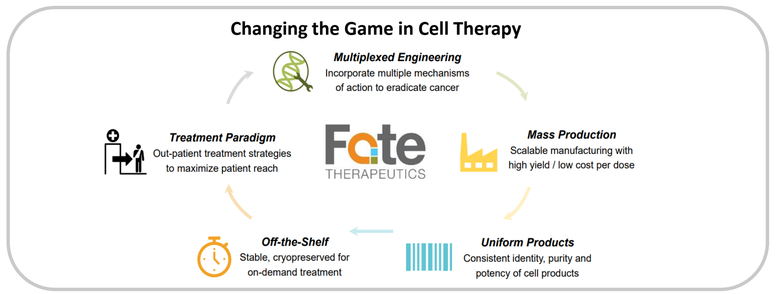
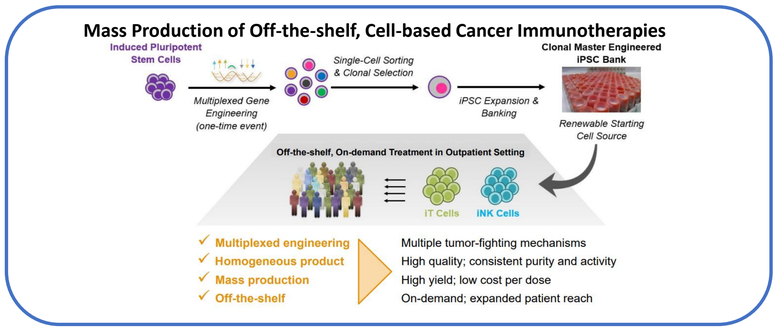
Robust T-cell Cellular Reprogramming and Single-cell Engineering Platform Overcomes Inconsistencies and Heterogeneity Associated with Engineering Primary T cells

Yi-Shin Lai, Greg Bonello, Angela Macia, Jonathan Lee, Brent Wilkinson, Lauren Fong, Mobina Roshandell, Janel Huffman, Raedun Clarke, Tom Lee, Ramzey Abujarour and Bahram Valamehr*

Fate Therapeutics, Inc., San Diego, CA, USA *Corresponding author: Bob.Valamehr@fatetherapeutics.com

GRAPHICAL ABSTRACT & INTRODUCTION





Introduction

- Adoptive transfer of primary T cells expressing chimeric antigen receptors (CARs) has shown great promise in treating hematologic malignancies.
- Application of CAR T-cell therapy is limited by several challenges, including drug product inconsistencies and heterogeneity resulting from engineering primary T-cell populations as part of drug product manufacture.
- We report here the derivation and qualification of cGMP T cells from consented and diverse donors for cellular reprogramming
- T cell banks were used to generate clonal iPSC lines which were evaluated for critical quality attributes including viability, purity and potency.

cGMP donor-derived T cell banks

- Derived from eligible and fully consented donors
- Compliant with US and international donor eligibility requirements
- Banks characterized for purity, safety, identity, and viability

Optimized cell reprogramming and engineering

- Fate proprietary non-integrating reprogramming system resulted in naïve pluripotency from diverse donors
- Precisely-engineered T cell-derived iPSC clones generated and showed robust recovery, viability, genomic stability, and differentiation potential

SUMMARY

- Donor T cells were sourced from leukapheresis blood collections following consent and screening for infectious disease markers
- αβ T-cell population was isolated and banked in animal component-free cryopreservation medium
- Robust reprogramming of T cell banks (n= 8 independent donors) demonstrated using Fate proprietary reprogramming platform (Valamehr et al., 2014)[1] regardless of donor background
- T cell-derived iPSCs (from 4 parental banks) were efficiently engineered (20-60% knock-in efficiency) and maintained uniform pluripotent phenotype and genomic stability post engineering
- This study demonstrates the potential to use clonally-derived, master engineered TiPSC lines as a renewable source for the consistent and uniform manufacture of off-the-shelf CAR T cells for therapeutic applications

1. Stem Cell Reports. Valamehr et al., 2014 Mar 6;2(3):366-81.

RESULT

Derivation and testing of cGMP T cell banks from diverse consented healthy donors

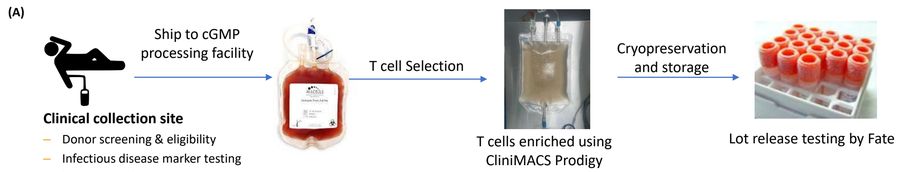


Figure 1. Generation and testing of donor-derived T cell banks. (A) Workflow of T cell banking process including donor tissue procurement by clinical collection sites, TCR αβ cells isolation and cryopreservation in the cGMP processing facility and testing by Fate. A full characterization was performed prior to release of the qualified banks for MCB generation. (B) Testing attributes of T cell banks. (C) T cell bank testing summary.

