Multi-Antigen Targeting by Novel Combination of CAR-T Cells and hnCD16 Transgene Yields in Complete Tumor Clearance via Antibody Dependent Cellular Cytotoxicity

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INTRODUCTION

Tumor heterogeneity and antigen escape are primary obstacles to the durable and long-term efficacy of chimeric antigen receptor (CAR) T-cell therapy across multiple tumor indications. Antibody dependent cellular cytotoxicity (ADCC) is a critical innate mechanism of natural killer (NK) cells designed to target and eliminate target cancer cells bound by therapeutic monoclonal antibodies (mAbs). This process is directed through the FcγRIII CD16a, a potent activating receptor found on human NK cells.

We previously demonstrated the high-affinity (158VV) variant of CD16 combined with a point mutation that prevents activation-induced surface cleavage, termed high-affinity non-cleavable CD16 (hnCD16), results in the enhancement of ADCC mediated by iPSC-derived NK cells. Here we combined hnCD16 with an off-the shelf, allogeneic CAR T cell strategy to enable flexible and robust targeting of secondary tumor associated antigens (TAA) with therapeutic mAbs, a T-cell multi-antigen targeting strategy that has not yet been fully investigated.

METHODS

Induced pluripotent stem cells (iPSCs) were specifically engineered to express (i) tumor specific CAR from the T cell receptor alpha constant chain (TRAC) locus and (ii) a CAG driven hnCD16 from the CD38 locus. In addition to supporting the transgene expression, the knockout of the TRAC removes the risk of graft versus host disease, while CD38 knockout enables compatibility with anti-CD38 mAb targeting strategies. The multiplexed-engineered iPSCs were differentiated into alpha-beta T (iT) cells, and unlike other cellular engineering platforms, demonstrates uniform and high levels of both CAR and hnCD16 (>99%, **Fig1**).

RESULTS

Generation of off-the-shelf iPSC derived CAR-T cells uniquely engineered to express high affinity, non-cleavable CD16a (hnCD16)

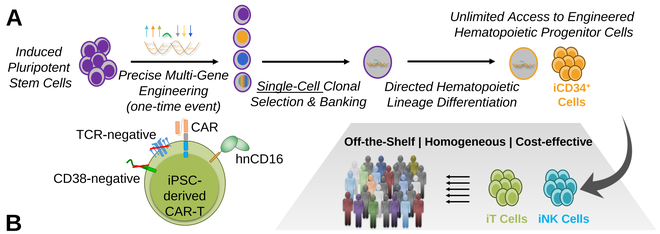

Fig 1. (A) Overview of Fate's proprietary iPSC platform for the generation of precisely and uniformly engineered effector NK and T cells to express multiple transgenes, such as hnCD16. (B) Flow cytometry of fully differentiated and engineered CAR iT cells expressing uniform and high levels of both CAR and hnCD16.

hnCD16 is functionally active in iPSC derived CAR-T cells, signaling through CD3ζ and activating both proximal and distal components of TCR signal transduction pathway

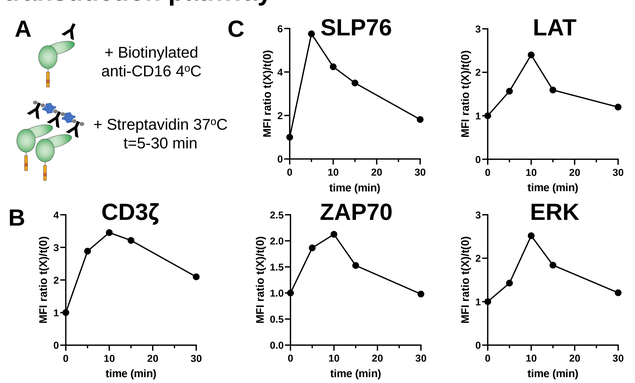

Fig 2. (A) hnCD16 crosslinking to evaluate (B) CD3ζ phosphorylation and (C) downstream signaling pathways. Indicated protein phosphorylation was assessed via phospho-flow cytometry following CD16 crosslinking for 0-30 minutes.

hnCD16 is compatible, complementary, and independent of CAR signaling, enhancing anti-tumor activity in a synergistic manner

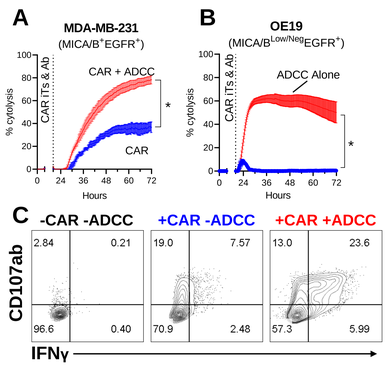

Fig 3. hnCD16 engineered CAR-iTs expressing a MICA/B CAR were co-cultured with (A) MICA/B$^+$ MDA-MB-231 or (B) MICA/B$^{low/neg}$ OE19 target tumor cells [1:1 E:T, stress test] with (red) or without (blue) anti-EGFR therapeutic antibody. Cell cytotoxicity was evaluated via xCELLigence assay. (C) hnCD16$^+$MICA/B CAR-iTs were co-cultured with CD38$^+$ NALM6 target cells engineered to over-express MICA/B with (right panel, red label) or without (middle panel, blue label) anti-CD38 therapeutic antibody and short-term (4 hr) intracellular cytokine production and degranulation assessed via ICCS (*P<0.05).

