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Blood 142 (2023) 335–337

The 65th ASH Annual Meeting Abstracts

ORAL ABSTRACTS

651.MULTIPLE MYELOMA AND PLASMA CELL DYSCRASIAS: BASIC AND TRANSLATIONAL

Mezigdomide Reverses T-Cell Exhaustion through Degradation of Aiolos/Ikaros and Reinvigoration of Cytokine Production Pathways

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Introduction: T cell exhaustion (Tex) is characterized by progressive decline in activation and proliferation and has been implicated as a major resistance mechanism of T cell immunotherapies such as BCMA directed bispecifics approved for the treatment of multiple myeloma (MM). Understanding and overcoming Tex is key for relapsed patient populations facing drug resistance. Here, by using the next generation cereblon E3 ligase modulator (CELMoD) Mezigdomide (Mezi), a specific and potent Aiolos/Ikaros degrader to date, we revealed for the first time through comprehensive genomic/epigenomic and functional analyses that Aiolos/Ikaros are two major transcription factors that contribute to the regulation of the Tex phenotype and their degradation reverses the negative impact on activation, proliferation and tumor cell killing.
Methods: Tex were generated *ex vivo* from normal donors through multiple rounds of anti-CD3/CD28 dynabead stimulations over the course of 14 days. Unstimulated (Tn) or transiently stimulated T cells (Tact) from the same donors were used for differential comparisons. Comprehensive genomic analyses were conducted to compare the three groups of T cells using RNAseq, ATACseq, whole genome bisulfite sequencing and ChIPseq for Aiolos/Ikaros and histone modifications (H3K27ac, H3K27me3, H3K4me3 H3K4me1), with and without Mezi treatment for 72 hrs. Cytokine/chemokines were measured by Luminex multiplex cytokine assays. Exhaustion markers were determined by flow cytometry. Mezi effects on Tex mediating tumor cell killing in combination with anti-CD3/BCMA bispecific antibody Alnuctamab (Alnuc) was also assessed via both incucyte and flow cytometry.
Results: After multiple rounds of stimulation, the viability of Tex remained over 90%, with proliferation decreased from 3-6-fold in initial stimulation to 1-1.4-fold in the last stimulation, as well as 98% reduction in IL-2 secretion. RNAseq analysis showed more proinflammatory cytokine expression from Tex was significantly down-regulated compared to Tact, such IL-1/6/9/17/21/23 and TNF. Exhaustion markers CTLA4, PD1, TIM3, LAG3, TOX, PDL1, and ENTPD1 (CD39) were analyzed via flow cytometry and confirmed by RNAseq to be significantly increased in expression after initial activation, then maintained and/or increased further with subsequent re-stimulations. Treatment of Mezi significantly down regulated PD1, TIM3, TIGIT, and up-regulated proinflammatory cytokines/chemokines such as IL-1/23/3/6/7, CCL1/2/17/20, CXCL1/3/11/12 and TNF, as well as cell surface interaction related integrins, RAS signaling and proliferation related pathways, as revealed by both Luminex multiplex cytokine analysis and RNAseq (Fig.A).
Using ATAC-seq, we found that the peaks gained or lost in Tex at promoters and distal sites were significantly correlated with gene expression changes from the RNAseq analysis, with the strongest effect seen at promoters. We identified that canonical RUNX1/2/3 binding motifs were significantly enriched in the gained peaks in Tex compared to Tact, with RUNX2/3 themselves also showing increased mRNA expression. After treatment of Mezi, Tex gained peaks were enriched with NFATC2 and RUNX3 binding sites whose gene expression levels were also increased (Fig. B). Enrichment analysis of the cis-regulated genes of the gained peaks in Tex after treatment highlights biological processes related to cell-cell adhesion, cytokine production and other T cell activation processes. Notably, TF analysis also showed that, after treatment, Ikaros motifs were enriched in the Tex gained peaks. Flow cytometry analysis revealed that Tex cells have the highest expression of Ikaros, especially in PD1 $^{+}$ TIM3 $^{+}$ CD4 subsets.
To investigate the effect of Aiolos/Ikaros in Tex functionally, we set up co-culture assays using Tex cells with BCMA expressing tumor cells in the presence of Alnuc. We found that Mezi treatment to Tex significantly enhanced killing of tumor cells and reduced the EC_{50} of Alnuc. 5-10 fold compared to DMSO controls.
Conclusion: We demonstrated here that Aiolos/Ikaros regulate and maintain T cell exhaustion. Mezi mediated degradation of both transcription factors at the same time resulted in enhanced proinflammatory cytokine expression and reduction of

