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Generation of functional BCMA CAR-iNKT cells from clinical-grade iPSCs via a GMP-compliant manufacturing process with capacity for linear scale-up

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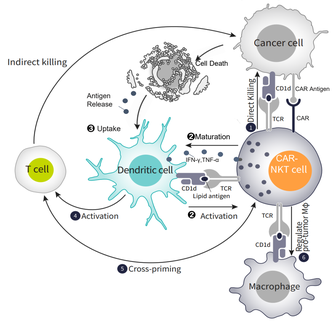
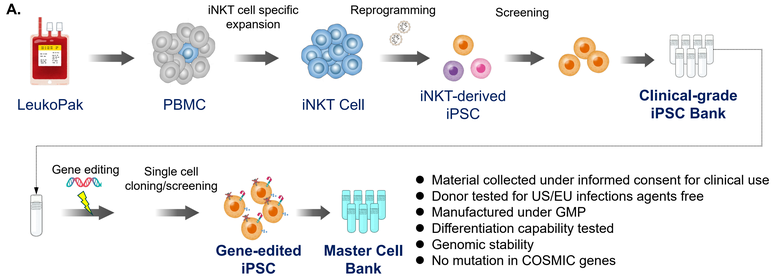
Introduction

A Novel Allogenic CAR-T Cell Therapy Platform using iPS Cell-derived Natural Killer T (NKT) Cells

- Invariant natural killer T (iNKT) cells are a rare subset of T lymphocytes that exert not only direct but indirect anti-tumor effects by activating CD8$^+$T cells and other immune populations.
- In our previous mouse model study, we demonstrated that the iPS cell-derived CAR-engineered iNKT (CAR-ipsNKT) cells retain the native iNKT cells' capacity to activate antigen-specific CD8$^+$T cells *in vivo*. These findings suggest that allogeneic CAR-ipsNKT cells can induce host CD8$^+$T cells to target broadly spread tumor antigens, thereby enhancing immune compatibility and potentially extending the durability of clinical responses.
- Induced pluripotent stem cell (iPSC) technology provides a scalable platform for generating this rare T cell subset while preserving their functional properties, addressing a major challenge in clinical-scale manufacturing.



Generation of gene-modified, clinical-grade iPS cells derived from iNKT cells



- Material collected under informed consent for clinical use
- Donor tested for US/EU infections agents free
- Manufactured under GMP
- Differentiation capability tested
- Genomic stability
- No mutation in COSMIC genes

Gene-edited iPSC retained a normal diploid karyotype and pluripotency

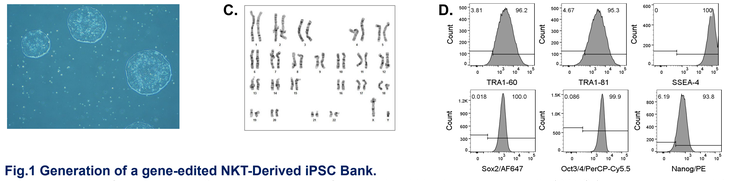


Fig.1 Generation of a gene-edited NKT-Derived iPSC Bank.
A. The schematic of the process. iNKT cells were enriched from peripheral blood mononuclear cells (PBMCs) and reprogrammed into iPSCs. Resulting colonies were screened, and selected clones were cryopreserved to establish an unmodified iPSC bank. Using CRISPR-Cas12a technology, gene editing (knock-in/knock-out) was performed to target specific loci. Edited iPSCs were cloned, validated, and expanded to establish a master cell bank. **B.** Representative microscopic image of gene-edited iPSCs. **C.** The karyotype of the gene-edited iPSCs was analyzed by G-banding. Representative cytogenetic analysis is shown. **D.** Expression of surface and intracellular pluripotency markers was analyzed by flow cytometry.

