#315 iPSC-derived CAR-iNKT cells targeting HER2 show prolonged tumor control and promote durable survival in a tumor xenograft model.

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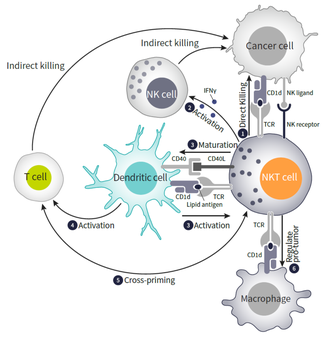
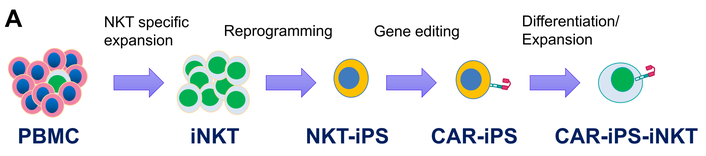
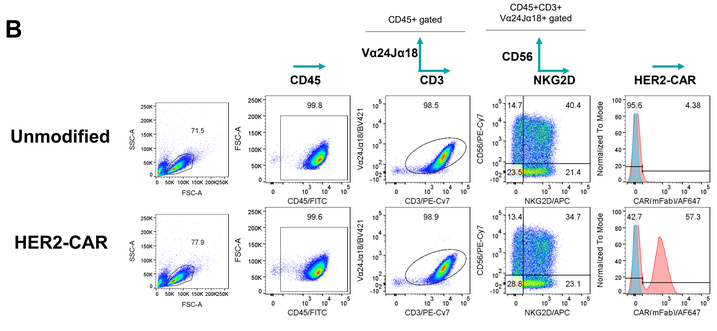
Introduction



Invariant natural killer T (iNKT) cells are a rare subset of T lymphocytes that express an invariant TCR which recognizes glycolipids presented by the monomorphic MHC like molecule CD1d. iNKT cells can directly kill tumor cells through TCR, while also indirectly exert antitumor activities by prompting dendritic cell maturation, priming tumor-specific CD8+T cells, and reprograming pro-tumor myeloid cells. iNKT cells do not induce graft-versus-host disease, which makes them an ideal cell source for “off-the-shelf” cell product. However, the rarity of iNKT cells in human blood poses a challenge in manufacturing large quantities of iNKT cells. Induced pluripotent stem cells (iPSCs) offer a promising strategy to overcome this hurdle by their potential to generate thousands of doses of iNKT cells from a single manufacturing campaign. In the present study, taking advantage of gene editing technology, we established a CAR-iPS cell line and differentiated it into CAR-iPS-iNKT cells with enhanced in vitro and in vivo anti-tumor activities.

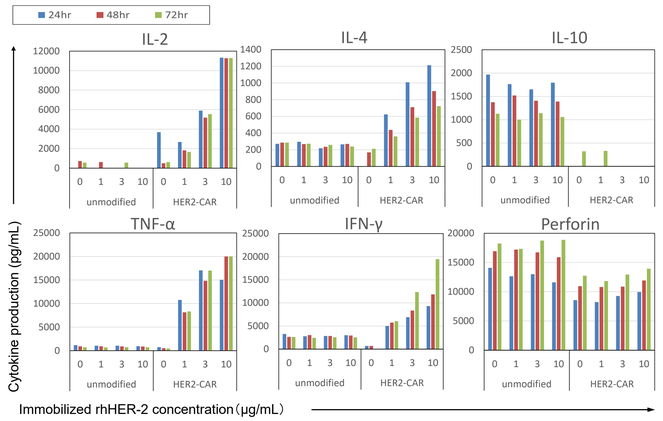
Generation of CAR-iPS-iNKT cells





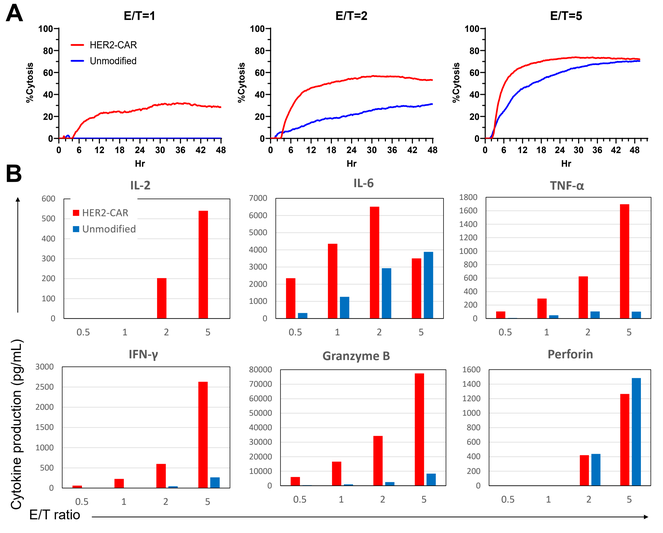
A. Schematic strategy to generate CAR-iPS-iNKT. B. Phenotypic analysis of iPS-iNKT cells with no gene editing (Unmodified) or HER2-CAR introduced iPS-iNKT cells (HER2-CAR) by Flow cytometry.

