Engineering of Synthetic Chemokine Receptors into iPSC-derived CAR-T cells to Increase Homing and Enhance Trafficking into Solid Tumors

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Introduction

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.

Summary of results:

I. The CXCR2 ligand CXCL8 is expressed by various tumor cell lines and is specifically upregulated following radio- and chemo-therapy preconditioning (**Fig 1**).

II. Specific engineering of CXCR2 into iPSC derived CAR-T cells enables their ability to specifically migrate to the CXCR2 ligand CXCL8 without impacting their differentiation, phenotype, or anti-tumor efficacy **(Fig 2 & 3)**.

III. CXCR2 expression by CAR iTs enhances their homing to and retention within multiple tumor models expressing CXCL8, either naturally or chemotherapy-induced, leading to enhanced tumor control *in vivo* (**Fig 4 & 5**).

CXCL8 (IL-8) is upregulated following radio- and chemo-therapy treatment *in vitro*

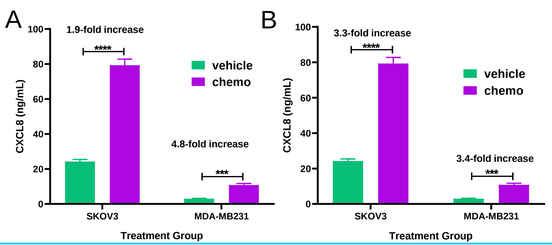

Figure 1. *In-vitro* analysis of CXCL8 levels following (A) radiation and (B) chemotherapy treatment. Ovarian (SKOV3) and Triple negative breast cancer lines (MDA-MB-231) were treated either with radiation (21Gy) or chemotherapy (Cisplatin, IC_{50}). Supernatants were collected 48hrs post treatment and IL-8 levels were determined by ELISA. (**** P<0.0001,***P<0.001**P<0.01).

Generation of CXCR2 engineered off-the shelf iPSC derived CAR-T cells

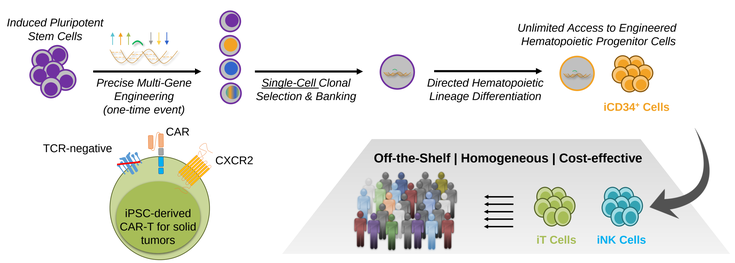

Figure 2. Overview of Fate's proprietary IPSC platform for the generation of precisely and uniformly engineered effector NK and T cells to express chemokine receptors such as CXCR2.

CXCR2 engineering enables specific migration to the CXCR2 ligand CXCL8 without altering CAR iT differentiation or anti-tumor efficacy

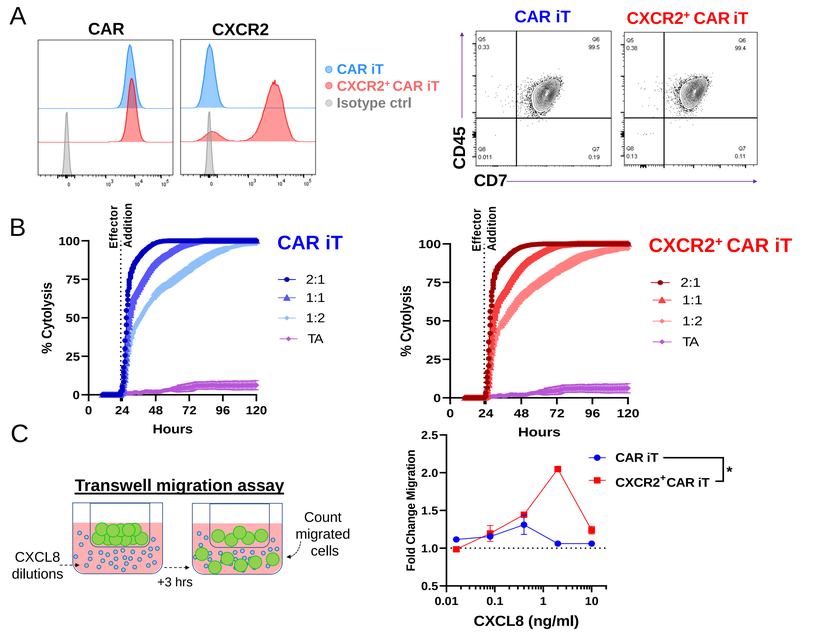

Figure 3. (A) Phenotypic evaluation of iPSC derived CAR iT cells. All CAR iT cells express uniform levels of CAR (99%) along with high levels of CXCR2 (90%) and are more than 99% positive for CD45 and CD7. (B) Both CAR iT cells with/without CXCR2 exerted robust cytolytic targeting of tumor targets at the indicated [Effector:Target] ratios, compared to tumor alone (TA) controls as evaluated in an xCELLigence assay. (C) Transwell migration assays demonstrated that CXCR2 expressing CAR iTs were chemotactic to the CXCR2 ligand CXCL8.* P<0.05

Enhanced intratumoral infiltration/retention and tumor control by CXCR2+ CAR iT cells in CXCL8 rich tumors

