Engineering Human Induced Pluripotent Stem Cells with Novel Chimeric Antigen Receptors to Generate Natural Killer (NK) Cell Cancer Immunotherapies with Targeted Anti-Tumor Activity

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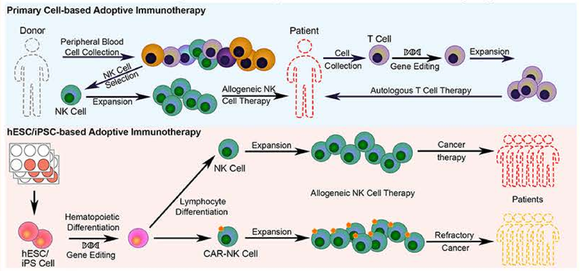
Introduction: CARs iPSC-NK Cell, and Cell Therapy

Chimeric antigen receptors (CARs) provide a powerful strategy to direct and enhance anti-tumor activity of immune effector cells. While most studies have evaluated CAR-expression in T cells, here we evaluate and optimize CAR constructs that are specifically designed with natural killer (NK) cell transmembrane and signaling domains. Since NK cell-mediated cytotoxicity does not require self-HLA expression, derivation of NK cells from human induced pluripotent stem cells (iPSCs), combined with the use of NK cell-specific CARs, enables production of a standardized, targeted allogeneic effector cell population.



We identified a CAR construct containing the transmembrane domain of NKG2D, the 2B4 co-stimulatory domain, and the CD3ζ signaling domain that mediates a strong increase in intracellular NK cell signaling and cytotoxicity in iPSC-derived NK cells. Together, the strategies provide a comprehensive approach to utilize NKCAR-iPSC-NK cells as a novel strategy to produce "off-the-shelf", targeted, allogeneic lymphocytes suitable to treat refractory solid tumor and hematologic malignancies.

Screen Novel NK-specific CARs in NK92 Cells

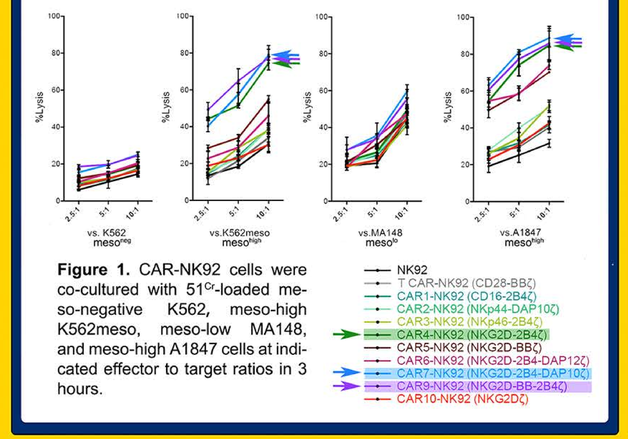


Figure 1. CAR-NK92 cells were co-cultured with 51^{Cr}-loaded meso-negative K562, meso-high K562meso, meso-low MA148, and meso-high A1847 cells at indicated effector to target ratios in 3 hours.

Differentiation and Phenotype of CAR-iPSC Derived NK Cells

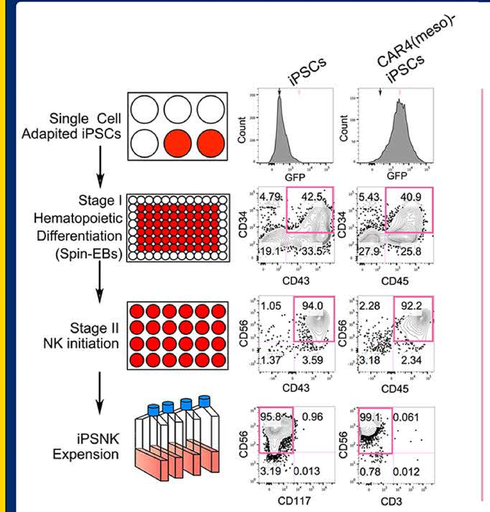


Figure 2. Derivation of NK cells from CAR-expressing iPSCs. CAR transfected iPSCs were dissociated and plated in spin-EB conditions for 11 days. After 11 days in spin EB culture, EB were transferred to conditions supporting NK cell development for 4 weeks in NK cell differentiation culture.NK cells were then expanded via artificial antigen presenting cell+ IL-2 co-culture. EB: embryonic body.

