ABSTRACT

Adaptive 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, multiple therapeutic antibodies have shown clinical efficacy in solid tumor settings. Here we report the development of an antibody-dependent cellular cytotoxicity (ADCC) 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 inducible pluripotent stem cell (iPSC) platform to engineer multiple modalities into a chimeric T-cell, which can serve as the starting cell source for mass production of off-the-shelf iPSC-derived CAR T (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.

RESULTS

Graphical Abstract

Figure 1. Illustration of Fate Therapeutics off-the-shelf platform. It cells primary T cells iPSCs NK cells Monocytes Macrophages

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 TCRb and CD3 complex. (B) Flow cytometry assessment based on CD69 and CD107a reveal memory phenotype of CAR-iT cell. (C) RNAseq 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 by BLU 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 TNFα and IFNγ upon antigen engaging from a tumor library. (B) MICA CAR iT-cell effectively control tumor growth in an in vivo long-term killing assay.

Novel Trimodal iT Cells (CAR- TCR, hNCD16) Demonstrate the Compatibility Between CAR, TCR, and hCD16 in Enhancing Anti-Tumor Activity and Mitigate Tumor Heterogeneity

Figure 5. (A) Cytoxicity releasing and degradation of iPSC derived T cells equipped with engineered M1R-TCR and NYESO1-TCR upon antigen encounter. (B) M1R-TCR mediated tumor killing by iT cells in an antigen specific manner

Tricodal iT Cells (BCMA-CAR, NYESO1-TCR, hNCD16) Show Unique Ability to Control a Xenograft Model Consisting of a Heterogeneous Population of Three Different Tumor cells

Figure 6. (A) iPSC derived T cells expressing 1) BCMA-CAR, 2) NYESO1-TCR and 3) hNCD16 show additive effect to inhibit tumor growth in in vivo tumor studies. (B) Scheme of syngeneic NYESO1 expressing Ad5 vector 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 model (A549).

Graphical Abstract

Graphical Abstract

Dendritic Cell

Summary

• The use of clonally derived, master induced pluripotent stem cell (iPSC) lines is an attractive source for the renewable manufacture of precisely-engineered, homogeneous 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.

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.

GRAPHICAL ABSTRACT

CAR

TCR Complex

hnCD16

Neo-Antigen

Cell Surface Antigen

Maximize mAb activity

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