COMMON TRAJECTORIES OF HIGHLY EFFECTIVE ANTI-CD19 CHIMERIC ANTIGEN RECEPTOR-MODIFIED T CELLS IDENTIFIED BY ENDOGENOUS T CELL RECEPTOR LINEAGES

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Background Chimeric antigen receptor modified (CAR) T cells have revolutionized the treatment of blood cancers, though some patients still show a poor response in either CAR expansion, effector response, or persistence.¹ In this study, we determined the features of pre-infusion CAR-transduced T cells that generated optimally functional responses after infusion.

Methods Using both the pre-infusion product and PBMCs isolated at weeks 1–4, 8, and 3-months post-infusion from 15 patients undergoing experimental anti-CD19 CAR T cell treatment for refractory or relapsed B-ALL, we generated a comprehensive single cell gene expression and T cell receptor (TCR) sequencing dataset on over 180,000 CAR T cells (figure 1).

Results As expected, pre-infusion CAR T cells tend to highly express genes associated with proliferation, while post-infusion CARs show signs of either cytotoxic effector differentiation or dysfunctional terminal differentiation. Sequencing of the endogenous TCR, at the single cell level, allows us to track the trajectories of clonally and transcriptionally related cells (figure 2). Post-infusion cells with significant cytotoxic effector function share TCRs with a statistically defined subset of CARs in the pre-infusion sample (figure 3). Using a machine learning approach, we found that potent effector precursor CAR T cells have a specific transcriptional profile distinct from the other pre-infusion CAR T cells, including markers of early effector function such as increased EOMES, GNLY, GZMH, GZMK, KLRD1, and IFN γ . Formalizing this signature, we have developed a robust classifier that can predict with 82.8% accuracy whether a CAR T is likely to become a favorable effector based on its pre-infusion profile (figure 4). This prediction model can be used to evaluate the extent to which a patient's generated CAR product will be able to mount a robust response after encountering its target. Additionally, there are a number of genes, as a part of this signature, that are expressed on the cell surface and can be utilized as a method to differentiate the effector precursor pre-infusion CAR T cells from other pre-infusion CARs, including CD52, CD74, CD86, and LAG3, among others.

Conclusions Our findings suggest a therapeutic approach that enriches these cells prior to infusion resulting in superior per cell CAR effector activity.

Abstract 152 Figure 1 Clustering of 184,791 CAR-transduced T cells based on gene expression

Abstract 152 Figure 2 Alluvial plot depicting CAR T cell lineage tracing using the endogenous T cell receptor

Abstract 152 Figure 3 Visualization of CAR T cell clusters with arrows indicating the shared TCRs between pre-infusion and post-infusion cells

Abstract 152 Figure 4 Machine learning classifier of pre-infusion, early effector CAR T cell phenotype

REFERENCE

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Ethics Approval This study was approved by St. Jude Children's Research Hospital's Institutional Review Board (IRB); IRB number Pro00007661. All patients consented to the use of materials for the research study.

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