#6105 iPSC-derived HER2 CAR-iNKT cells enhance the activity of immune cells against cancer cells

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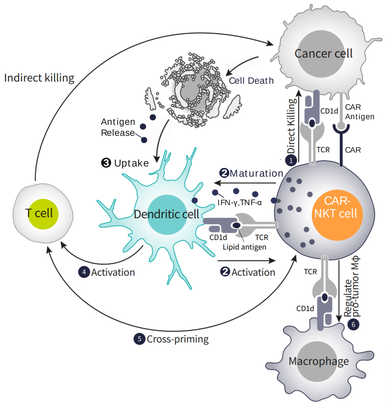
Introduction

Allogeneic invariant natural killer T (iNKT) cell therapy is known to elicit indirect anti-tumor effects by activating endogenous cytotoxic T lymphocytes (CTLs), represent ing a unique mechanism of action distinct from conventional T cell-based therapies. To harness this intrinsic immuno-modulatory potential, we have been developing induced pluripotent stem cell (iPSC)-derived CAR-iNKT cells as a novel allogeneic CAR-T cell platform.

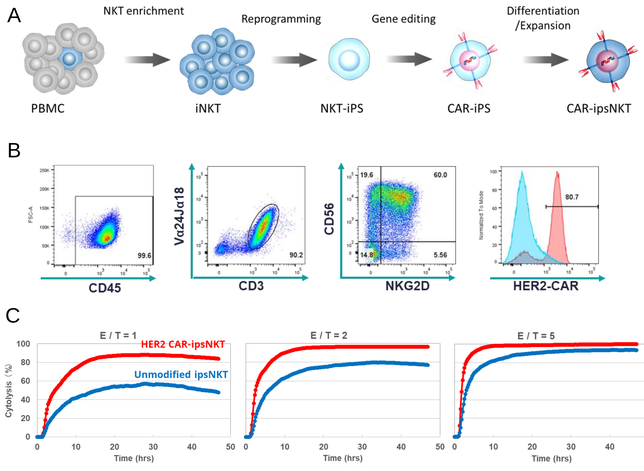
Unlike unmodified iNKT cells, which are activated through their invariant TCR recognizing glyco-lipid antigens presented by CD1d on antigen-presenting cells, CAR-iNKT cells are activated directly by tumor-associated antigens through CAR engagement.



Upon CAR-mediated activation, CAR-iNKT cells exert cytotoxic effects against tumor cells, resulting in the release of endogenous tumor antigens. These antigens are subsequently taken up by endogenous dendritic cells (DCs), which are themselves activated through interactions with CAR-iNKT cells. Activated DCs then present the antigens to CD8⁺ T cells, thereby priming a secondary, endogenous anti-tumor immune response.

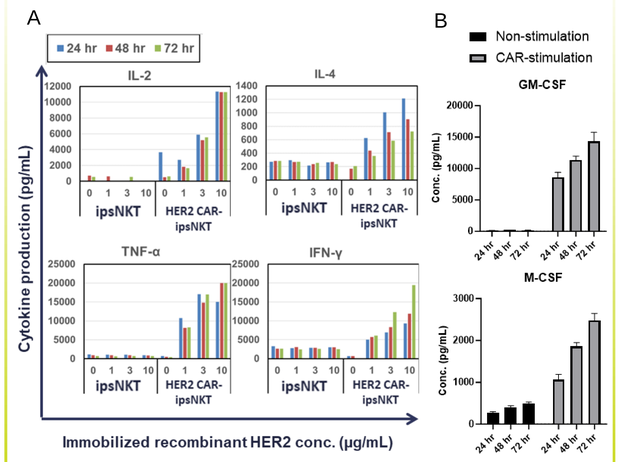
Thus, CAR-iNKT cells serve not only as direct effectors via CAR signaling, but also as initiators of endogenous adaptive immunity. Here, we demonstrate this mechanism of action for the first time using iPSC-derived HER2 CAR-iNKT (HER2 CAR-ipsNKT) cells in an allogeneic in vivo model, extending beyond prior studies conducted in syngeneic settings or using non-CAR transfected cells.

