

ENGINEERING OF SYNTHETIC CHEMOKINE RECEPTORS INTO iPSC-DERIVED CAR-T CELLS TO INCREASE HOMING AND ENHANCE TRAFFICKING INTO SOLID TUMORS

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Background Despite the success of chimeric antigen receptor (CAR)-T cells in treating hematological malignancies, the efficacious treatment of solid tumors has been hampered by lack of CAR-T cell persistence, tumor-associated antigen heterogeneity, and the immuno-suppressive tumor microenvironment (TME). In addition, trafficking of CAR-T cells to solid tumors, potentially due to a chemokine-chemokine receptor mismatch between the tumor and the CAR-T cells, has proven ineffective. Early and sustained detection of T cells within a solid tumor has been associated with better outcomes across several clinical trials, suggesting that strategies focused on enabling CAR-T cell homing and trafficking can generate significant therapeutic benefit.

Methods IL8/CXCL8, a ligand for the chemokine receptor CXCR2, has been detected in many cancer types including ovarian, breast, prostate and renal and is often associated with poor prognosis and overall survival. We have previously shown high baseline expression and inducible upregulation of IL8 following chemo- or radiotherapy conditioning in multiple tumor lines. We therefore engineered CXCR2 into induced pluripotent stem cell (iPSC)-derived CAR-T (CAR iT) cells to express a synthetic CXCR2 transgene.

Results CXCR2-engineered iPSCs were differentiated to manufacture alpha-beta CAR iT cells expressing uniform and high levels of the chemokine receptor (>90% CXCR2 expression). Importantly, CXCR2 expression did not affect CAR-dependent effector function. CXCR2 engineered CAR iT cells demonstrated specific and functional *in vitro* chemotactic migration to recombinant IL8 and tumor preconditioned media (up to 3 fold increase compared to control CAR iT cells). In preclinical *in vivo* assessment with either (i) an aggressive ovarian xenograft model that produces high levels of CXCL8, or (ii) a triple negative breast cancer xenograft model that expresses CXCL8 following cyclophosphamide/fludarabine preconditioning, CXCR2-engineered CAR iT cells demonstrated enhanced infiltration (SKOV3 model; 6.01e6 cells/gr tumor vs 0.94e6 cells/gr tumor with CXCR2+ CAR iT cells vs control CAR iT cells, respectively) and increased retention specifically within the solid tumor microenvironment, resulting in improved tumor control (SKOV3 model; 81.3% TGI vs 60.9% TGI with CXCR2+ CAR iT cells vs control CAR iT cells, respectively).

Conclusions Preclinical data demonstrate that the engineering of synthetic chemokine receptors to further direct off-the-shelf CAR iT cells to the tumor site is an exciting strategy to improve anti-tumor activity, including as part of a multiplexed-engineering strategy for overcoming challenges in treating solid tumors.

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