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Hypoxia Induced Impairment of NK Cell Killing against MM can be Overcome by IL-2 Activation of NK Cells

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We aim to develop allogeneic Natural Killer (NK) cell immunotherapy for Myeloma. As myeloma cells are present in hypoxic regions and the tumor environment can be immunosuppressive, we hypothesized that hypoxia inhibits NK cell anti-MM responses. NK cells were isolated from healthy donors by negative selection and NK cell function and phenotype were examined at oxygen levels representative of the BM using flowcytometry. Additionally, NK cells were activated with IL-2 to enhance NK cell cytotoxicity under hypoxia. Hypoxia reduced NK cell killing of MM cell lines in an oxygen dependent manner. NK cell degranulation was not influenced by hypoxia indicating that NK cells had been activated. Adaptation of NK- or MM cells to hypoxia was not required, hence, the oxygen level during the killing process was critical. Hypoxia did not alter surface expression of NK cell ligands (HLA-ABC, -E, MICA/B and ULBP1-2) and receptors (KIR, NKG2A/C, DNAM-1, NCRs and 2B4). It did, however, decrease expression of the activating NKG2D receptor. Pre-activation of NK cells by IL-2 abrogated the detrimental effects of hypoxia and increased NKG2D expression. This emphasized that activated NK cells can mediate anti-MM effects, even under hypoxic conditions. Hypoxia abolishes killing potential of natural killer cell against multiple myeloma, which can be restored by IL-2 activation. Our study shows that for the design of NK cell-based immunotherapy it is necessary to study biological interactions between NK- and tumor cells also under hypoxic conditions.

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HDACi and IMiDs Enhance Anti-myeloma Activity of the Anti-KMA Monoclonal Antibody MDX-1097

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MDX-1097 is a monoclonal antibody being assessed as a single agent in a Phase II clinical trial for the treatment of κ-type multiple myeloma (κ MM). MDX-1097 binds kappa myeloma antigen (KMA), a tumor specific antigen, and exerts its anti-tumour effects through multiple mechanisms including antibody-dependent cell cytotoxicity (ADCC). We investigated whether MDX-1097 could be combined with established or novel anti-MM therapeutic agents to improve the treatment of κ MM. Treatment of human κ MM cell lines (κ HMCLs) with immunomodulatory drugs (IMiDs) or histone deacetylase inhibitors (HDACi) significantly increased cell surface expression of KMA. These IMiD or HDACi-treated κ HMCLs, when spiked with MDX-1097, were more susceptible to ADCC-mediated cell death in the presence of peripheral blood mononuclear cells (PBMCs) compared to untreated κ HMCLs. The increase in KMA expression presumably allows more binding of MDX-1097, which in turn recruits more PB immune effector cells to κ HMCLs and thereby increases ADCC. PBMCs treated in vitro with IMiDs were more potent at inducing ADCC against MDX-1097 spiked κ HMCLs. Similarly, in vivo lenalidomide exposed PBMCs isolated from MM patients were more effective in killing MDX-1097 spiked κ HMCLs compared to PBMCs obtained from the same patients prior to treatment. Finally, combining IMiD-treated PBMCs with IMiD-treated, MDX-1097 spiked κ HMCLs resulted in a further increment in ADCC. This data provides a rationale for the clinical evaluation of a combination therapy involving IMiDs or HDACi and MDX-1097 for the treatment of κ MM.

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Targeting JAK1/2 with Ruxolitinib Blocks IL-6 Induced Plasma Cell Growth and Overcomes Resistance to Dexamethasone

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Malignant plasma cell growth and survival is regulated by cytokines in the tumor environment. Interleukin(IL)-6 plays a key role by activating important signalling pathways through its gp130 receptor associated Janus kinases (JAK). Ruxolitinib (INC424/INCB018424; Novartis/Incyte) is the first JAK inhibitor approved for patients with myelofibrosis and is selective for JAK1 and JAK2. Among human plasma cell lines, ruxolitinib showed a strong cytotoxic activity on the IL-6 dependent INA-6 line (IC50 0.23 μM), even in the presence of stromal cells. Stromal cell viability and IL-6 production were not affected. Consistent with the inhibition of IL-6 induced STAT3 phosphorylation, apoptosis was in-