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A novel iPSC-derived CAR-invariant natural killer T (iNKT) cell therapy platform for hematologic malignancies and solid tumors

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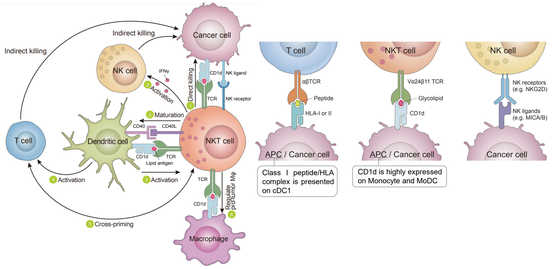
Introduction

Following the success of autologous chimeric antigen receptor (CAR)-T cells in hematologic malignancies, allogeneic CAR-transduced cells have been developed with various immune cells including induced pluripotent stem cell (iPSC)-derived NK cells and T cells. We have developed a novel platform of first-in-class iPSC-derived CAR-invariant natural killer T (iNKT) cells. To demonstrate that CAR-transduced iPSC-derived iNKT cells provide a novel platform for effective cancer immunotherapy, the killing activities of CD19-CAR or HER2-CAR-transduced iPSC-derived iNKT cells were investigated in this first set of studies.

Invariant NKT (iNKT) cells

- iNKT cells are a rare subset of innate lymphocytes that bridge innate and adaptive immune response.
- HLA-independent TCR: No risk of GvHD.
- Providing clinical durability through indirect anti-tumor effect by activating host endogenous T cells, dendritic cells and NK cells, and reprograming pro-tumor myeloid cells in the tumor microenvironment.
- Direct cytotoxic effect via NK receptors and/or endogenous TCRs



iPSC derived iNKT cells

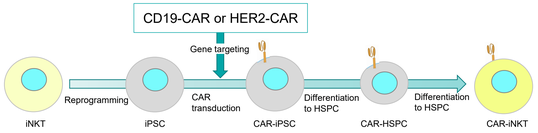
- Use of iPSC derived from iNKT cells is an ideal strategy to realize clinical scale production of functional iNKT cells from such a rare population.
- A Phase 1 study of the iPSC derived non-transduced (unmodified) iNKT cells is currently ongoing in patients with head and neck squamous cell carcinoma.

Advantages of iNKT over NK / αβT / γδT cells

	allo iNKT	allo αβT	allo γδT	allo NK
Innate - adaptive immunity bridging				
DC cross-talking	✓			
$CD8^+$ T cross-priming	✓			
Myeloid cell (TAM, MDSC) reprogram	✓			
Innate anti-tumor response	✓		✓	✓
HLA independency				
No need to TCR gene editing	✓		✓	n.a.
Low GvHD risk	✓		✓	✓
Proliferating capacity	✓	✓	✓	

Results

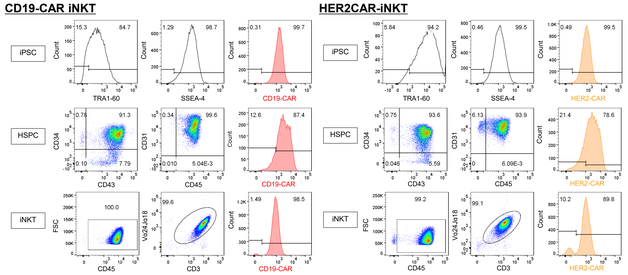
Strategy for development of CAR-iNKT from iNKT derived-iPSCs



iNKT-derived iPSC was prepared by reprogramming expanded iNKT cells from human PBMC. Next, iPSCs were engineered to express CD19-CAR or HER2-CAR by targeting the adeno-associated virus integration site-1 locus using genome editing.