hnCD16 enables potent and flexible targeting of secondary tumor associated antigens, and the magnitude of CAR complementation correlates with the level of target antigen expression

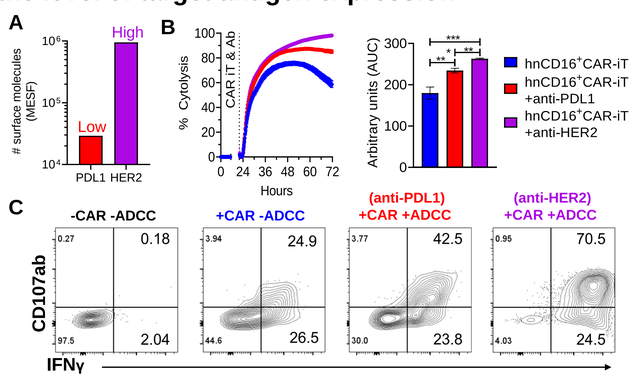

Fig 4.(A) Surface molecule quantification of PDL1 and HER2, as determined by flow cytometry, on ovarian tumor target cells. hnCD16$^+$ CAR iT cells were cocultured with ovarian tumor target cells (CAR Ag^{High}) along with therapeutic antibodies to PDL1 or HER2. (B) Cell cytotoxicity was evaluated via xCELLigence assay, and (C) intracellular cytokine production (16 hr) was evaluated via ICCS (**P<0.005, ****P<0.0001).

No evidence for functional exhaustion; CAR and hnCD16 co-activation sustains enhanced killing over multiple rounds of tumor target challenge

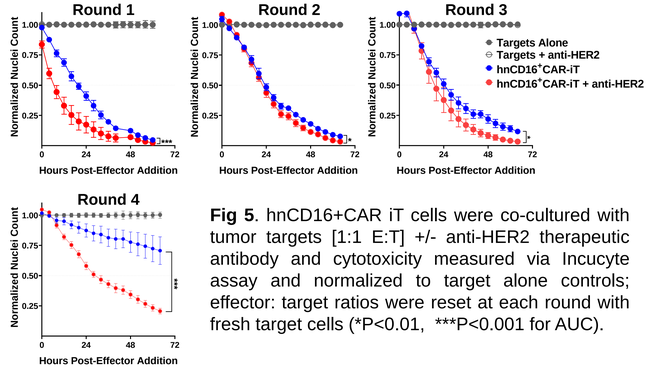

Fig 5. hnCD16+CAR iT cells were co-cultured with tumor targets [1:1 E:T] +/- anti-HER2 therapeutic antibody and cytotoxicity measured via Incucyte assay and normalized to target alone controls; effector: target ratios were reset at each round with fresh target cells (*P<0.01, ***P<0.001 for AUC).

CAR and hnCD16 coactivation *in vivo* leads to complete tumor clearance and control in an aggressive metastatic ovarian xenograft model

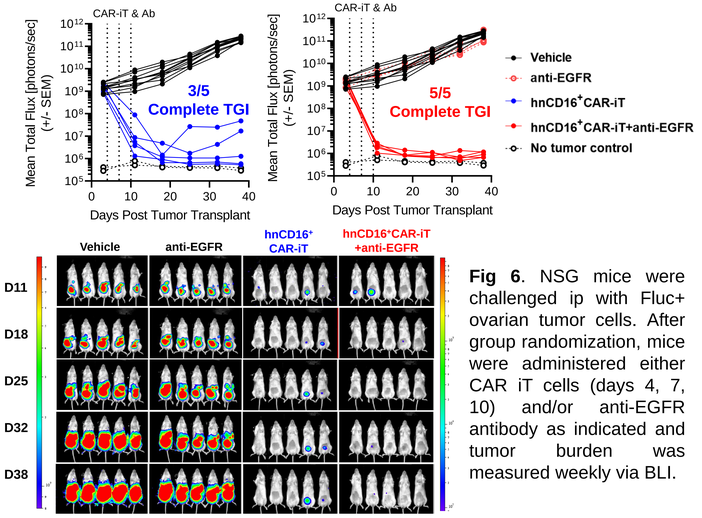

Fig 6. NSG mice were challenged ip with Fluc+ ovarian tumor cells. After group randomization, mice were administered either CAR iT cells (days 4, 7, 10) and/or anti-EGFR antibody as indicated and tumor burden was measured weekly via BLI.

CONCLUSIONS

Together, these results demonstrate that arming of CAR-iT cells with a unique Fc receptor, hnCD16, enables synergistic, potent, and flexible anti-tumor targeting through CAR activity and antibody directed cellular cytotoxicity, overcoming tumor heterogeneity and antigen escape. hnCD16 is currently being incorporated into multiple off-the-shelf iPSC-derived CAR-iT and CAR-iNK cells products to be combined with various therapeutic mAbs for the treatment of both liquid and solid tumors to provide more durable and long-lasting responses in cancer patients.