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POSTER ABSTRACTS

636.MYELODYSPLASTIC SYNDROMES-BASIC AND TRANSLATIONAL

Sting-Mediated Interferon Signaling Exerts Potent Antileukemic Effects in High-Risk Myeloid and Monocytic Malignancies

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Clinical outcomes of patients (pts) with high-risk (HR) myeloid neoplasms remain dismal despite available therapies. Interferons (IFN) regulate hematopoietic stem-progenitor cell (HSPC) differentiation, proliferation and self-renewal capabilities and preferentially impair clonal HSPC fitness. To evaluate if myelodysplastic syndrome (MDS) and chronic myelomonocytic leukemia (CMML) HSPCs activate IFN responses we performed RNA sequencing in bone marrow (BM) CD34 $^{+}$ cells obtained from pts with MDS (n=58), CMML (n=27) and age-matched healthy donors (HDs, n=17). This analysis revealed significant deregulation of IFN pathway genes in HSPCs from pts with CMML and to a lesser extent in those of pts with MDS.

Activation of the stimulator of interferon genes (STING) can inhibit stem-cell function and induce myeloid and monocytic cell death via IRF3-dependent and independent mechanisms. Therefore, we hypothesized that STING pathway activation might have therapeutic potential in high risk (HR) myeloid and monocytic (MyMo) neoplasms. In vitro treatment of monocytic MOLM13 and THP1 cell lines with the novel synthetic STING agonist dazostingag resulted in induction of IRF3 phosphorylation, caspase 3 cleavage and apoptosis. Dazostingag was also able to induce enhanced cell death in multiple myeloid cell line cultures.

Given the ability of dazostingag to induce apoptosis of monocytic cell lines, we sought to evaluate the activity of monocyte-directed STING activation using TAK-500, an IgG1 anti-CCR2 antibody linked to dazostingag as a STING agonist payload. To do so, we first analyzed the expression of the monocyte-specific cysteine-cysteine chemokine receptor 2 (CCR2) in primary pts samples and cell lines. Both CCR2 RNA and protein expression levels were higher in CMML compared to HDs or MDS. In accordance with this finding, monocytic cell lines (MOLM13, THP1) had significantly higher CCR2 expression compared to non-monocytic cell lines (SKM1, HL60, U937, KG1). Treatment of MOLM13 and THP1 cell lines with TAK-500 induced strong IRF3 phosphorylation, caspase 3 cleavage and apoptosis. In vitro treatment of BM mononuclear cells (MNCs) from pts with MDS (n=10), CMML (n=11) or AML (n=8) in co-culture with mesenchymal stromal cells with TAK-500 (0.5 mM) or dazostingag (1 mM) resulted in increased cell death of MNCs from pts with CMML and AML. TAK-500 (0.5 mM) significantly depleted CCR2 $^{+}$ BM MNCs from all disease groups but did not affect CCR2 $^{+}$ cells from HDs. Next, to evaluate whether STING activation impairs repopulation potential of neoplastic HSPCs we performed colony-formation studies in CD34 $^{+}$ BM cells from pts with CMML (n=8), MDS (n=3) and AML (n=4). Treatment with TAK-500 (0.5mM) and dazostingag (1mM) resulted in significantly impaired myeloid colony-formation in all disease groups without significantly impairing erythroid colony-formation in MDS or CMML samples. Taken together, these data suggests that STING activation might induce aberrant myelomonocytic cell death and deplete aberrant HSPCs in HR-MyMo neoplasms.

To evaluate the in vivo efficacy of TAK-500 and dazostingag, we established xenograft models by inoculating NOG-IL15 transgenic mice with MOLM13 cells. Treatment with TAK-500 and dazostingag resulted in reduction of tumor burden evaluated by bioluminescence with no observed toxicities. Administration of weekly doses of TAK-500 induced enhanced and sustained tumor burden suppression. To determine the in vivo effect of STING activation in an immune competent model of CMML, we then evaluated the effects of TAK-500 and dazostingag in *Vav-KDM6B/Tet2* $^{f/f}$*/Vav-Cre* mice, a previously reported (Wei et al Leukemia 2022) animal model with CMML phenotype. Treatment with TAK-500 led to enhanced apoptosis of Ccr2 $^{+}$ BM cells in these mice. Moreover, the number of colonies formed by lineage negative/Sca1 positive/cKit positive BM progenitor (LSK) cells isolated from TAK-500 treated mice was significantly lower than in vehicle treated control mice, suggesting a negative

regulation of repopulating capacity of *Tet2* deficient/ *KDM6B* overexpressing BM HSPCs by TAK-500. Evaluation of the efficacy of TAK-500 and dazostingag in patient-derived xenograft models of primary CMML samples is planned.
Taken together, our data suggests that CCR2-directed STING activation might have therapeutic potential in HR-MyMo neoplasms by depleting CCR2 $^{+}$ cells and impairing clonal HSPC repopulation potential.

Disclosures Appleman: *Takeda:* Current Employment. **Hatten:** *Takeda:* Current Employment. **Huang:** *Takeda:* Current Employment. **Raizer:** *Takeda:* Current Employment. **Parent:** *Takeda:* Current Employment. **Lineberry:** *Takeda:* Current Employment. **Garcia-Manero:** *Bristol Myers Squibb:* Other: Medical writing support, Research Funding; *Genentech:* Research Funding; *AbbVie:* Research Funding. **Montalban-Bravo:** *Takeda:* Research Funding; *Rigel:* Research Funding.

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