Multiplexed-engineered, iPSC-derived T Cells Expressing Three Unique Targeting Modalities Address Tumor Heterogeneity and Antigen Escape

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Fate THERAPEUTICS

Abstract 5044

ABSTRACT

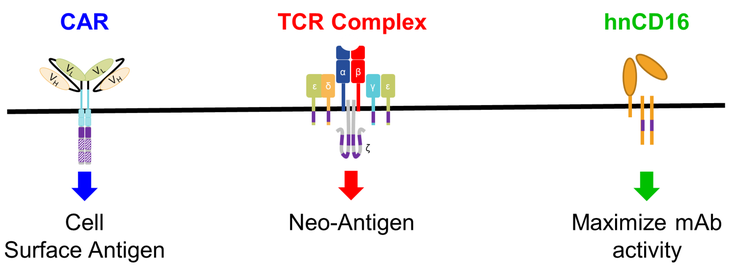
Adoptive T-cell therapy with chimeric antigen receptor (CAR) has shown promising results in cancer treatment, however, antigen escape and tumor heterogeneity are major causes for disease relapse. While CARs are known to trigger an effective immune response through surface antigen recognition many solid tumor cancer antigens are intracellular and presented by MHC molecules recognized by T cell receptors (TCRs). In addition, many therapeutic antibodies have shown clinical efficacy in solid tumor settings. However, antibody-dependent cellular cytotoxicity (ADCC) is mediated by the CD16 Fc receptor naturally expressed on NK cells although its application in T cells is yet not fully appreciated.

Utilizing our proprietary induced pluripotent stem cell (iPSC) platform to engineer multiple modalities into a clonal iPSC line, which can serve as the starting cell source for mass production of off-the-shelf, iPSC-derived CAR-T cells (CAR-iT cells), we aimed to study the combination of these three targeting modalities, CAR, TCR, and CD16, to determine whether challenges associated with the treatment of solid tumors, which are heterogeneous and challenging to treat, may be overcome.

To test the base line activity of CAR-iT cells in the solid tumor setting, we selected our anti-MICA/B CAR, previously shown to effectively target stress ligands found on transformed cells, to demonstrate effective anti-tumor activity against multiple solid tumor cell lines (72 hrs cytotoxicity: A2058 = 99%; 786-O = 98%; versus non-specific CAR-iT cells: A258 = 13%; 786-O = 17%). To test compatibility of TCR in our iT cell platform, we engineered MR1-TCR in iT cells to show increased cytokine release and degranulation upon stimulated with MR1 positive lung carcinoma epithelial cells line A549 (fold change compared to un-stimulated: IFNg = 210, p = 0.0032; TNFa = 76.9, p = 0.0005; CD107ab = 115.0, p=0.0013). Notably, with the engineering of tumor antigen specific TCR in TCR-less CAR-iT cells, CD3 complex can be re-established to provide an opportunity to combine with bispecific T cell engagers. Lastly, combining CAR-iT cells with MR1-TCR and hnCD16 uniquely demonstrated synergistic tumor growth inhibition and validated our approach to target multiple antigens at once for an effective anti-tumor response (A549 cytotoxicity: tumor only = 3.68±2.04%; effector+TCR = 41.31±2.27%; effector+TCR+ADCC = 90.28±1.87%).

In summary, using the unique approach to engineer iPSCs at the clonal level to create a distinct population of engineered iT cells, we successfully demonstrated the compatibility between CAR, TCR, and hnCD16 to mitigate tumor heterogeneity. This approach is an ideal strategy to create off-the-shelf cellular immunotherapy for a promising therapeutic approach to combat heterogeneous and difficult to treat solid tumors, including those that are resistant due to antigen escape.

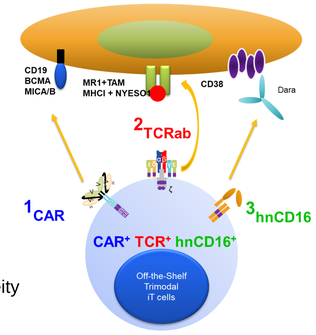
GRAPHICAL ABSTRACT



Enhance Specificity and Multi-antigen Targeting:
Use of synthetic biology to integrate multiple antigen-targeting modalities into iPSC derived T cells for enhanced anti-tumor activity

SUMMARY

- The use of clonally-derived, master induced pluripotent stem cell (iPSC) lines is an attractive source for the renewable manufacture of precisely-engineered, homogenous CAR T-cell products that can be fully characterized, stored, and administered on-demand for broad patient access.
- In addition to CAR, engineered tumor antigen specific TCR and hnCD16 modalities are compatible with iT cell platform to expand the tumor targeting capability
- Tri-modal (CAR + TCR + hnCD16) iT cells demonstrate additive tumor killing effect to address tumor heterogeneity for both liquid and solid tumor models



