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PD-L1 CHECKPOINT BLOCKADE ERADICATES RESIDUAL LEUKEMIA IN A MOUSE MODEL OF ACUTE LYMPHOBLASTIC LEUKEMIA BY COUNTERING EXHAUSTION OF CYTOTOXIC AND EFFECTOR CD4+ T-CELLS

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Background Phenotypic exhaustion of CD4+ T-cells is a strong negative prognostic factor in acute lymphoblastic leukemia (ALL).¹⁻³ Despite this, PD1/PD-L1 immune checkpoint therapy has shown little activity in this disease setting to date. Factors influencing the responsiveness of the T-cell compartment to checkpoint blockade are unknown.

Methods An established murine model of BCR-ABL+ ALL was used. Leukemia was established by tail vein injection, and mice were treated with the BCR-ABL tyrosine kinase inhibitor nilotinib with or without PD-L1 mAb therapy. scRNAseq/TCRseq was performed using multiple treatment groups.

Results Treatment of leukemia-bearing mice with a combination of the BCR-ABL tyrosine kinase inhibitor nilotinib and PD-L1 immune checkpoint blockade led to eradication of leukemia in 70% of treated mice (figure 1). Efficacy was dependent on the presence of CD4+ T-cells, while CD8+ T-cells appeared to play a lesser role. Direct cytotoxicity by CD4+ T-cells was confirmed in live cell-killing assays (figure 2). Mice that were treated with PD-L1 blockade and survived to day 100 were found to have no detectable residual leukemia. They were also protected from leukemia rechallenge, suggesting the elicitation of a memory response. scRNAseq analysis revealed that CD44^{hi} CD4+ T-cells were highly heterogeneous, with regulatory, effector, and stem-like TCF7+ precursor subsets present (figures 3-4). A unique population of CD4+ T-cells was elicited by live leukemia challenge (clusters 6 and 7 in figure 3) but not by vaccination with heat-killed leukemia cells. This subset was characterized by relatively low levels of expression of TCF7, but high levels of expression of Granzyme B, TOX, the effector cytokines IFN γ and TNF α , the inhibitory receptors PD1, TIM3, and LAG3, and the chemokine CCL5 (figure 5). PD-L1 checkpoint blockade was associated with early narrowing of the clonality of this population (figure 6), decreased markers of exhaustion, and more robust synthesis of TNF α .

Conclusions PDL1 immune checkpoint blockade is effective at eradicating residual disease in preclinical models of BCR-ABL + ALL. ALL elicits a unique CD4+ memory/effector subset characterized by the potential for both chemotactic and cytotoxic functions. Leukemia induces early exhaustion of this subset, which is countered by PDL1 blockade. Efforts to extend these observations to human specimens are underway and will be reported.

Abstract 321 Figure 1 Survival analysis. BCR-ABL+ ALL was established by tail vein injection on day 0. Nilotinib (75 mg/kg) was administered via oral gavage. mAbs targeting PD-L1 with or without depleting antibodies towards CD4 or CD8 were administered via intraperitoneal injection. p-value derived by log-rank analysis

Abstract 321 Figure 2 Analysis of the increase in the number of dead cells (y-axis) over time (x-axis) from a live killing assay (Incucyte) using splenic CD4+ or CD8+ T-cells from experimental arms as treated in figure 3. Control traces from separate wells with LM138 target cells only are included. Experiments were done using Cytotox NIR

Abstract 321 Figure 3 (Left) Experimental approach. 5 groups (n=4 mice/group) were treated in parallel with the indicated conditions. CD44^{hi} CD4⁺ T-cells from the spleen and bone marrow of mice in each group were labelled with oligo-conjugated hashtag antibodies (Biolegend) and CITE-SEQ antibodies towards PD1, TIM3, LAG3, CD25, and TIGIT, prior to FACS-sorting. scRNAseq/TCRseq analysis (10x Genomics) was performed on 5,349 individual cells after multiplet removal. (Right) UMAP plots of all cells combined. Clusters were identified by differential expression of canonical gene products

Abstract 321 Figure 4 Feature plots demonstrating expression of canonical gene products projected onto the UMAP plot in figure 3. Antibody derived tags (ADTs; bottom row) indicate expression level of surface proteins profiled using CITESEQ antibodies

Abstract 321 Figure 5 Heatmap of select gene product expression levels in exhausted (cluster 6) CD4⁺ T-cells across treatment conditions

Abstract 321 Figure 6 Simpsons diversity index of the TCR repertoire across treatment arms. Lower values indicate relatively decreased clonality

REFERENCES

- 1.. Hoftari H, Brück O, Blom S, *et al.* Immune cell constitution in bone marrow microenvironment predicts outcome in adult ALL. *Leukemia* 2019;**33**(7):1570–1582.
- 2.. Blaesckhe F, Willier S, Stenger D, *et al.* Leukemia-induced dysfunctional TIM-3. *Leukemia* 2020;**34**(10):2607–2620.
- 3.. Liu L, Chang YJ, Xu LP, *et al.* T cell exhaustion characterized by compromised MHC class I and II restricted cytotoxic activity associates with acute B lymphoblastic leukemia relapse after allogeneic hematopoietic stem cell transplantation. *Clin Immunol* 2018;**190**:32–40.

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