304 OFF-THE-SHELF IPSC-DERIVED CAR-T CELLS CONTAINING SEVEN FUNCTIONAL EDITS OVERCOME ANTIGEN HETEROGENEITY, IMPROVE TRAFFICKING, AND WITHSTAND IMMUNOSUPPRESSION ASSOCIATED WITH FAILED TUMOR TREATMENT

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Background Although chimeric antigen receptor (CAR) T-cell therapy has shown remarkable efficacy in liquid tumors, its wider application to solid tumors has been hampered by tumor-associated antigen (TAA) heterogeneity, inefficient CAR-T cell trafficking to the tumor, and immunosuppression inherent to the tumor microenvironment. Moreover, the often-dysfunctional and heterogenous yield of highly-edited (*e.g.* >2 transgenes) CAR-T cells necessary to address these obstacles has limited their efficacy and wider clinical investigation.

Methods T-cell derived induced pluripotent stem cells (TiPSCs) were engineered to express a CAR targeting a novel TAA domain and an interleukin-7 receptor fusion protein (IL7RF) under the regulation of the T-cell receptor alpha chain constant locus. Additionally, these TiPSCs were engineered to express TGFβ-signal redirection receptor (TGFβ-SRR), high-affinity non-cleavable CD16A (hnCD16), and CXCR2 within the CD38 locus enabling TGFβ resistance, secondary TAA targeting via antibody-dependent cell cytotoxicity (ADCC), and solid tumor specific trafficking, respectively. Engineered TiPSCs were differentiated into alpha-beta T (iT) cells, uniformly expressing all engineered transgenes and completely lacking both CD38 and T-cell receptor expression, avoiding the potential risk of graft-versus-host disease in an allogeneic setting.

Results Functional evaluation of these multiplexed-engineered CAR iT cells demonstrated broad, potent, and specific CAR based efficacy across multiple solid tumor indications. Importantly, hnCD16 activation with multiple therapeutic antibodies further augmented anti-tumor efficacy across target cell lines with heterogenous CAR antigen expression, underscoring the broad efficacy, applicability, and multi-antigen targeting capability of these CAR iT cells. Additionally, engineered CAR iT cells maintained high levels of anti-tumor efficacy over multiple rounds of tumor challenge, even in the presence of high and suppressive levels of TGFβ (Round 3 challenge with TGFβ: 90% cytolysis with TGFβ-SRR expressing CAR iT cells vs. 10% cytolysis with control CAR iT cells), demonstrating potent resistance to TGFβ-mediated effector suppression. CXCR2 expression enabled specific and potent migration to the CXCR2 ligand IL-8 (2-fold specific migration, 10ng/ml) that has been associated with, along with other CXCR2 ligands, poor outcomes in diverse tumor indications. Furthermore, we demonstrate robust anti-tumor efficacy in an aggressive ovarian cancer xenograft model which, when coupled with the activation of hnCD16 via therapeutic antibody, reached complete tumor clearance.

Conclusions Together these results demonstrate that a multiplexed-engineered CAR-iT cell product tailored for solid tumors can promote trafficking, overcome tumor microenvironment resistance, and elicit enhanced anti-tumor activity.

Ethics Approval These studies were approved by Fate Therapeutics Institutional Animal Care and Use Committee and were carried out in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals.

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