1. Generation of iPSC-derived HER2 CAR-iNKT cells



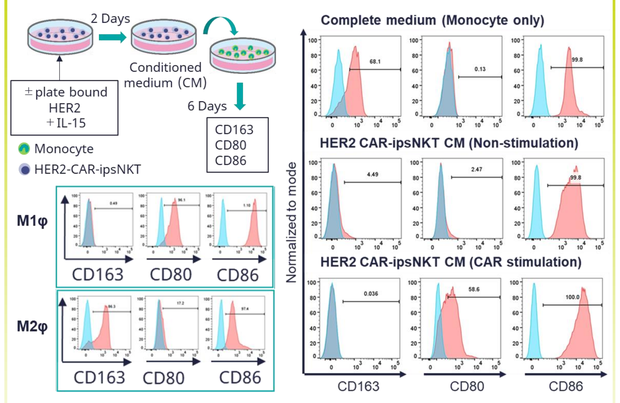
A) Schematic strategy to generate CAR-iPS-iNKT cells. B) Phenotypic analysis of HER2 CAR-ipsNKT by Flowcytometry. C) HER2 CAR-ipsNKT cells or Unmodified CAR-ipsNKT cells were co-cultured with CMVpp65 expressing HER2 positive breast cancer (HCC1954-CMVpp65) for cytotoxic activities using real-time cell analyzer xCELLigence.

2. HER2 CAR-ipsNKT cells release cytokines that promote DC maturation, differentiation, and M1 polarization CAR engagement



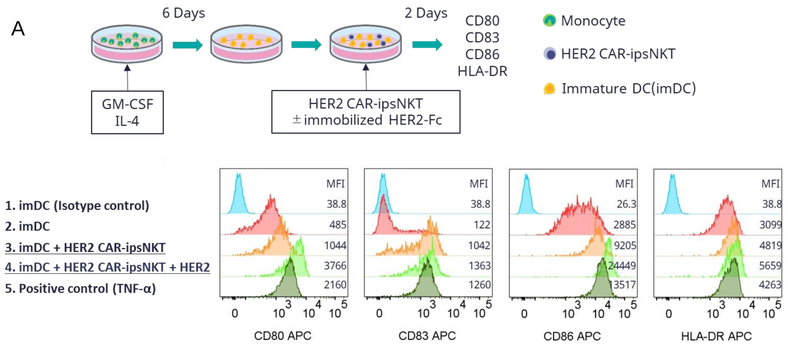
Cytokines in culture supernatant were assessed by LEGENDplex™. A) Unmodified ipsNKT or HER2 CAR-ipsNKT cells were cultured on recombinant HER2 protein coated plate for 24, 48 or 72hrs. B) HER2 CAR-ipsNKT cells were cultured on 0 or 1 μg/mL recombinant HER2 protein coated plate for 24, 48 or 72hrs.

3. CAR-stimulated HER2 CAR-ipsNKT cells promote the M1 macrophage polarization



Conditioned medium was collected from culture of HER2 CAR-ipsNKT for 2 days with 1 μg/mL recombinant HER2 protein. Monocytes were then cultured in these conditioned medium for 6 days. The expression of M1 or M2 macrophage markers CD80, CD86 and CD163 on these monocytes were assessed by flow cytometry.