CAR signal enhanced production of Th1 cytokines to promote immune response whereas decreased immune suppressive cytokine



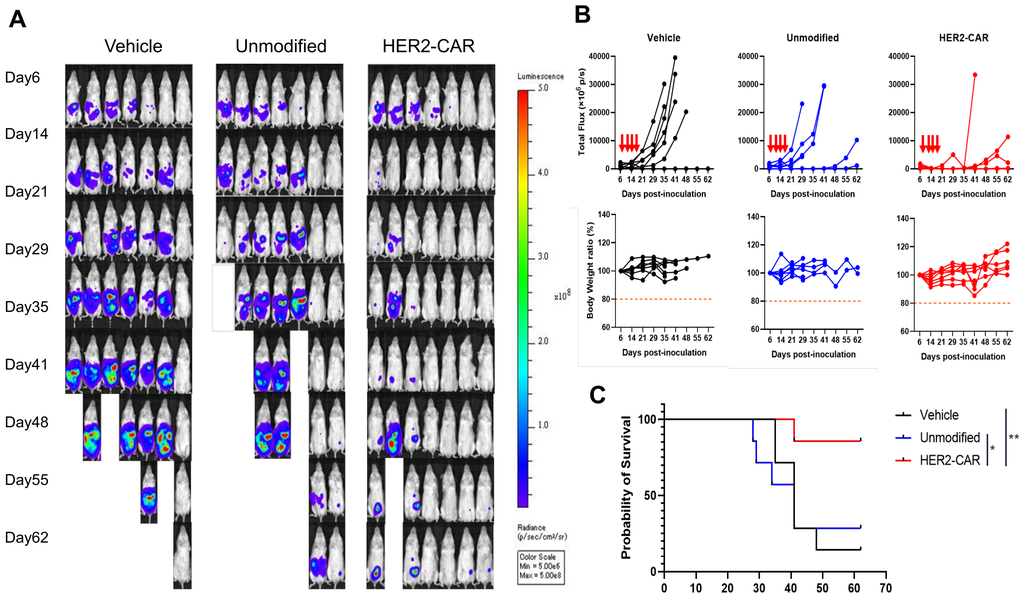
Unmodified or HER2-CAR-iPS-iNKT cells were cultured on recombinant HER2-His protein coated plate for 24, 48 or 72 hrs. Culture supernatant were assessed by LEGENDplex™ Human CD8/NK Panel.

Introduction of CAR increased killing and cytokine production of iPS-iNKT cells in vitro



Unmodified or HER2-CAR-iPS-iNKT cells were co-cultured with HER2 expressing tumor cell line SK-OV-3. A. Cytotoxic activities were measured by real-time cell analyzer xCELLigence. B. Cytokine secretion into culture supernatant after 48hr of co-culture were measured by LEGENDplex™ Human CD8/NK Panel. IL-4 and IL-10 were not detected.

CAR-iPS-iNKT shows strongly prolonged survival with its target specific anti-tumor activity without body weight loss for a long-term



NSG-hIL15/hIL7KI mice were i.p. inoculated with 2M SK-OV-3 expressing luciferase cells (SK-OV-3-luc) on day 0. Lactated ringer (vehicle), 10M iPS-iNKT cells (Unmodified) or 10M HER2-CAR-iPS-iNKT cells (HER2-CAR) were i.p. administered to each group mice on day 7, 11, 14 and 18. A; Tumor burden of SK-OV-3-luc bearing mice was acquired as bioluminescence imaging once a week. B; Spider plot of total flux and body weight ratio based on day 6 body weight were shown each groups. Time points of administration are indicated by red arrows. C; Survival rate (n = 6) for SK-OV-3-luc bearing mice was shown as Kaplan–Meier survival curves. Survival estimates were assessed by log-rank tests.
*: P<0.05, **: P<0.01.

Conclusions

This study demonstrated that differentiated CAR-iPS-iNKT cells derived from CAR-iPSC showed potent anti-tumor effects and promoted survival in a tumor xenograft model. Results from in vitro analysis suggest that target-specific killing activity of HER2-CAR-iPS-iNKT cells were derived from increased secretion of Th1 and inflammatory cytokines and NK phenotype such as NKG2D for direct killing.

These findings suggest that iPSC-derived CAR-iNKT cells would be a novel allogeneic cell therapy platform because of enhanced innate and adapted immunity.

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