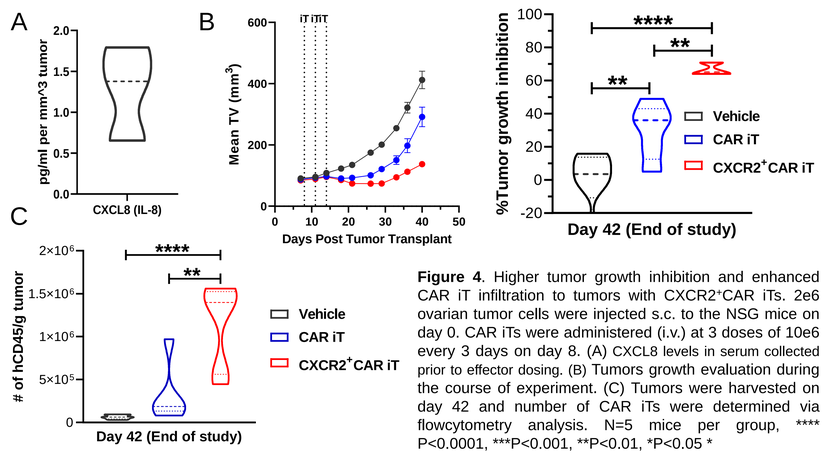

Figure 4. Higher tumor growth inhibition and enhanced CAR iT infiltration to tumors with CXCR2+CAR iTs. 2e6 ovarian tumor cells were injected s.c. to the NSG mice on day 0. CAR iTs were administered (i.v.) at 3 doses of 10e6 every 3 days on day 8. (A) CXCL8 levels in serum collected prior to effector dosing. (B) Tumors growth evaluation during the course of experiment. (C) Tumors were harvested on day 42 and number of CAR iTs were determined via flowcytometry analysis. N=5 mice per group, **** P<0.0001, ***P<0.001, **P<0.01, *P<0.05 *

CXCL8 upregulation following chemotherapy, specifically enhances intratumoral infiltration and retention of CXCR2+ CAR iT cells leading to improved tumor control in a CAR T cell resistant tumor xenograft model, consisting of aggressive tumor growth

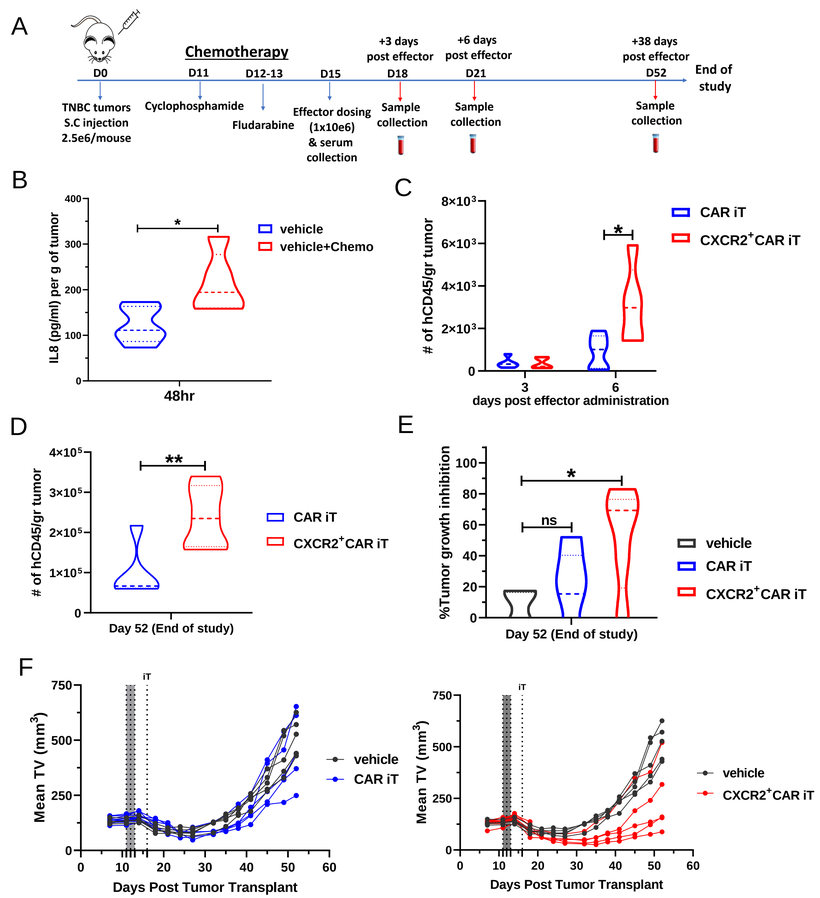

Figure 5 (A) Experimental design for *in vivo* evaluation of CXCR2- and CXCR2+ CAR iT cells with and without (data are not shown) chemotherapy (Cyclophosphamide/Fludarabine) preconditioning. (B) CXCL8 levels in serum collected 48hrs post chemo-preconditioning. (C-D) Intratumoral CAR iT infiltration & retention was evaluated by flow cytometry in tumors collected at 3, 6 and 38 days post CAR iT administration. (E-F)Tumor measurements (avg +/- SEM) for CAR iT and CXCR2+ CAR iT with chemo preconditioning. **P<0.01, *P<0.05.

Conclusion

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