RESULT

Fate iPSC Platform for Mass Production of Universal NK and T-Cell Products

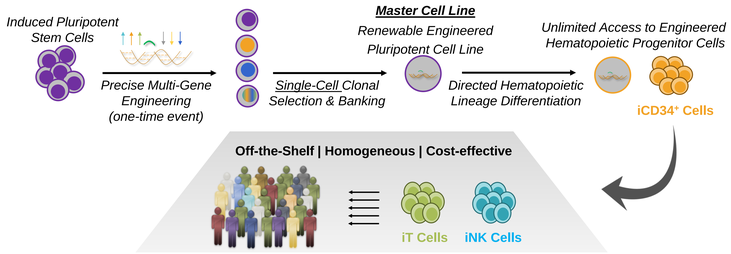


Figure 1. Illustration of Fate Therapeutics off-the-shelf iPSC platform.

Phenotype of iPSC Derived CAR-T Cell

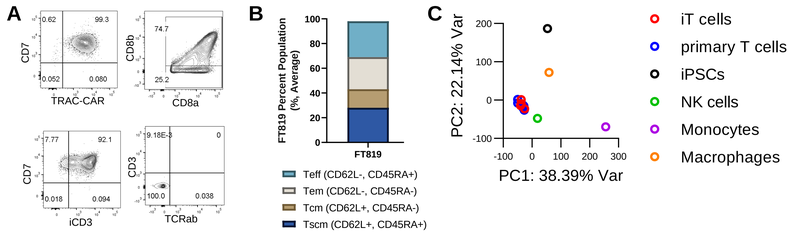


Figure 2. (A) Representative FACS plots show iPSC derived CAR-T cells express high percentage of standard T cell markers while knock-out of surface TCRab and CD3 complex. (B) Flow cytometry assessment based on CD62L and CD45RA reveal memory phenotype of CAR-iT cell. (C) RNA seq profiling analysis shows iPSC derived CAR-T cells closely cluster with primary T cells.

Durable and Consistent Tumor Growth Inhibition in Comparison to Primary CAR T-cells

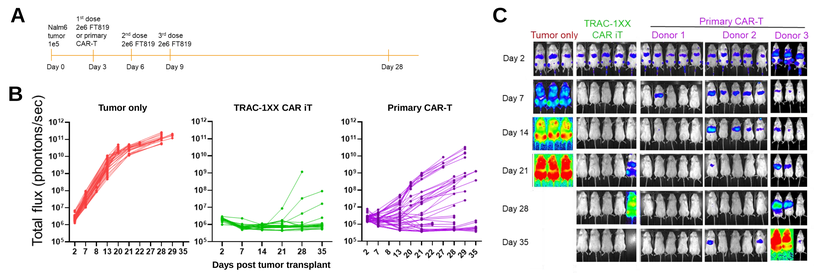


Figure 3. (A) In vivo design of disseminated xenograft model of leukemia. (B and C) CAR iT-cells demonstrate consistent capacity to control tumor growth in vivo as detected in BLI tumor burden assessment.

Novel Pan-Tumor Targeting Anti-MICA/B CAR iT-cells Exhibit Potent Immune Response to Multiple Cancer Cell Lines and Effectively Inhibit Tumor Growth

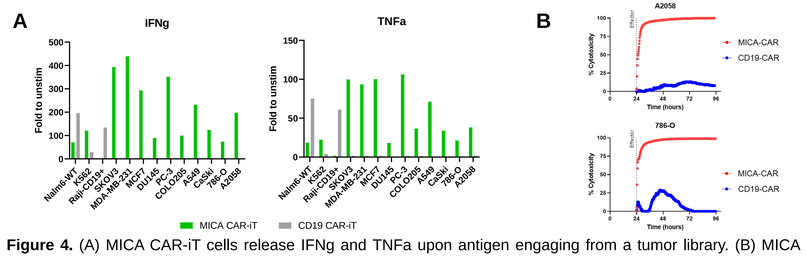


Figure 4. (A) MICA CAR-iT cells release IFNg and TNFa upon antigen engaging from a tumor library. (B) MICA CAR-iT cell effectively control tumor growth in an in vitro long-term killing assay. (A2058=melanoma ; 786-0= renal cell carcinoma)

iPSC Derived T cells With Engineered Tumor Antigen Specific TCRs Against MR1 or NYESO1 Expand the Scope of Tumor Antigen Targeting

