Long-term Stability Assessment of Cryopreserved iPSC-derived T and NK Cells Supports Mass Production and Off-the-shelf Therapeutic Applications

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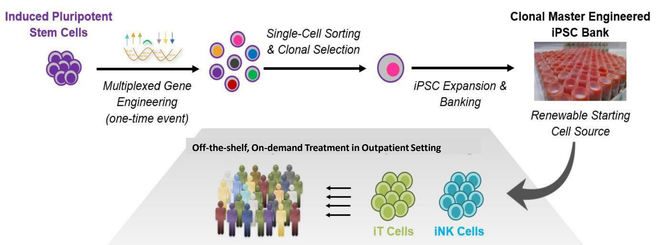
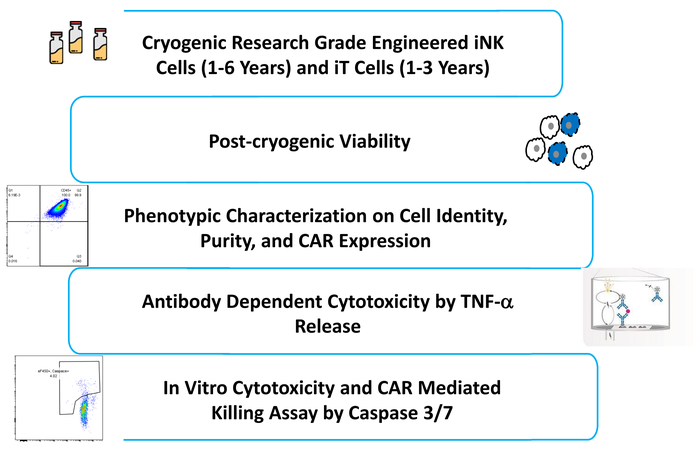
GRAPHICAL ABSTRACT & INTRODUCTION

Engineered iPSC–derived T (iT) and NK (iNK) Cell Products for Immunotherapy



Cell-based immunotherapies have shown remarkable promise in the fight against various cancers. Induced pluripotent stem cell (iPSC)-derived natural killer (NK) and T (iNK and iT, respectively) cells can be mass produced and administered off-the-shelf to patients, and several iPSC-derived cell-based cancer immunotherapies are now undergoing clinical testing. Our iPSC product platform leverages the use of clonal master iPSC lines that serve as the starting material for the manufacture of multiplexed-engineered, cell-based cancer immunotherapies that can be fully characterized, stored, and administered on-demand to patients. Here we demonstrate long-term stability (up to 6 years) of our engineered iNK and iT cell drug product candidates following cryogenic storage.

Schematic of Long-term Cryogenic Study Workflow



SUMMARY

In this study, we demonstrate that iPSC -derived engineered-NK cells and T cells are stable over long-term cryogenic storage.

- iNK and iT cells demonstrated consistent cell viability, identity and purity with uniform expression of engineered modalities, including hnCD16 and CAR, regardless of the duration of cryopreservation tested in this study.
- Cryopreserved iNK and iT cells maintained robust cytolytic effector function as demonstrated by ADCC-mediated proinflammatory cytokine production and potent CAR-mediated anti-tumor cytotoxicity
- Collectively, the data illustrate that multiplexed engineered iNK and iT cell products can be mass produced, cryopreserved, and stored long-term for use as off-the-shelf cell-based cancer immunotherapy

RESULTS

iNK Cells Maintain Durable Viability and Uniform Phenotype of Multiplexed Edits after Long-term Cryopreservation

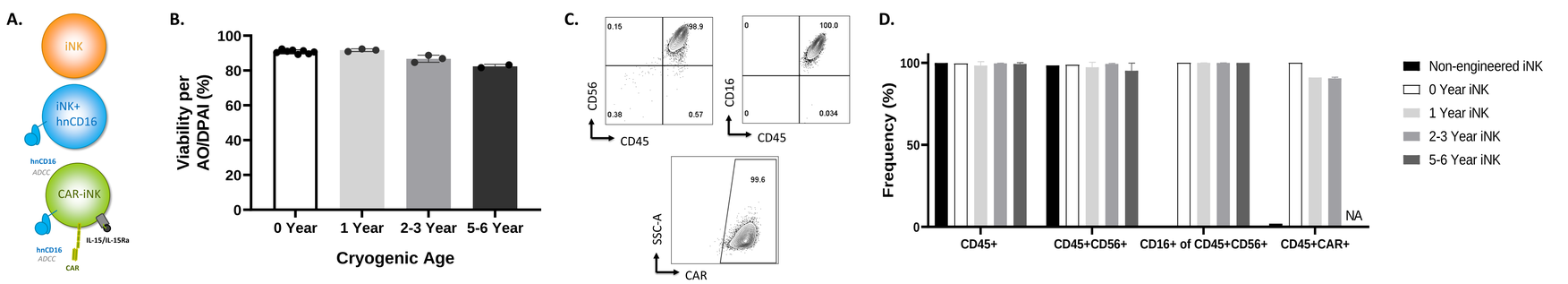


Figure 1. (A) Non-engineered and engineered iNK products examined in the study. (B) Cryopreserved iNK cells maintain high viability post-thaw. Cryopreserved iNK cells were thawed in culture medium and the viability was assessed by Acridine orange and DAPI staining using a NucleoCounter® NC-200. (C) Representative post-cryopreservation flow cytometry profiles (D) Post-thaw cellular phenotypes were analyzed by flow cytometry. NK Identity ($CD45^+/CD56^+$) and the transgene (human non-cleavable CD16, hnCD16) and CD19 targeting chimeric antigen receptor (CAR) expression were > 90%. NA indicates the iNK were not engineered with the designated modality.