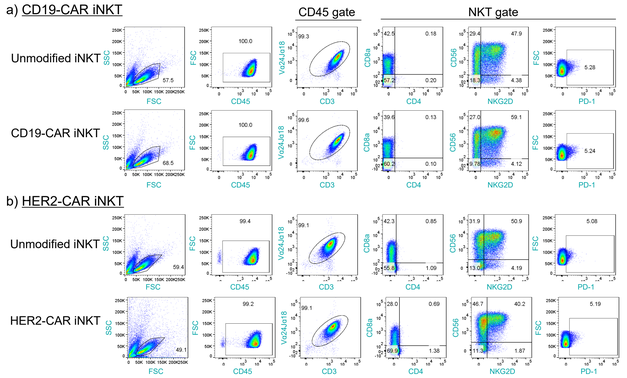
CAR-introduced iPSCs can differentiate to CAR-iNKT cells under feeder cell free culture conditions.

Production of CD19-CAR and HER2-CAR iNKT cells differentiated from iPSCs in feeder cell-free culture system without losing CAR expression



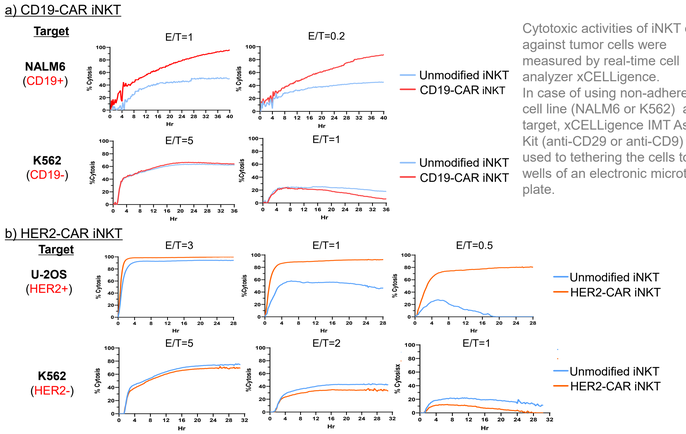
CAR positive cells were measured by flow cytometry using target antigen (rhCD19 or rhHER2) followed by detection antibody treatment. These data were derived from independent experiment.

Phenotype analysis of CD19-CAR and HER2-CAR iNKT cells showed CAR-iNKT cells have similar phenotypic properties to iPSC-derived non-transduced iNKT cells



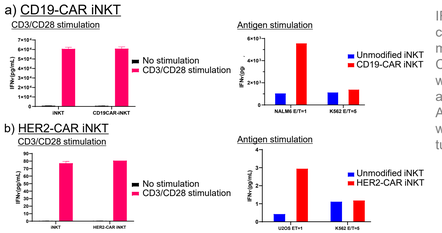
Unmodified iPSCs and CAR transduced iPSCs were differentiated into iNKT cells by the same differentiation protocol. a) and b) data were derived from independent experiment.

CD19-CAR iNKT and HER2-CAR iNKT cells showed target-specific anti-tumor effects *in vitro*



Cytotoxic activities of iNKT cells against tumor cells were measured by real-time cell analyzer xCELLigence.
In case of using non-adherent cell line (NALM6 or K562) as a target, xCELLigence IMT Assay Kit (anti-CD29 or anti-CD9) were used to tethering the cells to the wells of an electronic microtiter plate.

Interferon-γ release from CAR-iNKT cells



IFN-γ concentration of iNKT cells culture supernatants were measured by ELISA.
CD3/CD28 stimulation: iNKT cells were treated with anti- CD3 and anti-CD28 antibodies for 24 hours.
Antigen stimulation: iNKT cells were mixed with indicated target tumor cells for 48 hours.

Conclusion

- **This study showed the first successful delivery of a CAR construct into iPS cells that differentiate precisely into iNKT cells with enhanced cytotoxicity.**
- **iPSC-derived CAR-iNKT cells are demonstrated to become a novel allogeneic cell therapy platform.**

Disclosure: Urakami A.: Employee of BrightPath Biotherapeutics

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