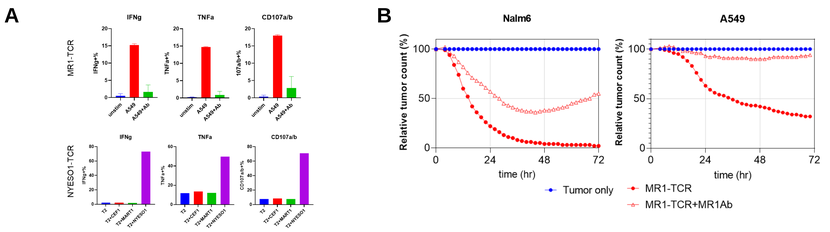


Figure 5. (A) Cytokine releasing and degranulation of iPSC derived T cells equipped with engineered MR1-TCR and NYESO1-TCR upon antigen encounter. (B) MR1-TCR mediated tumor killing by iT cells in an antigen specific manner

Novel Trimodal iT Cells (CAR+, TCR+, hnCD16+) Demonstrate the Compatibility Between CAR, TCR, and hnCD16 to Enhance Anti-Tumor Activity and Mitigate Tumor Heterogeneity

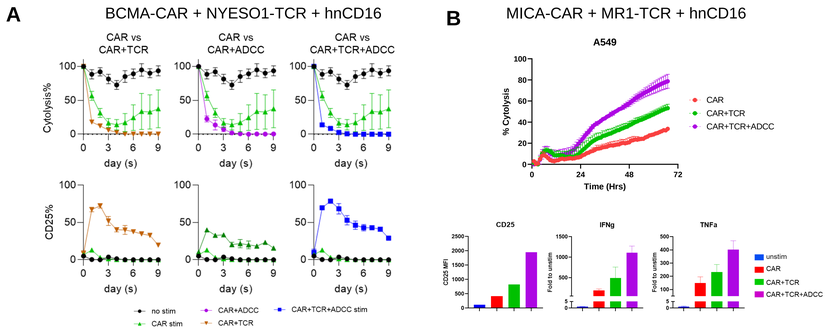


Figure 6. (A) iPSC derived T cells expressing 1) BCMA-CAR, 2) NYESO1-TCR and 3) hnCD16 show additive effect to enhanced tumor clearance in an acute lymphoblastic leukemia Nalm6 tumor model. (B) iPSC derived T cells expressing 1) MICA-CAR, 2) MR1-TCR and 3) hnCD16 reveal an additive effect to better control tumor growth with an adenocarcinoma solid tumor line, A549.

Trimodal iT Cells (BCMA-CAR, NYESO1-TCR, hnCD16) Show Unique Ability to Control a Xenograft Model Consisting of a Heterogenous Population of Three Different Tumor cells

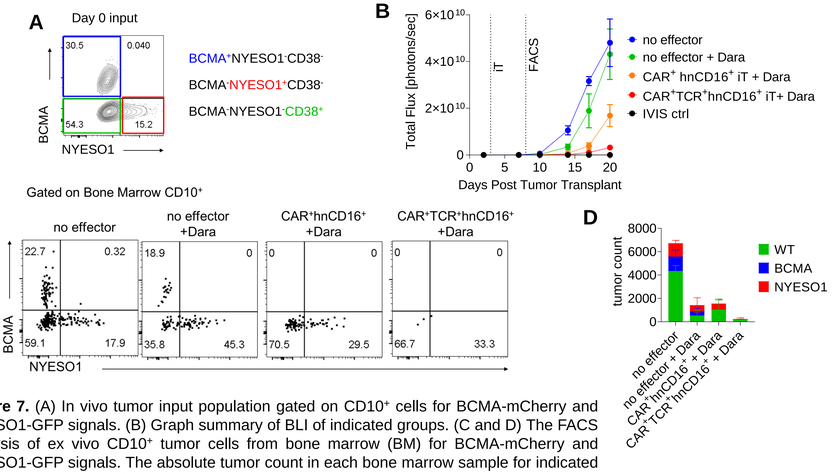


Figure 7. (A) In vivo tumor input population gated on CD10+ cells for BCMA-mCherry and NYESO1-GFP signals. (B) Graph summary of BLI of indicated groups. (C and D) The FACS analysis of ex vivo CD10+ tumor cells from bone marrow (BM) for BCMA-mCherry and NYESO1-GFP signals. The absolute tumor count in each bone marrow sample for indicated tumor lines are plotted for all groups.