BET INHIBITORS SYNERGIZE WITH ANTI-PD1 BY ENHANCING TCF7 ACCESSIBILITY IN LEUKEMIA-DERIVED TERMINALLY EXHAUSTED CD8+ T CELLS

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Background Our lab and others have shown that a proportion of blood and bone marrow specimens from AML patients have an immunosuppressive microenvironment and have hallmarks of immune exhaustion: increased frequencies of regulatory T cells (Tregs)1 and myeloid-derived suppressor cells (MDSCs),2 decreased T-cell proliferation,3 elevated expression of immune checkpoint molecules4 and increased TEx vs. TPEX5–7 populations. Importantly, a subset of these patient samples containing dysfunctional T cells can be rescued by ICB.[3]. We have identified the same immune-related features, in a mouse model of AML. BET inhibitors (BETi) have been shown to positively affect CD8+ T cells, indirectly via reduction of PD-L1 expression on myeloid cells8,9 and directly by inhibition of chronic TCR activation genes, increasing stem cell like memory CD8+ T cells.[10,11] We hypothesized that BETi may synergize with anti-PD1 therapy in AML through promoting T cell stemness.

Methods Our AML mouse model bears FLT3-ITD and deletion of TET2 restricted to the myeloid lineage (LysM-CRE). For in vitro studies, splenocytes were stimulated with anti-CD3 and either JQ1, anti-PD1 or both and proliferation and differentiation status were assessed by flow cytometry. For in vivo studies, treatment consisted of 2 weeks. s3-ATAC-seq: Cells were prepared as described.12 Libraries were sequenced on a NextSeq 2000. Data was then analyzed and visualized using the ArchR. GSE Accession: GSE205386.

Results We show that inhibitors which target bromodomain and extra-terminal domain (BET) proteins rescue T cell exhaustion. Ex vivo treatment of cells from AML mice and AML patients with BET inhibitors (BETi) reversed CD8+ T cell exhaustion by restoring proliferative capacity and expansion of the more functional TPEX (figure 1). This reversal is enhanced by combined BETi and anti-PD1 treatment. BETi synergized with anti-PD1 in vivo, resulting in the reduction of circulating leukemia cells, enrichment of CD8+ T cells in the bone marrow, and increased expression of Tcf7, Slamf6, and Cxcr5 in CD8+ T cells (figure 2). Finally, we profiled the epi-genomes of in vivo JQ1 treated AML-derived CD8+ T cells by single-cell ATAC seq and find that JQ1 increases Tcf7 accessibility specifically in TEx cells, suggesting that BETi likely mechanistically acts by relieving repression of progenitor programs in TEx CD8+ T cells and maintaining a pool of anti-PD1 responsive CD8 T cells (figure 3).

Conclusions Using an AML mouse model that exhibits leukemia-induced immune exhaustion, we demonstrate the pre-clinical efficacy of combining BETi and anti-PD1 therapy in the treatment of AML.

REFERENCES

Abstract 899 Figure 1 BETi partially rescues T cell function in vitro Splenocytes were isolated from 7 AML (a) or 6 WT mice (b), stained with CFSE, and cultured for 72 hours without TCR stimulation (HIgG), anti-CD3 alone, anti-CD3 with anti-PD1 or titrations of anti-CD3 with JQ1 or both JQ1 and anti-PD1. Cells were stained and analyzed by flow cytometry. Plots represent the fold-change in CD8 T cell proliferation treatment of cells from AML mice and AML patients with BET inhibitors (BETi) reversed CD8+ T cell exhaustion by restoring proliferative capacity and expansion of the more functional TPEX. Cells were prepared as described. Libraries were sequenced on a NextSeq 2000. Data was then analyzed and visualized using the ArchR. GSE Accession: GSE205386. Significance determined by Kruskal-Wallis multiple comparisons T-tests.
Abstract 899 Figure 2  In vivo treatment with BETi and anti-PD1.

a.) Schematic detailing in vivo BETi + anti-PD1 treatment strategy and functional readouts of efficacy. b.) Mice treated with RlgG, JQ1, JQ1 with anti-PD1, or anti-PD1 alone were bled periodically over a two-week period and assessed for WBC. Data displays fold-change WBC (k/mL) normalized per mouse in comparison to pre-treatment bleed WBC. Significance determined by one-way ANOVA. Timepoints are Pre-Bleed, Mid-bleed (day 7), Endpoint (day 14). c, d.) Bone marrow cells were isolated from treated AML and WT mice and assessed by flow cytometry. Graph denotes %CD3+ T cells in the bone marrow (c) and %CD8+ T cells in the bone marrow as a percent of all T cells (d). Significance derived from combining two experimental replicates and determined by one-way ANOVA. e.) CD8+ T cells were isolated from JQ1-treated and RlgG-treated AML mice and RNA harvested and analyzed by Nanostring. Volcano plot shows the fold-change in normalized transcript levels of JQ1-treated mice vs RlgG treated AML mice vs. –log10 P-value as determined by multiple t-tests. Hits of interest are highlighted in red. Dashed red line denotes significance threshold (0.05).

Abstract 899 Figure 3  ATAC-seq of CD8 T cells from animals treated with BETi.

a.) (Top) cartoon schematic describing the cell types fit to the differentiation trajectory (bottom). Colors represent pseudotime values throughout TEx differentiation. b-c.) Pseudotime trajectory plot with corresponding TCF7 gene score throughout TEx differentiation for b.) Vehicle treated cells and c.) JQ1 treated cells. Vertical red dashed marks the third quartile throughout pseudotime (late TPEx/early Tex) and the horizontal red dashed line markers the TCF7 accessibility at pseudotime 100. Significance was calculated by mann-whitney T-test comparing the aggregate TCF7 gene scores for each cell throughout pseudotime in vehicle and JQ1 treated groups. d.) Schematic of the ATAC signal peaks vs treatment and cluster which were generated in ArchR. (Top) Vehicle treated Tex, Naive, Late TPEx, and early Tex cells. (Bottom) Matching clusters for JQ1 treated cells. Grey boxes highlight greatest differential peaks in the TEx cells between Vehicle and JQ1 treated cells. e.) A portion of splenocytes derived from the vehicle or JQ1 treated AML mice were analyzed by flow cytometry. Data represents TCF1 median fluorescence intensity of TCF1 on all CD8+ T cells. Significance determined by Mann-Whitney T-test. f-h.) Volcano plot describing the mean difference in gene scores (JQ1 – Volcano treated) from f.) Tex cells only (Cluster 5), g.) late TPEx cells only (Cluster 7), and h.) early TPEx cells only (Cluster 8). Significance calculated via multiple corrected t-tests. Genes with a mean difference >.25 are highlighted in red and genes < -.25 are highlighted in blue. Genes of interest (Tcf7, TcF3, Pdcd1, and Cxcr3) are highlighted in black. The dashed horizontal line marks the significance cutoff. Genes below the line are not significant.