, the antibody combination CD38PC7/CD138PC5.5/CD45KO/CD56PE/CD19PB was mixed with 100 μL of EDTA PB dispensed with reverse pipetting, added with 500 μL of lysing solution. After 15 min, 100μL of flow count were dispensed with reverse pipetting and acquired with Navios flow cytometer. In order to reduce the acquisition of cellular debris, a "live gate" was set on CD45/CD38 dot plot and all the events CD38 and CD45 negative were excluded. The CPC clonality was confirmed, in a second tube, by the determination of kappa and lambda light chains of intracytoplasmic immunoglobulins.
Results: CPC were detected in 390 of 413 samples (94.4%): median values were 0.03% (range 0%>51%), 2.37/mm^3 (range 0/mm^3-6272/mm^3), number of absolute CPC was 58 (range 0-441000); cellular events acquired 190000 (range 4428-1300000). Statistically significant higher values of CPC were found in samples from patients with poor prognosis features: Hb <10 g/dL, ISS stage III, R-ISS stage III, Albumin <3.5 g/dL, b2-microglobulin >5.4 mg/dL, LDH >upper limit, PC in biopsy ≥60%, presence of del13, ECOG 3 (all with a p value <0.001); ampl1, High Fonseca cytogenetic risk, High Morgan cytogenetic risk all with a p value <0.05.
A linear correlation was found between CPC and hemoglobin (r=-0.46 p<0.001), bone marrow aspirate plasma cells (r=0.36 p<0.001), plasma cells in biopsy (r=0.38 p<0.001), b2-microglobulin (r=0.25 p<0.001). CPC absolute values were sorted in quartiles (0/mm^3-0.86/mm^3, 0.86/mm^3-2.37/mm^3, 2.37/mm^3-11.2/mm^3, 11.2/mm^3-6272/mm^3) and associated with poor prognosis features. Significant differences expressed by Cramer's V >0.2 were observed between CPC and hemoglobin (V= 0.41 p<0.001), ISS (V= 0.26 p<0.001), R-ISS (V= 0.24 p<0.001), ≥60% of plasma cells in biopsy (V= 0.23 p<0.001), bone marrow aspirate plasma cells sorted in quartiles (V= 0.21 p<0.001), LDH upper the high limit (V= 0.24 p<0.001), del13q14 (V= 0.21 p=0.002), 1q gain (V=0.21 p=0.001).
Summary/Conclusion: The single platform cytometric method quantified CPC in 94.4% of PB samples from NDMM patients. Higher CPC number significantly correlated with poor clinical and laboratory features, confirming that CPC are an indicator of more aggressive disease, as showed by other studies. This method allows a high recovery of CPC, needs a small amount of PB sample, is a fast procedure, does not need cell separation, and is accurate. Moreover, it can be performed in all patients and can be particularly useful when cytogenetic score cannot be defined.