confirmed by ELISA for both cell lines. The in vitro co-culture of PBMCs with the B7-1/IL-2 expressing MM cells resulted in greater than 50 fold expansions in the number of NK cells, increased expression of cell surface activation markers, enhanced secretion of IFN-γ, and increased cytolytic activity against both K562 and the myeloma cell lines. This comprehensive stimulation of both NK cell number and functional activity was dependent on the combined expression of B7-1 and IL-2, and on the presence of T cells and/or monocytes, involving both cell contact dependent/and independent mechanisms. **Conclusion:** This study shows that myeloma cells that are genetically modified to express B7-1 and IL-2 can induce both the numerical expansion and functional activation of NK cells. Therefore, B7-1/IL-2 transduction could provide a suitable strategy for immune gene therapy of residual myeloma.

B447

Pomalidomide Inhibits MM Proliferation In Vitro via Enhanced Expression of Tumor Suppressor Genes

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Pomalidomide, an oral anti-proliferative and immunomodulatory drug, is currently in phase I/II studies for relapsed/refractory multiple myeloma (MM) and other diseases including myelofibrosis, amyloidosis, and sickle cell disease. Pomalidomide appears to be highly active in MM. Here, we investigated direct anti-MM effects of pomalidomide in vitro using human MM cell lines with various cytogenetic backgrounds (NCI-929, Karpas-620, U266, KMS-12BM, JJN-3, OPM2, LP-1, RPMI-8226, EJN and SKMM2). We also assessed the effect of pomalidomide on production of pro-angiogenic factors such as VEGF and PDGF, and expression of tumor suppressor and enhancer genes such as p21cip1, SPARC, ING1/4, p57 kip2, p53, cyclin D1/2, and IRF4/MUM1. Pomalidomide can directly inhibit proliferation of these human MM cells at levels attainable in the plasma of treated patients. The pro-angiogenic factors VEGF, PDGF and IL-8, which are constitutively expressed at high levels, were strongly reduced by pomalidomide, although there was no appreciable effect on VEGF and PDGF production for 2 cells lines (U266 and LP-1), despite dose-dependent inhibition of proliferation by pomalidomide. Pomalidomide strongly increased the expression of p21cip1, SPARC, ING4 and p57 kip2. In all MM cell lines tested, pomalidomide had weak but consistent inhibitory effects on expression of IRF4, an important MM survival factor. However, pomalidomide had no marked effect on gene expression of tumor enhancers VEGF, PDGF, IL-8, cyclin D1/2 and MAF or tumor suppressors activin A and p53. This suggests that the antiproliferative and anti-growth factor effects of pomalidomide on MM cells may be related to up-regulation of tumor suppressor expression and posttranscriptional control of VEGF production and other growth factors. The antiproliferative effect of pomalidomide is significantly correlated with upregulation of SPARC expression (P = .021, r^2 = 0.77) and inversely correlated with the constitutive level of cyclin D2 (P = .014, r^2 = 0.81) in these MM cell lines. In conclusion, pomalidomide demonstrates direct inhibitory effect on proliferation and growth factor production against various MM cells. These dual anti-myeloma activities may help explain the clinical efficacy seen in MM patients treated with pomalidomide. Pomalidomide-induced up-regulation of SPARC mRNA and baseline level of cyclin D2 are associated with sensitivity of MM cells, which may have biomarker potential for MM therapy with pomalidomide.

B452

Impact of Lenalidomide on Bone Marrow Microenvironment: Implications for Stem Cell Mobilization

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Introduction: There is an ongoing debate about the impact of lenalidomide on the efficacy of stem cell mobilization with granulocyte-colony stimulating factor (G-CSF) alone. Up to 43% of patients who have received lenalidomide therapy prior to stem cell mobilization failed to mobilize sufficient number of CD34+ cells with granulocyte-colony stimulating factor (G-CSF) alone (Mazumder, et al. 2008) raising concerns that lenalidomide therapy specifically prevents G-CSF mobilization or exerts stem cell toxicity to hematological progenitors. Therefore, this analysis focused on the effect of lenalidomide, pomalidomide, and thalidomide on CD34+ hematopoietic progenitors. **Methods and Results:** In standard colony formation assays, as well as in long term culture-initiating cell (LTC-IC) tests, it was shown that lenalidomide, pomalidomide and thalidomide were not toxic to hematopoietic stem cells and did not inhibit the self-renewal capacity of stem cells. This excludes a direct toxic effect of lenalidomide on hematopoietic progenitors as a reason for the limited stem cell collection. It was further shown that these compounds promoted myelopoiesis with a concomitant maturation stop of neutrophil granulocytes by down-regulation of the critical transcription factor PU.1 in vitro as well as in bone marrow patient samples. With the down-regulation of PU.1, an accumulation of immature granulocytes within the bone marrow compartment and neutropenia in the peripheral blood was observed. Cytokine arrays revealed that G-CSF secretion was highly up-regulated in cultures of CD34+ cells treated with lenalidomide (800 pg/mL) and with pomalidomide (1500 pg/mL) versus control treatment (140 pg/mL). The biological reason for the strong up-regulation of G-CSF is not fully elucidated, but it is likely that by a self-regulatory mechanism the maturation stop of granulocytes within the bone marrow should be overcome by higher levels of G-CSF. **Conclusions:** Higher levels of G-CSF in response to lenalidomide within the bone marrow microenvironment might lead to a tachyphylactic response resulting in resistance to G-CSF mobilization. This is in accordance with the observation that other mobilization regimens, such as cyclophosphamide, did mobilize sufficient CD34+ cell numbers following lenalidomide-based therapy (Mark T, et al. 2008). These results suggest that lenalidomide has no toxic effects on hematopoietic progenitors.