iPSC-DERIVED CD4+, CD8+ αβT CELLS THAT EXPAND UPON ANTIGEN STIMULATION AND EXHIBIT IN VIVO TUMOR CONTROL

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INTRODUCTION

The clinical success of autologous CAR-T cells relies upon acute cytotoxicity, expansion upon target engagement, cytokine secretion, and persistence but so far iPSC-derived cells cannot recapitulate adult αβT cell functionality. Notably, autologous CAR-T cells are a mix of mature CD4+ and CD8+ cells, where each plays a supporting or direct cytotoxic role, respectively.

We report the scalable generation of feeder-free CD4+ and CD8+ αβ iPSC-CAR-T cells that specifically expand upon target engagement, secrete the critical cytokine IL-2, and successfully control tumor cells in both BCMA and CD19 systemic tumor models. In addition to rapid tumor control, iPSC-derived CAR-T cells showed temporary control of tumor re-challenge 36 days after initial tumor clearance and persistence in the bloodstream three weeks after infusion. In sum, we have developed a process that can generate engineered iPSC CAR T cells that perform comparably to primary T cells in a full suite of preclinical assays, unlocking the potential of the platform to solve key challenges faced by allogeneic cell therapies.

TUNABLE GENERATION OF CD4+ AND CD8+ αβT CELLS

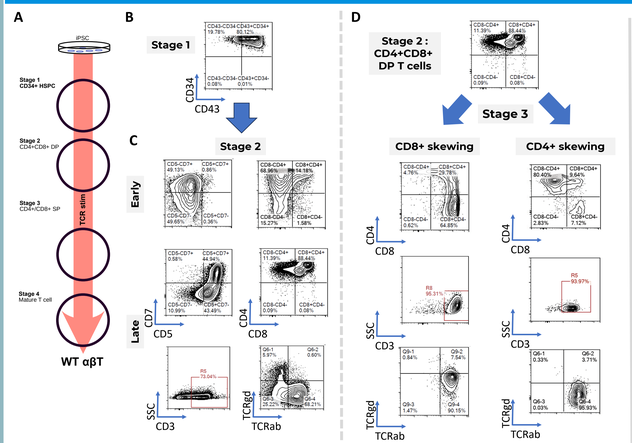
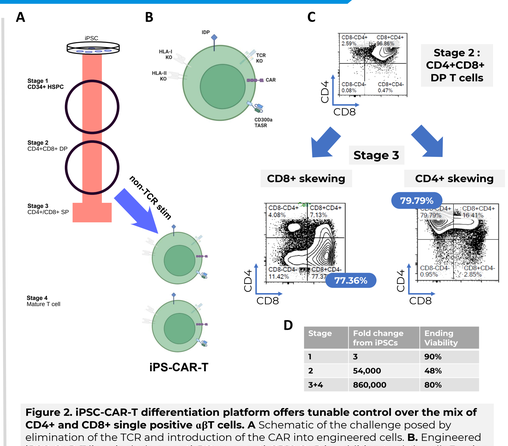


Figure 1. Century differentiation platform generates TCRαβ+ CD4+ and CD8+ T cells from WT iPSCs in a scalable, feeder-free culture system. A. Schematic of differentiation from unengineered (WT) iPSCs. **B.** iPSC differentiation through Stage 1 produces CD34+CD43+ progenitors. **C.** WT iPSC-derived T cells robustly generate CD4+/CD8+ immature T cells that express CD3 and TCRab. **D.** Through a controllable process, WT iPSC-derived T cells mature into CD8SP and CD4SP αβT cells.



Stage	Fold change from iPSCs	Ending Viability
1	3	90%
2	54,000	48%
3+4	860,000	80%

Figure 2. iPSC-CAR-T differentiation platform offers tunable control over the mix of CD4+ and CD8+ single positive αβT cells. A Schematic of the challenge posed by elimination of the TCR and introduction of the CAR into engineered cells. **B.** Engineered iPSC-CAR-T lines include an anti-BCMA or anti-CD19 CAR in addition to a) the AlloEvasion 5.0 pair of IDP and CD300a TASR, b) knockout of HLA class I and II, and c) knockout of the TCRab complex. **C.** CAR engineered, Stage 2 CD4+CD8+ DP T cells are differentiated to single positive CD4+ or CD8+ T cells in a process that can be purposely shifted by alteration of conditions.

iPSC-CAR-T DEMONSTRATE αβT CELL FUNCTION IN VITRO

The Century iPSC-CAR-T differentiation platform generates cells with an anti-BCMA or anti-CD19 CAR that create a controlled balance of CD4+ and CD8+ cells. These cells a) access recognizable cell states in biological T cell development, b) activate transcriptional signatures upon reaching the end of Stage 4 maturation, and c) express a repertoire of extracellular receptors that match primary T cells.