The newly developed manufacturing process enabled high-yield production of BCMA CAR-ipsNKT cells (BP2202) with proven feasibility for clinical-scale expansion

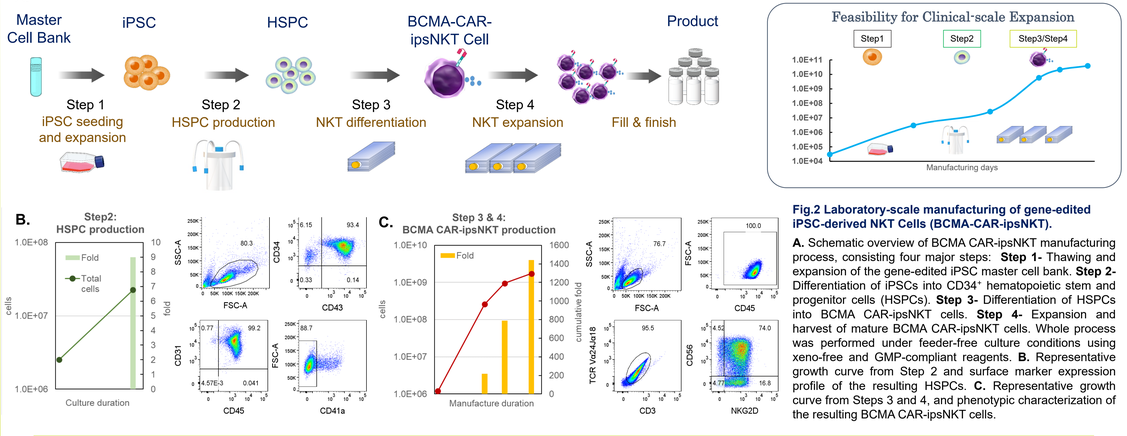
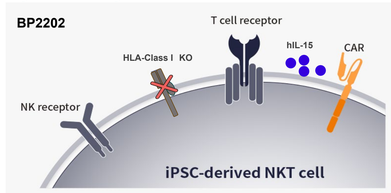
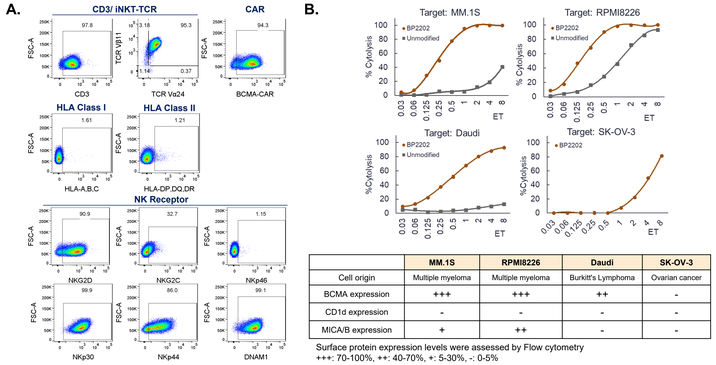


Fig.2 Laboratory-scale manufacturing of gene-edited iPSC-derived NKT Cells (BCMA-CAR-ipsNKT).
A. Schematic overview of BCMA CAR-ipsNKT manufacturing process, consisting four major steps: **Step 1-** Thawing and expansion of the gene-edited iPSC master cell bank. **Step 2-** Differentiation of iPSCs into CD34$^+$ hematopoietic stem and progenitor cells (HSPCs). **Step 3-** Differentiation of HSPCs into BCMA CAR-ipsNKT cells. **Step 4-** Expansion and harvest of mature BCMA CAR-ipsNKT cells. Whole process was performed under feeder-free culture conditions using xeno-free and GMP-compliant reagents. **B.** Representative growth curve from Step 2 and surface marker expression profile of the resulting HSPCs. **C.** Representative growth curve from Steps 3 and 4, and phenotypic characterization of the resulting BCMA CAR-ipsNKT cells.