exhaustion associated markers, as well as enhanced Tex killing of BCMA expressing tumor cells in combination with Alnuctamab.

Disclosures Chiu: *Bristol Myers Squibb:* Current Employment, Current equity holder in publicly-traded company. **Zhao:** *Bristol Myers Squibb:* Current Employment, Current equity holder in publicly-traded company. **Ortiz Estevez:** *Bristol Myers Squibb:* Current Employment, Current equity holder in publicly-traded company. **Hagner:** *BMS:* Current Employment, Current equity holder in publicly-traded company. **Gandhi:** *Bristol Myers Squibb:* Current Employment, Current equity holder in publicly-traded company.

https://doi.org/10.1182/blood-2023-189445

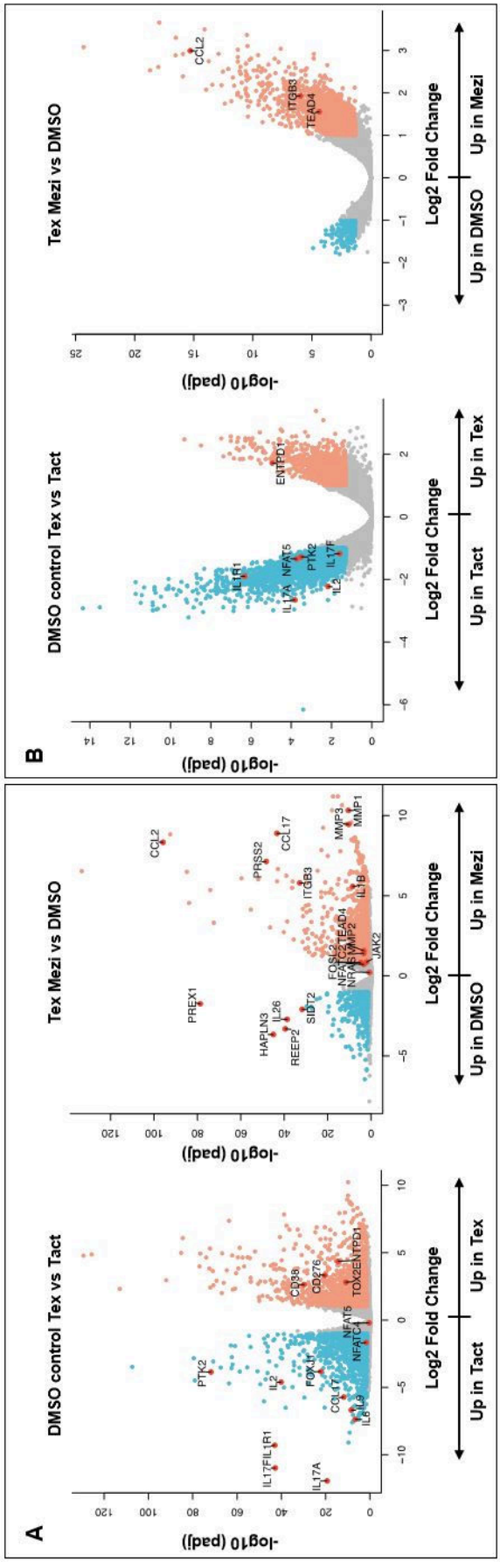

Figure 1