A functional iPSC-CAR-T cell product requires three critical in vitro attributes to maximize the chances of clinical efficacy:

Therapeutic efficacy requires:	Cytotoxicity: Effector function	Cytokine (IL2) production	Cell expansion
1° CAR-T	✓	✓	✓
Century's αβ iPSC-CAR-T	✓	✓	✓

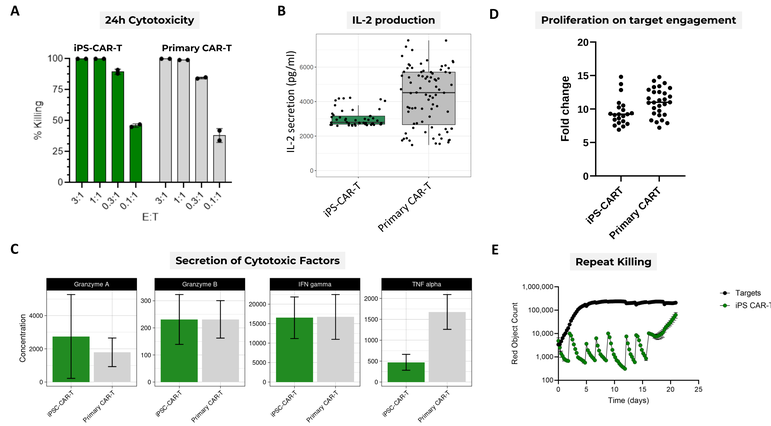


Figure 5. Century iPSC-CAR-T cells demonstrate robust T cell function in vitro. A. 24-hour cytotoxicity assay demonstrates short-term cytotoxicity on par with primary CAR-T in a cell engineered with anti-BCMA CAR. **B.** IL-2 production detected by ELISA after 48-hour exposure to targets is similar to primary T cell levels. **C.** Quantification of secreted proteins important for cytotoxicity show similar levels between iPS-CAR-T and primary CAR-T. Data shown for effectors with CD19 CAR challenged with Nalm6 targets for 48 hours. **D.** Antigen-mediated effector expansion evident after 5-day target exposure by cell counts after verification that targets are absent. **E.** IPSC-CAR-T cells kill multiple rounds of tumor challenge at an E:T 0.5:1 in an assay without cytokine support.

SINGLE iPS-CAR-T INFUSION CONTROLS TUMOR, PERSISTS IN VIVO

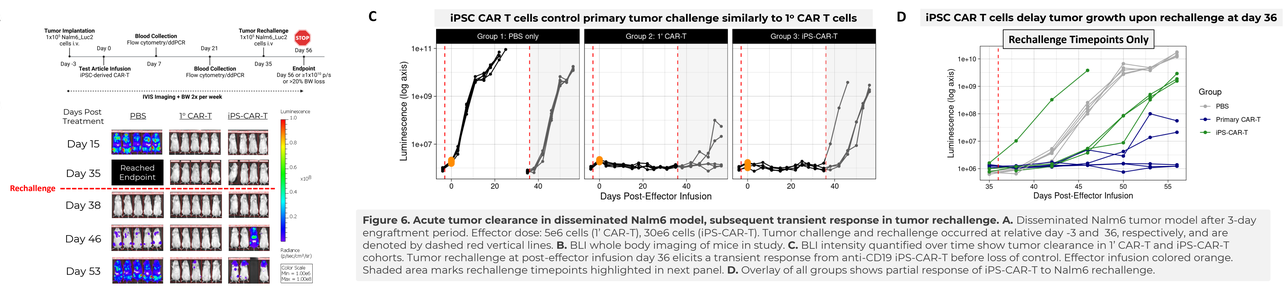


Figure 6. Acute tumor clearance in disseminated Nalm6 model, subsequent transient response in tumor rechallenge. A. Disseminated Nalm6 tumor model after 3-day engraftment period. Effector dose: 5e6 cells (1' CAR-T), 30e6 cells (iPS-CAR-T). Tumor challenge and rechallenge occurred at relative day -3 and 36, respectively, and are denoted by dashed red vertical lines. **B.** BLI whole body imaging of mice in study. **C.** BLI intensity quantified over time show tumor clearance in 1' CAR-T and iPS-CAR-T cohorts. Tumor rechallenge at post-effector infusion day 36 elicits a transient response from anti-CD19 iPS-CAR-T before loss of control. Effector infusion colored orange. Shaded area marks rechallenge timepoints highlighted in next panel. **D.** Overlay of all groups shows partial response of iPS-CAR-T to Nalm6 rechallenge.