iT Cells Exhibit Robust Viability and Uniform Phenotype of TCR-null TRAC-CAR Expression after Long-term Cryopreservation

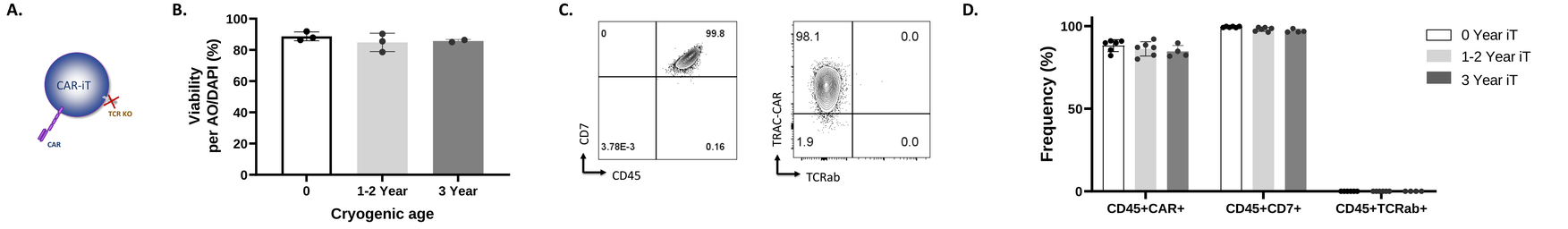


Figure 2. (A) Engineered iT products examined in the study. (B) Cryogenic storage does not affect iT viability. Cryopreserved iT cells were thawed in culture medium and the viability was assessed by Acridine orange and DAPI staining using a NucleoCounter® NC-200. (C) Representative post-cryopreservation flow cytometry profiles (D) Post-thaw cellular phenotypes were analyzed by flow cytometry. Engineered iT cell identity as measured by the expression of $CD45^+CD7^+$, CD19 targeting chimeric antigen receptor (CAR) and the lack of expression of TCRab.

hnCD16 iNK Cells Demonstrate Enhanced Antibody Dependent Cellular Cytotoxicity

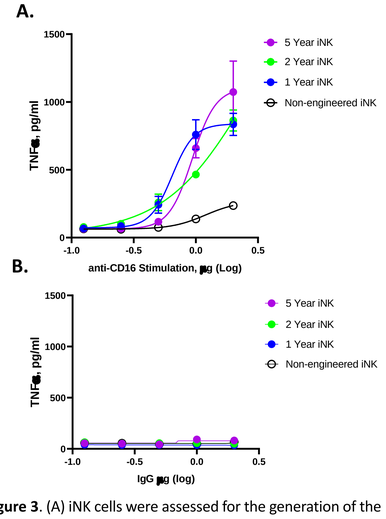
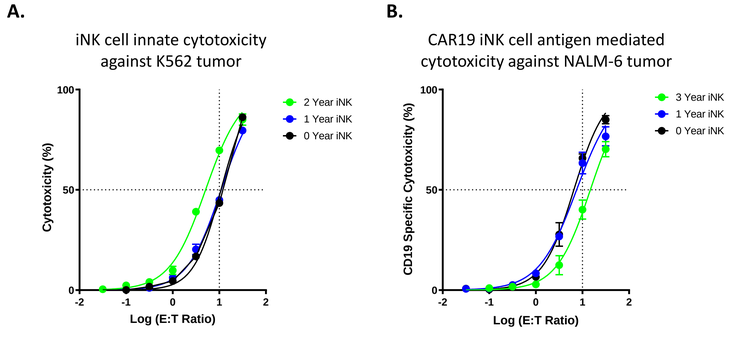


Figure 3. (A) iNK cells were assessed for the generation of the proinflammatory cytokine TNFα in response to anti-CD16 stimulation for 24 hours in a dose-dependent manner. (B) Non-specific IgG at the same concentration elicits minimal spontaneous cytokine release.

iNK Cells Exhibit Potent Innate and Antigen Specific Cytotoxicity against Tumor Targets

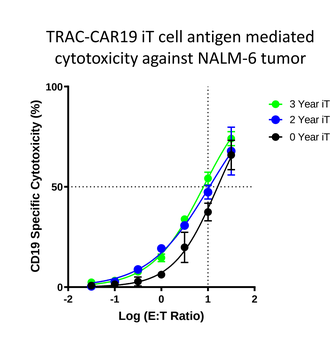


Cryogenic Age	0	1	2
EC50 of K562 Killing	10.9	11.0	5.1

Cryogenic Age	0	1	3
EC50 of Nalm6 Killing	6.5	7.5	14.8

Figure 4. (A) Innate iNK cytotoxicity was assessed via an *in vitro* cytotoxicity assay using K562 tumor cells as targets. Caspase3/7 on K562 tumor targets was measured via flow cytometry after a 4-hour coculture. (B) CAR mediated iNK cytotoxicity was assessed via an *in vitro* cytotoxicity assay using NALM-6 tumor cells as targets. Caspase3/7 on NALM-6 tumor targets was measured via flow cytometry after a 4-hour coculture.

iT Cells Exhibit Potent Antigen Specific Cytotoxicity against Tumor Targets



Cryogenic Age	0	2	3
EC50 of Nalm6 Killing	16.1	10.8	8.0

Figure 5. CAR mediated iT cytotoxicity was assessed via an *in vitro* cytotoxicity assay using NALM-6 tumor cells as targets. Caspase3/7 on NALM-6 tumor targets was measured via flow cytometry after a 4-hour coculture.