Development of Next-Generation, Off-the-Shelf CAR T-cell Immunotherapies for Solid Tumors

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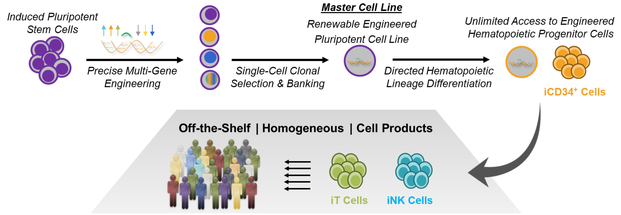
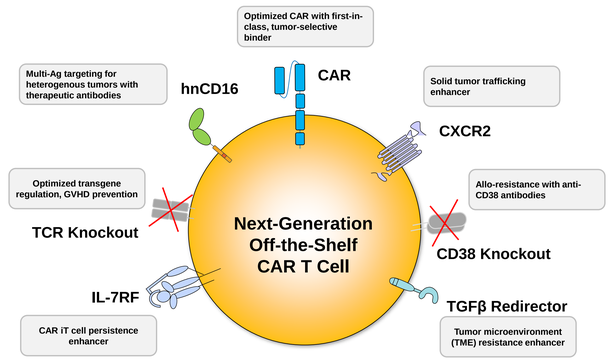
GRAPHICAL ABSTRACT & INTRODUCTION

A Novel Platform for Creating Off-the-shelf Multiplex-Engineered Hematopoietic Cell Products



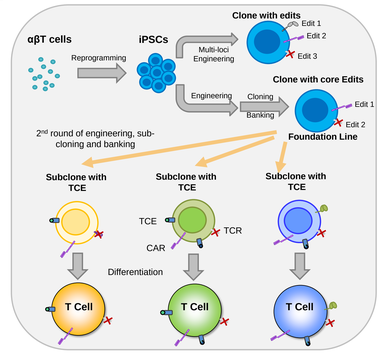
- Induced pluripotent stem cells (iPSCs) are a unique renewable starting material for the manufacture of homogenous off-the-shelf immune cells.
- FT819 is a first-of-kind off-the-shelf chimeric antigen receptor (CAR)-T cell therapy derived from a renewable master iPSC line engineered to uniformly express a novel CD19 1XX-CAR driven by the endogenous (TCR) promoter at the T-cell receptor α constant (TRAC) locus, to provide access to off-the-shelf availability, antigen specificity and improved safety.
- Despite promising clinical outcomes in patients with hematologic malignancies, the adoptive transfer of engineered T cells armed with a chimeric antigen receptor (CAR) has been less effective against advanced solid tumors. Specific challenges that have emerged include lack of tumor-exclusive antigen targets, antigen heterogeneity, and functional suppression resulting from the tumor microenvironment.



- To further enhance the effectiveness of iPSC-derived CAR-T cells (CAR-iTs) in solid tumor settings, we sought to develop a novel off-the-shelf CAR-T cell product engineered to express multiple **T** **C**ell **E**nhancers (**TCE**s, e.g. a cytokine receptor signaling complex) at desired loci.
- The novel multiplex-engineered, off-the-shelf CAR T-cell incorporates seven functional modalities: 1) a CAR for direct targeting of tumor antigen; 2) a cytokine receptor fusion protein for enhanced T-cell activity; 3) a high-affinity, non-cleavable CD16 (hnCD16) for enhanced antibody-dependent cellular cytotoxicity (ADCC) in combination with tumor-targeting monoclonal antibodies; 4) an engineered modality for enhanced trafficking; 5) a novel chimeric protein for enhanced functionality in response to tumor microenvironment resistance signaling; 6) CD38 deletion for enhanced metabolic fitness; and 7) TRAC-targeted TCR deletion for eliminating the risk of GVHD.

RESULTS

Precision Engineering at the Single-Cell Level Provides Unprecedented Capacity to Test the Functional Impact of Six Functional Edits



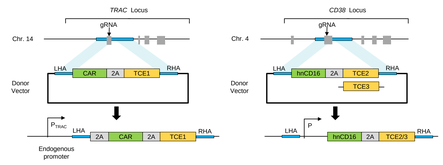
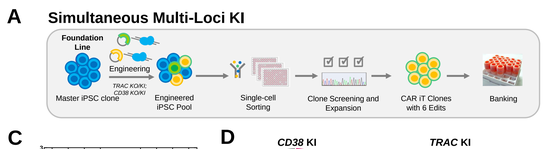
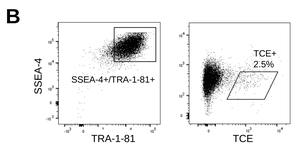
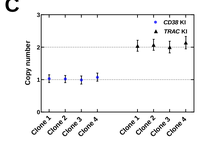


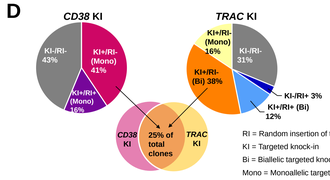
Figure 1. Multi-loci engineering of six functional edits. **(A)** Versatile engineering platform for iPSC-based single cell engineering and cloning. **(B)** Engineering strategy. Molecular engineering design for CAR and TCE1 knock-in into *TRAC* locus (left); hnCD16 and TCE2/3 knock-in into *CD38* locus (right). For each locus, Top: Locus depiction; Middle: donor plasmid containing the transgene cassette flanked by homology arms; Bottom: edited locus.