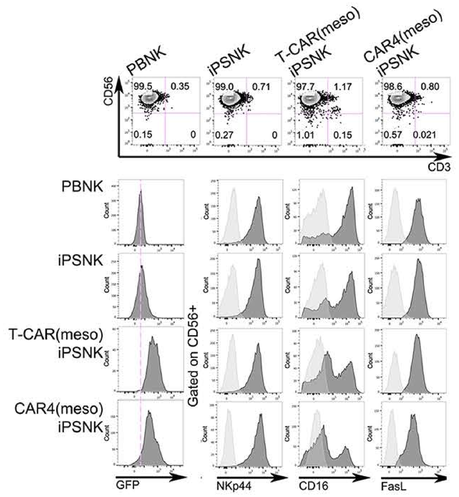


Figure 3. Phenotype of CAR-expressing iPSC-NK cells. Flow cytometric analysis of surface markers of CD56 and CD3 and the expression of transcriptional marker GFP, and NK cell surface receptors NKp44, CD16, FasL in the gate of CD56+ NK cell population.

Activation Signaling in Novel NK-specific CAR Expressing NK Cell Populations

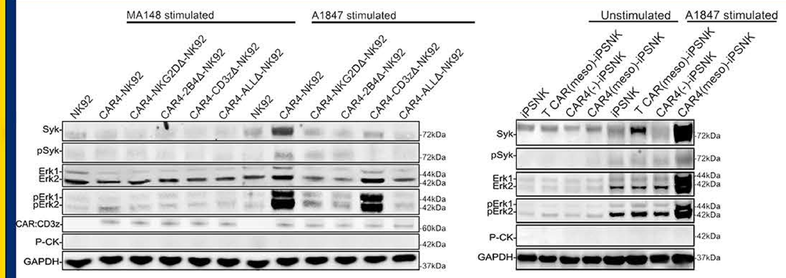


Figure 4. Protein analysis of total Syk, phosphorylated Syk (pSyk), total Erk1 and Erk2, and phosphorylated Erk1/2 (pErk1/pErk2) in cell lysate of CARs expressing NK92 cells (left) and iPSCNK cells (right).

Increased Cytotoxicity of Novel NK-specific CAR-iPSC-NK Cells

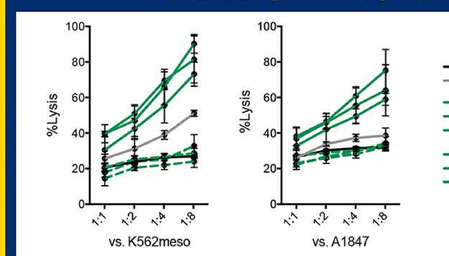
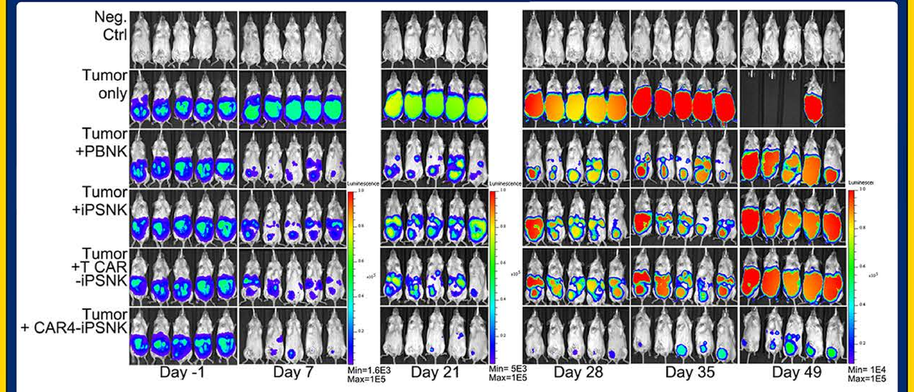


Figure 6. Anti-tumor activity of CAR4 expressing iPSC-NK cells. NK cells derived from pooled or clonal [CAR4(meso)-iPSC #1/4, CAR(scFv-)-PSC #2/3] were co-cultured with europium-loaded meso-high target cells of meso-high K562meso and A1847 cells at different effector to target ratios.