BP2202, produced using the developed manufacturing process, exhibited high purity and potent *in vitro* cytotoxicity



BP2202 Key Features
- **BCMA-CAR Knock-in**
  ⇒ Enables targeted killing of BCMA$^+$ tumor cells
- **Soluble hIL-15 expression**
  ⇒ Enhances iNKT cell survival and persistence
- **HLA-class I Knock-out**
  ⇒ Reduces immunogenicity and promotes persistence
- **Invariant TCR expression**
  ⇒ Enables CD1d-restricted cytotoxicity, Promotes DC maturation, Facilitates M1φ polarization
- **NK receptor expression**
  ⇒ Boost innate cytotoxicity



	MM.1S	RPMI8226	Daudi	SK-OV-3
Cell origin	Multiple myeloma	Multiple myeloma	Burkitt's Lymphoma	Ovarian cancer
BCMA expression	+++	+++	++	-
CD1d expression	-	-	-	-
MICA/B expression	+	++	-	-

Surface protein expression levels were assessed by Flow cytometry
+++: 70-100%, ++: 40-70%, +: 5-30%, -: 0-5%

Fig. 3 *In vitro* Characterization of BCMA CAR-ipsNKT (BP2202)
A. Phenotypic characterization of BP2202 was assessed by flow cytometry. **B.** Luciferase-expressing target cells were co-cultured with BP2202 at various effector-to-target (E:T) ratios. Cytotoxicity was assessed 24 hours later by measuring luciferase activity after target cell lysis. Surface expression of BCMA, CD1d and MICA/B on target cells was analyzed by flow cytometry.

BP2202 treatment led to significantly prolonged survival in mice

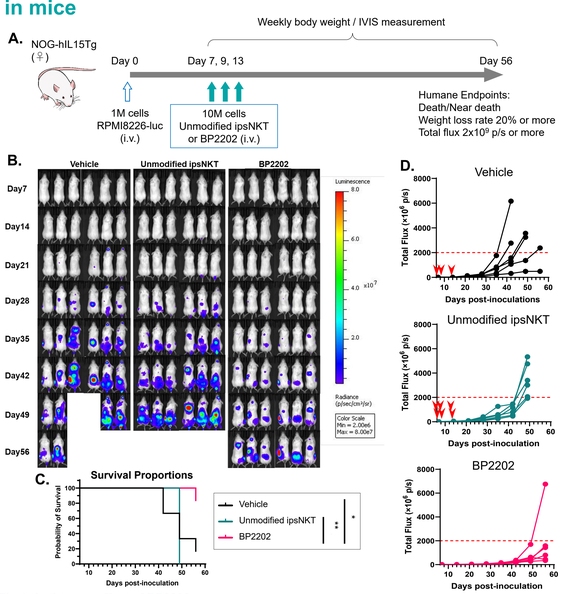


Fig.4 Anti-tumor effect of BP2202
A. NOG-hIL15Tg mice were i.v. inoculated with 1M RPMI8226-luciferase (RPMI8226-luc) cells on Day 0. Mice were then i.v. administrated with either Lactated Ringer (vehicle), 10M unmodified ipsNKT cells, or 10M BCMA CAR-ipsNKT cells(BP2202) on Days 7, 9 and 13. **B.** Tumor burden of RPMI8226-luc bearing mice was acquired as bioluminescence imaging once a week. **C.** Spider plots show the total flux over time for each group. Time points of dosing are indicated by red arrows. **D.** Survival rate (n = 6 per group) for RPMI8226-luc inoculation mice was shown as Kaplan–Meier survival curves. Survival estimates were assessed by log-rank tests. *P<0.05, ** P<0.01.

Conclusion

- Clinical-grade iNKT cell-derived iPSC lines have been successfully established and banked.
- A GMP-compliant gene editing process has been successfully developed.
- A scalable manufacturing process enabling large-scale production of CAR-ipsNKT cells has been developed.
- This study highlights the potential of iPSC-derived CAR-iNKT cells as a versatile and scalable platform for allogeneic cell therapies.

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