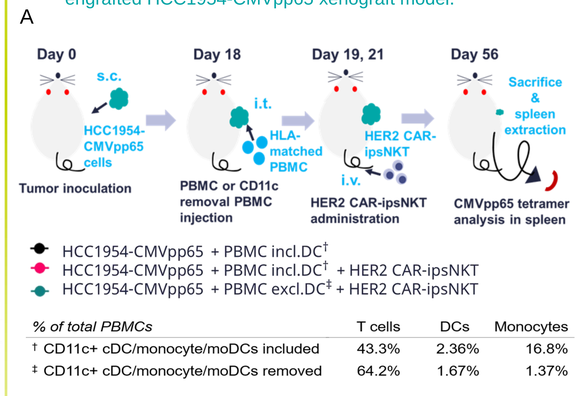
4. HER2 CAR-ipsNKT cells drive DC maturation and boost antigen-specific CD8⁺ T cell activation



A) Immature DCs were generated by stimulating monocytes with GM-CSF and IL-4. These immature DCs were then co-cultured with either HER2 CAR-ipsNKT cells or antigen-activated HER2 CAR-ipsNKT cells for 48 hours. The expression of DC maturation markers CD80, CD83, CD86, and HLA-DR were assessed by flow cytometry. B) HLA-matched PBMC was co-cultured with HCC1954-CMVpp65 cells and HER2 CAR-iPS-NKT cells for seven days. HLA-matched PBMC also co-cultured with UV irradiated HCC1954-CMVpp65 cells, LPS and IFN-γ as positive control. CMVpp65-specific cytotoxic T lymphocytes were then detected using HLA-A*24:02 CMVpp65 tetramer assay. One-way ANOVA followed by Tukey's multiple comparison test was used to assess significance ; *p< 0.05

5. HER2 CAR-ipsNKT cells elicit anti-tumor response of antigen-specific CD8⁺ T cells in HLA-matched PBMC

- HER2 CAR-ipsNKT cells drove maturation of CD11c⁺ dendritic cells and enhanced CMVpp65-specific CD8⁺ T cells in HLA-matched PBMC-engrafted HCC1954-CMVpp65 xenograft model.



% of total PBMCs	T cells	DCs	Monocytes
† CD11c+ cDC/monocyte/moDCs included	43.3%	2.36%	16.8%
‡ CD11c+ cDC/monocyte/moDCs removed	64.2%	1.67%	1.37%

A) NOG-hIL15Tg mice were s.c. inoculated with 5M HCC1954-CMVpp65 cells on day 0. HLA-matched PBMC or CD11c+ cells removal PBMC were engrafted on day 18. CP-1/lactated ringer (vehicle) or 10M HER2 CAR-ipsNKT cells were i.v. administered to each group mice on day 19 and 20. B) Mean tumor volume of HCC1954-CMVpp65 was acquired as twice a week. Data are shown as mean values of 5 or 6 mice ± SEM. PBMC engraftment indicated by blue arrow. Administration points are indicated by red arrows. Two-way ANOVA followed by Tukey's multiple comparison test was used to assess significance. *; p<0.05, ***; p<0.001 (vs vehicle group). Body weight ratio based on day 18 body weight were shown each groups. C) CMVpp65-specific CD8+ T cells in spleen on day 56 were detected using HLA-A*24:02 CMVpp65 tetramer assay. One-way ANOVA followed by Tukey's multiple comparison test was used to assess significance. *p< 0.05

Conclusion

1. **iPSC-derived CAR-NKT cells, even after the source NKT cells undergo reprogramming into iPSCs, CAR gene editing, and subsequent redifferentiation back into NKT cells, retain the native ability of NKT cells to activate endogenous T cells.**
2. **Immune modulatory effects of HER2 CAR-ipsNKT cells, in addition to their direct tumor cell killing, include:**
   - **Promotion of Dendritic Cell (DC) Maturation and M1 Macrophage Polarization upon CAR Stimulation**
   - **Activation of DCs, leading to tumor-specific T cell responses through the presentation of antigens released following tumor cell killing by HER2 CAR-ipsNKT cells**
3. **The direct anti-tumor effects mediated by allogeneic CAR-NKT cells, followed by the indirect anti-tumor activities driven by NKT cell-induced endogenous CD8⁺ T cell, are anticipated to enhance the durability of clinical responses for this novel allogeneic CAR-T approach utilizing iPSC-derived NKT cells as effector cells.**

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