Novel NK-specific CAR Displays Superior Anti-Tumor Activity in vivo



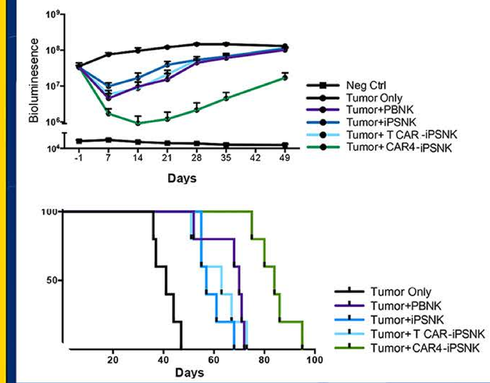


Figure 7. Luciferase (luc)-expressing A1847 cells (meso^{high}) in a mouse xenograft model with indicated NK cell. NSG mice were inoculated intraperitoneally with 2 × 10^5 luc+A1847 cells. 3 days later, mice received 225cGy radiation and 1 day later, one dose of 1.5 × 10^7 of NK population intraperitoneally. Cytokines hIL-2 and hIL-15 were administrated for the following 21 days. Tumor burden was determined by weekly bioluminescent imaging (BLI). Kaplan-Meier curve representing the percent survival of the experimental groups, n=5 for all groups: Tumor only, median survival (MS) 41 days; PB-NK: MS 70 days; iPSC-NK: MS 57 days; T-CAR[28bbz]-iPSC-NK: MS 63 days; CAR4-iPSC-NK: MS 84 days.

Statistics:
iPSC-NK vs. CAR4-iPSC-NK, P=0.0017, HR=0.2236;
T-CAR-iPSC-NK vs. CAR4-iPSC-NK, P=0.0018, HR=0.2153.

Enhanced Persistence of Novel NK-specific CAR-iPSC-NK Cells in vivo

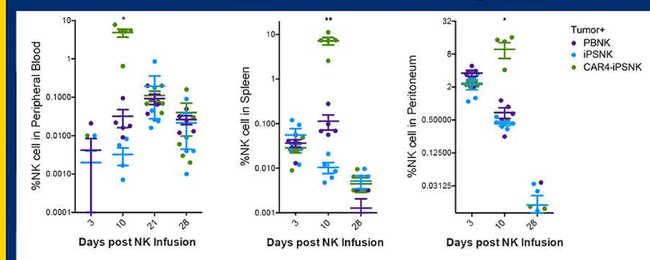


Figure 8. Measurement of percentage of CD45+CD56+CD3- NK population from cells collected from peripheral blood, spleen, and peritoneal fluid, assessed by flow cytometery. Each dot represents one recipient mouse. Median ± SEM is shown. Statistical significance was determined using two-tailed one-way ANOVA. *P<0.05.

Conclusion

1. NK-CAR-iPSC-NK cells have a phenotype similar to NK cells isolated from peripheral blood (PB-NK) and unmodified iPSC-derived NK cells.

2. Antigen (meso)-expressing targets induced phosphorylation of Syk and Erk in CAR4 expressing NK cells, that robust specific tumor cell lysis mediated by CAR4 expressing NK cells.

3. Single dose of CAR4-iPSC-NK cells markedly inhibited tumor growth, and mediated significantly enhanced survival

4. CAR4 expressing iPSCNK cells significantly increase proliferation and persistence in vivo.

Acknowledgement: We appreciate funding support from CIRM (DISC2-09615), NIH (R01CA203348 and U01CA217885) and Fate Therapeutics.