Title: *Genome-wide In vivo CRISPR Screens to Uncover Regulators of CAR-T Cell Antitumor Activity in DLBCL*

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Introduction:

Chimeric antigen receptor (CAR) T-cell therapy has revolutionized the treatment of hematological malignancies, including lymphomas. Despite remarkable initial responses, up to 60% of patients eventually relapse, mainly driven by complex transcriptional networks that push CAR-T cells toward dysfunctional fates. To systematically identify genes involved in CAR-T cell functionality, we performed in vivo CRISPR screens in a clinically relevant DLBCL mouse model.

Methods:

We designed a pooled single-guide RNA (sgRNA) library targeting ~21,000 genes implicated in CAR-T cell function. This library was engineered in a plasmid construct expressing an anti-CD19 CAR cassette (CD19 scFv, 28z) and the ALFA-tag as a surface marker for transduction detection and selection. T cells isolated from Rosa26-Cas9 knock-in mice were transduced, bypassing the need for electroporation. In vivo screens were conducted by adoptive transfer of CAR-T cells into autochthonous Myd88/Bcl2-driven DLBCL mice.

To validate screening hits, we generated a transgenic mouse model carrying a Cre-Lox-regulated anti-CD19 CAR construct for constitutive expression in naïve T cells, a Cas9 knock-in allele for efficient gene editing, and a CD45.1 (Ptprc) background for unambiguous in vivo tracking of CAR-T cells.

Results:

In vitro expansion screens demonstrated robust counter-selection of pan-essential genes after 14 days, confirming library performance. Enrichment of genes involved in sphingosine metabolism suggests a fitness advantage under IL-2/IL-7-supplemented culture conditions. Regulators involved in mTOR recruitment to the lysosome were also enriched, consistent with known CAR-T cell biology.

Characterization of the transgenic model revealed efficient CAR expression on $CD3^+$ splenocytes. These CAR-T cells specifically recognized and killed $CD19^+$ lymphoma cells, showing similar cytotoxicity and cytokine production profiles compared to retrovirally transduced CAR-T cells expressing the same construct. CRISPR/Cas9-mediated editing and CAR-T cell differentiation profiling are currently underway.

Outlook:

Building on these results, we have initiated genome-wide in vivo screens by treating DLBCL-bearing mice with 10^7 library-transduced CAR-T cells. Enriched hits from these screens will be validated using our transgenic platform, which, combined with the autochthonous Myd88/Bcl2-driven DLBCL model, offers a powerful system for dissecting cellular and genetic determinants of CAR-T cell efficacy. Together, these tools provide a unique framework to uncover mechanisms of therapy resistance and, ultimately, to develop strategies that improve patient outcomes.

Keywords:

CAR-T cell therapy, cellular therapy, lymphoma, diffuse large B-cell lymphoma