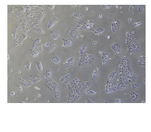
Novel Process to Facilitate the Derivation of Mono- and Bi-allelic Integration of Selected Transgenes into Multiple Loci at the Single-Cell Level

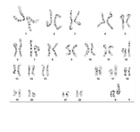


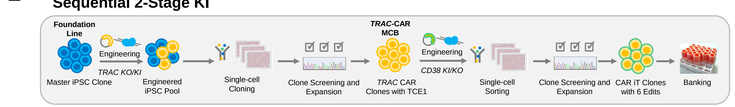


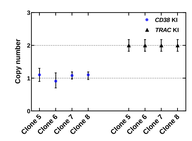


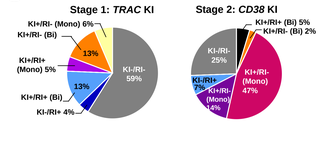












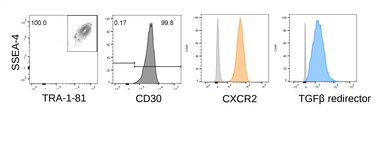


Figure 2. Confirmation of specific bi-allelic and mono-allelic integration of selected transgenes into the *TRAC* and *CD38* loci. **(A, E)** Workflow for the generation of a CAR-TiPSC master cell bank containing 6 edits with simultaneous multi-loci engineering strategy or sequential knock-in engineering strategy. **(B)** Representative flow cytometry profile depicts selection strategy of cells expressing pluripotency markers and TCE. **(C, F)** Transgenes for *CD38* KI (hnCD16) and *TRAC* KI copy number was determined using ddPCR. **(D, G)** Molecular characterization of KI edited clones shows the distribution of engineered iPSC clones with targeted knock-in (KI) integration and no random insertion (RI) at each locus in both strategies. **(I)** Representative cytogenetic analysis of engineered iPSC clones demonstrates a normal, diploid karyotype. **(J)** Representative flow cytometry profile of clonal iPSCs demonstrates expression of pluripotency markers TRA-1-81, SSEA-4, CD30 and transgenes (grey depicts non-engineered iPSC clone, orange depicts CXCR2, blue depicts TGFBβ redirector in engineered iPSC clone).

Multiplex Engineered CAR-iT Cells Demonstrate Optimized Fitness and Enhanced Efficacy for Solid Tumor Cells

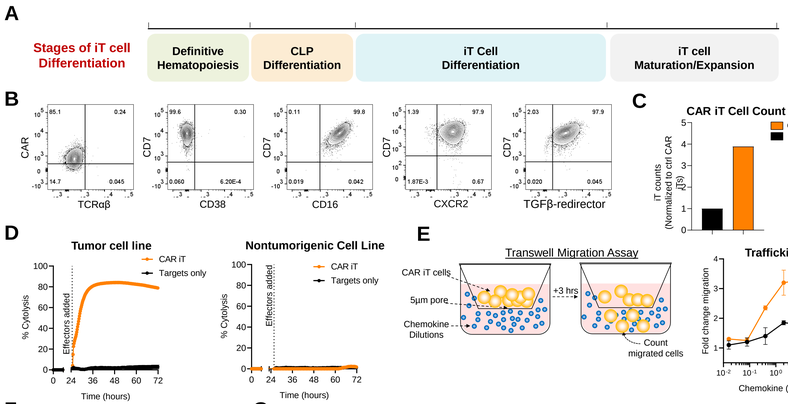


Figure 3. Multiplex engineered CAR-iTs show improved fitness and anti-tumor efficacy in vitro. **(A)** Schematic of iPSC-derived T cell (iT) differentiation protocol. **(B)** Representative flow cytometry profile demonstrates uniform expression of transgenes, as well as depletion of CD38 and TCR in CAR-iT cells. **(C)** Fold expansion of engineered CAR iT cells compared with control CAR-iTs without the modality following cognate antigen activation demonstrates improved persistence. **(D)** Cellular cytotoxicity of CAR-iT cells against malignant tumor cells (left panel) and non-tumorigenic cell lines that express the antigen (right) demonstrates the high efficacy, selectivity and specificity of the CAR binder. **(E)** Transwell migration assays (left panel) demonstrate that multiplex engineered CAR-iT cells have significantly higher chemotaxis to the chemokine compared with control CAR-iT cells without the modality (right panel). **(F)** Cellular cytotoxicity of CAR-iT clones with hnCD16 in combination with therapeutic antibodies demonstrates that Antibody Dependent Cellular Cytotoxicity (ADCC) supports CAR iT effector function compared with control CAR-iT cells without the modality. **(G)** Mixed lymphocyte reaction (MLR) assays (left panel) demonstrate that Daratumumab (DARA) protects CAR-iT cells with CD38 KO when co-cultured with PBMCs (right panel) by selectively depleting alloreactive CD38+ lymphocytes (middle panel, 9 days post co-culture). **(H)** Cellular cytotoxicity of engineered CAR-iT cells against tumor cells in the presence of immune antagonist component TGFβ demonstrates enhanced efficacy and TME resistance of CAR-iT cells.

SUMMARY

- We have demonstrated that through precisely controlled genetic editing at the clonal level, specific genes can be introduced into multiple loci with high efficiency and versatility.
- Using this method, a first-of-kind iPSC-derived CAR-T cell was generated containing seven unique functional modalities at defined loci and exhibited superior anti-tumor efficacy, including enhanced persistence and in vitro anti-tumor efficacy, improved trafficking potential and TME resistance, as well as protective effect from allo-rejection.
- Our data validates the potential of engineering multiple modalities at desired loci of iPSCs to enable the creation of functionally enhanced CAR-T cells in a consistent and reproducible manner. This unprecedented capacity to engineer cells at the clonal-level is achieved by our iPSC platform which uniquely facilitates the generation of single cell-derived multiplexed engineered iPSCs.
- Ongoing work is focused on extending these studies into disease specific in vivo models, as well as further investigating other synthetic candidates and genomic loci for the development of next-generation iPSC derived CAR-T therapies for solid tumors.