T cell-optimized stage-specific media and reprogramming system for generation of footprint-free naïve TiPSCs

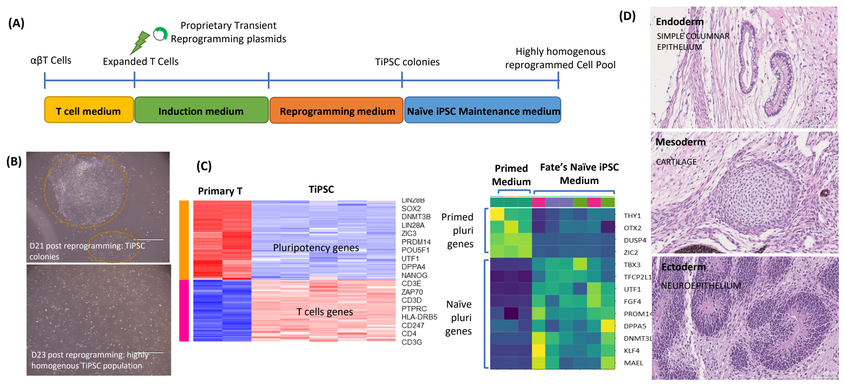


Figure 2. Generation of footprint-free and feeder-free naïve iPSCs from T cells. (A) Workflow of T cell reprogramming process. Expanded T cells were transfected with Fate's proprietary transient reprogramming plasmids that includes master regulator of pluripotency and dedifferentiation factors. Stage-specific media system promotes efficient T cell reprogramming and drives naïve pluripotency. (B) Highly homogenous TiPSC populations generated in 4 weeks. Naïve iPSC platform enables multiplex engineering, clonality and single cell expansion. (C) Fate naïve iPSC medium promotes expression of key regulators and naïve pluripotency genes. (D) Naïve TiPSCs exhibit pluripotency and propensity to differentiate into cells of the three germ layers as determined by the teratoma formation assay.

Robust generation of T cell-derived iPSCs from diverse donors regardless of background or attributes

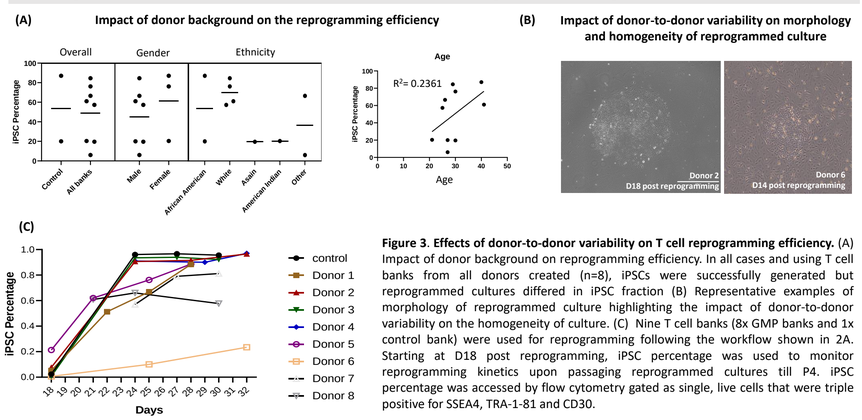
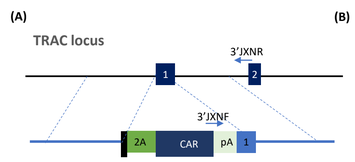


Figure 3. Effects of donor-to-donor variability on T cell reprogramming efficiency. (A) Impact of donor background on reprogramming efficiency. In all cases and using T cell banks from all donors created (n=8), iPSCs were successfully generated but reprogrammed cultures differed in iPSC fraction (B) Representative examples of morphology of reprogrammed culture highlighting the impact of donor-to-donor variability on the homogeneity of culture. (C) Nine T cell banks (8x GMP banks and 1x control bank) were used for reprogramming following the workflow shown in 2A. Starting at D18 post reprogramming, iPSC percentage was used to monitor reprogramming kinetics upon passaging reprogrammed cultures till P4. iPSC percentage was accessed by flow cytometry gated as single, live cells that were triple positive for SSEA4, TRA-1-81 and CD30.

Robust CRISPR-mediated engineering of TiPSCs from multiple donors

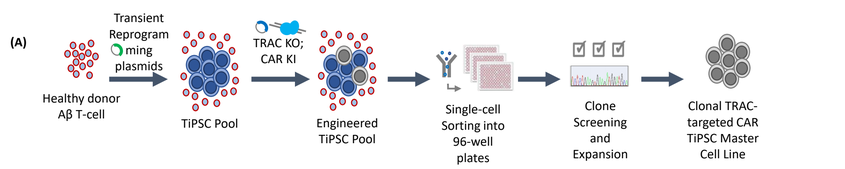


(B)