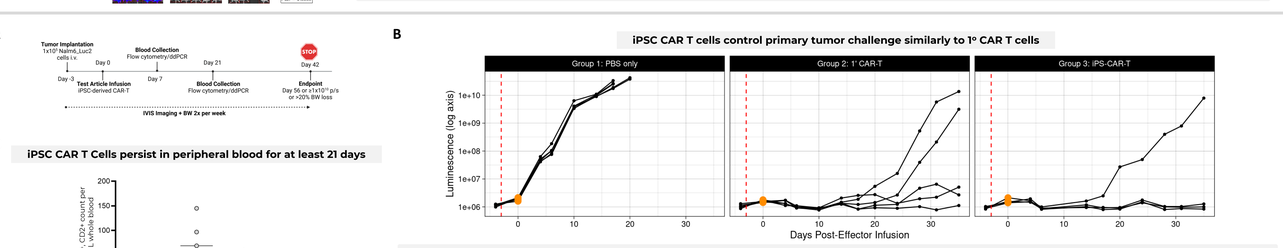


Figure 7. Extended persistence of circulating iPS-CAR-T cells in vivo. A. Disseminated Nalm6 tumor model after 3-day engraftment period. Effector dose: 5e6 cells (1' CAR-T), 30e6 cells (iPS-CAR-T). **B.** BLI traces quantified over time show sustained tumor control in iPS-CAR-T cohorts compared to 1' CAR-T cell cohort. Dotted red line indicates time of tumor challenge. Effector infusion colored orange. **C.** Circulating effector cells quantified by flow cytometry at day 7 and 21 post-effector infusion. iPS-CAR-T and 1' CAR-T cohorts have detectable circulating effectors at both time points. No data available for post-effector infusion day 7 cells in Group 4 due to failure to collect blood sample.

iPS-CAR-T CELLS TRAVERSE THYMOCYTE DEVELOPMENT

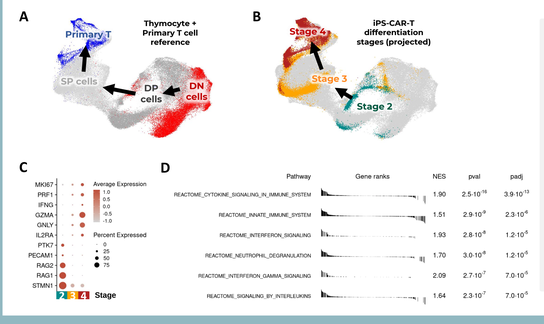


Figure 3. Century iPSC-CAR-T differentiation traverses biologically recognizable path. A. UMAP projection of a subset of a public thymocyte development atlas (Park et al.) integrated with internal adult primary T cell data. Colored by development stage. **B.** Projection of iPS-CAR-T data into reference UMAP space. **C.** Differential expression of progenitor versus mature T cell genes across differentiation stage. **D.** Gene set enrichment analysis of Stage 4 versus Stage 2 cells reveals enrichment of cytokine, interleukin, and interferon gamma signaling.

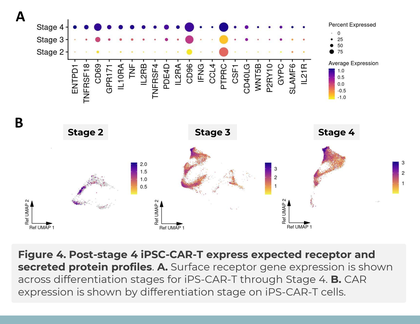


Figure 4. Post-stage 4 iPSC-CAR-T express expected receptor and secreted protein profiles. A. Surface receptor gene expression is shown across differentiation stages for iPS-CAR-T through Stage 4. **B.** CAR expression is shown by differentiation stage on iPS-CAR-T cells.