Sample	TRAC targeting efficiency (3'JXN CN)	Post-engineering karyotyping
iPSCs - control	0.47	TBD
iPSCs - donor 2	0.44	Normal
iPSCs - donor 3	0.61	Normal
iPSCs - donor 4	0.29	Normal
iPSCs - donor 5	0.26	TBD

Figure 4. CRISPR-mediated knock-in of CAR in TiPSCs derived from independent donors. (A) TRAC locus targeting of CAR transgene. CRISPR ribonucleoprotein (RNP) complex and donor plasmid were transfected into TiPSCs to mediate specific integration of CAR transgene into the TRAC locus. (B) TiPSCs derived from five T cell donors (one control and four GMP banks) were used to test TRAC engineering. Since CAR expression is not detectable at iPSC stage, engineering efficiency was determined by assessing CAR copy number (CN) of integrated transgene (3'JXN primers) by droplet digital PCR (ddPCR). A non-engineered clone was used as negative control (CN = 0) and a bi-allelic clone was used as positive control (CN = 2); not shown. G-banding karyotype analysis was performed for after engineering.

Generation of multiplexed engineered TiPSC clonal cell lines that maintain quality and genomic stability



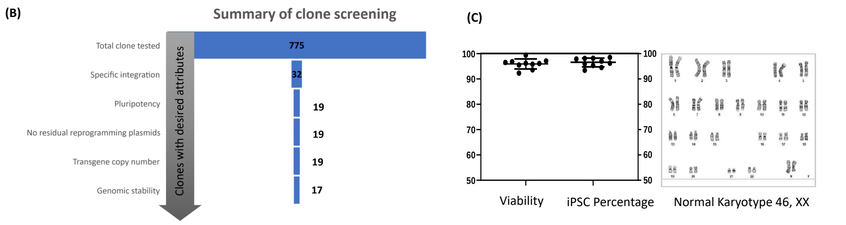


Figure 5. Engineered TiPSC clones maintain high quality and genomic stability. (A) Schematic presentation of TRAC-targeted CAR TiPSC cell line generation. CRISPR-mediated engineering was performed on the reprogrammed TiPSC pool from Donor 2, followed by single cell sorting into 96-well plates and clone screening for indicated test attributes. Qualified TiPSC clones were expanded and cryopreserved. (B) Summary of clone screening. Specific engineering was determined by on-target integration (flanking and junction PCRs) without random insertion (PCR for plasmid backbone). Pluripotency was determined by cell morphology and flow cytometry of iPSC marker expression. Residual reprogramming plasmid was determined by quantitative PCR. Transgene copy number was determined by ddPCR. Genomic stability was determined by G-banding karyotype analysis. (C) Characterization of engineered TiPSC clones post cryopreservation. Viability upon thaw and iPSC percentage (left, n=10) and representative karyotype analysis from a post-cryo clone (right). All clones maintained normal karyotype post-cryo (n=10).

Precise targeting of 1XX CAR19 into the TRAC locus for improved CAR T cell function, phenotype and avoidance of GvHD.

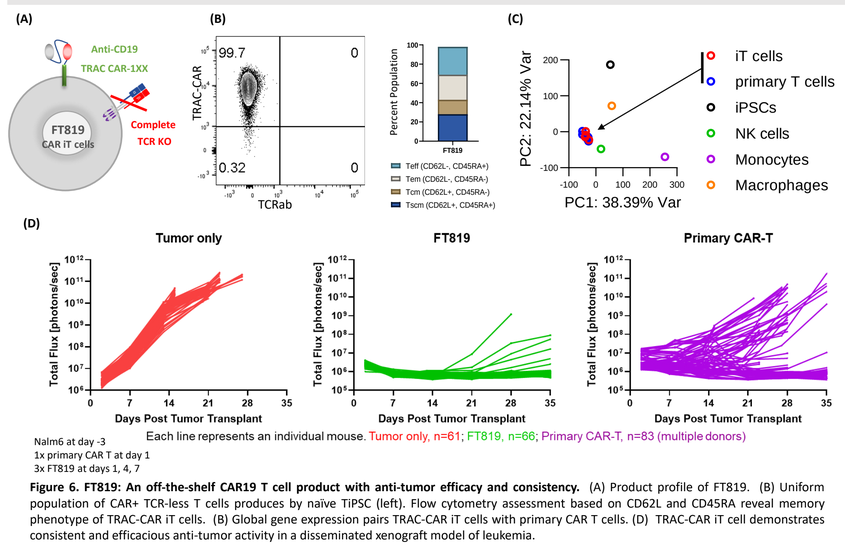


Figure 6. FT819: An off-the-shelf CAR19 T cell product with anti-tumor efficacy and consistency. (A) Product profile of FT819. (B) Uniform population of CAR+ TCR-less T cells produces by naïve TiPSC (left). Flow cytometry assessment based on CD62L and CD45RA reveal memory phenotype of TRAC-CAR iT cells. (B) Global gene expression pairs TRAC-CAR iT cells with primary CAR T cells. (D) TRAC-CAR iT cell demonstrates consistent and efficacious anti-tumor activity in a disseminated xenograft model of leukemia.