High-Density Cryopreservation of Off-the-Shelf CAR Cells Facilitates On-demand Treatment Access

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Abstract #2045

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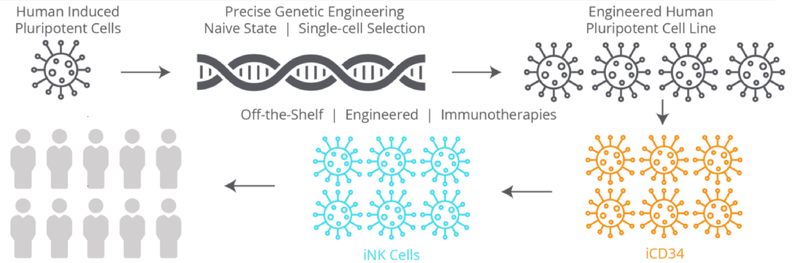
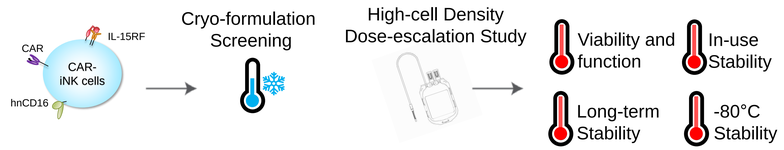
INTRODUCTION



iPSC Product Platform - Disruptive Approach Enabling Mass Production of Universal NK Cell and T-Cell Products. Multiplexed engineered, clonal master induced pluripotent stem cell (iPSC) lines are a renewable source for the routine, mass production of immune effector cells that address many shortcomings associated with current autologous and allogenic donor-derived cell-based immunotherapies, including off-the-shelf availability for broad patient access and multi-dose administration.



Feasibility Study of iNK Dose-escalation. To attenuate patient administration time at clinics, we screened multiple cryo-formulations for iNK cell products and generated testing products at three (3) cell densities, 1.5E+07 (standard-cell density, STD), 5.6E+07 (high-cell density, HD 1), and 1.1E+08 (HD 2) viable cells/mL, from two separate iNK products, FT596 (anti-CD19 CAR) and FT536 (anti-MICA/B CAR) for feasibility and stability evaluations.

RESULTS

Cryo-formulation Screening Identifies Lead Formulation

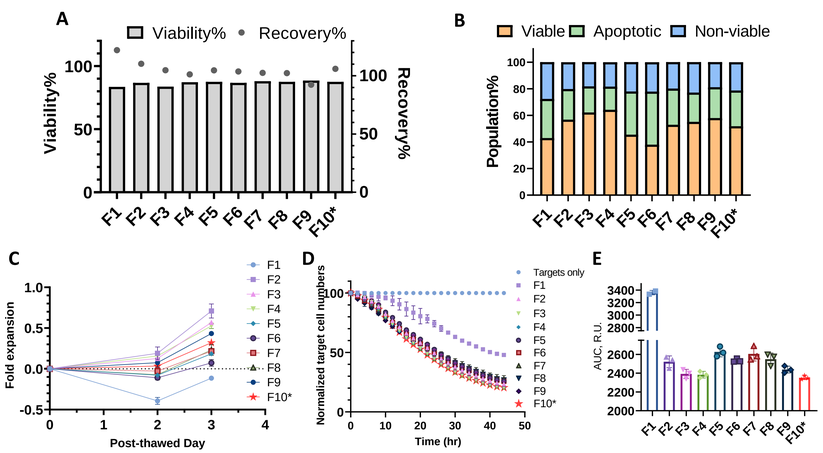
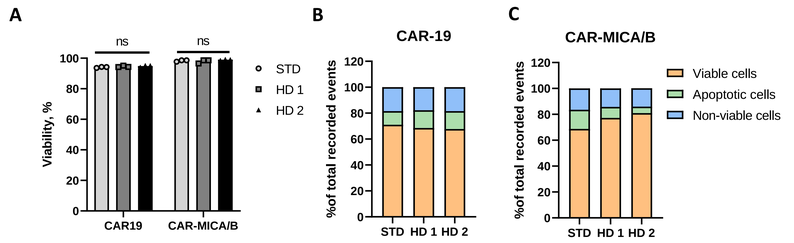


Figure 1. Formulation F10 is identified as the lead formulation by orthogonal assays.

iNK cells were cryopreserved in 10 candidate formulations, thawed and assessed for **A**. viability and recovery by cellular enumeration (AO/DAPI) **B.** Apoptosis via 7-AAD and Annexin V staining and **C.** cell proliferation. **D.** Tumor target cell killing assay (E/T 3:1) showing in the cytotoxicity curve and **E.** area under the curve (AUC) with E:T of 3:1. F10* was identified as top formulation and utilized for all subsequent studies.

High-Density Cryopreserved iNK Cells Retain Robust Viability and Uniform Phenotype



D

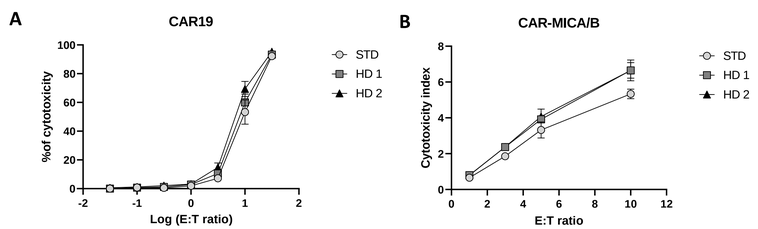
	CAR-19			CAR-MICA/B		
Dose / Expression	STD	HD 1	HD 2	STD	HD 1	HD 2
CD45CD56% of viable	98.0	98.3	98.4	98.1	98.5	98.4
CD16 % of CD45CD56	99.8	99.9	99.9	99.9	99.9	99.9
CAR % of CD45CD56	94.8	96.3	95.8	99.3	99.5	98.4

Figure 2. Comparable cell viability, health, and phenotype in HD-fill iNK cell drug products.

A. Cell viability of HD-fill CAR-19 and CAR-MICA/B iNK cell products is comparable to the STD condition after cell thawing at 37°C. One-way ANOVA. **B,C.** Flow cytometry-based assay indicated that high viability and low apoptotic (7-AAD$^-$Annexin V$^+$) or non-viable cells (7-AAD$^+$) across all cryopreservation densities. **D.** Post-thaw phenotypes were analyzed by flow cytometry. NK cell identity (CD45$^+$CD56$^+$) and the transgene (human non-cleavable CD16, hnCD16) and chimeric antigen receptor (CAR-19, CAR-MICA/B) expressions were >95%.

High-Density Cryopreserved iNK Cells Exhibit Potent Cytotoxicity

HD-fill iNK cells exhibit potent CAR-mediated cytotoxicity against tumor targets



HD-fill iNK cells demonstrate potent antibody-dependent cellular cytotoxicity (ADCC)

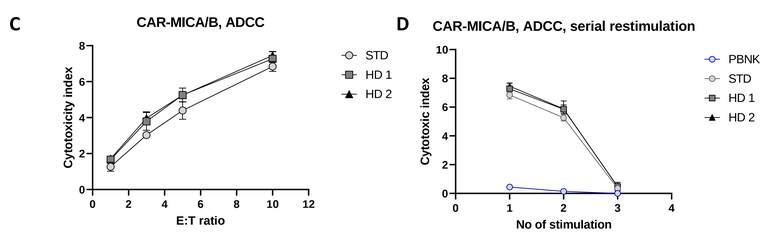
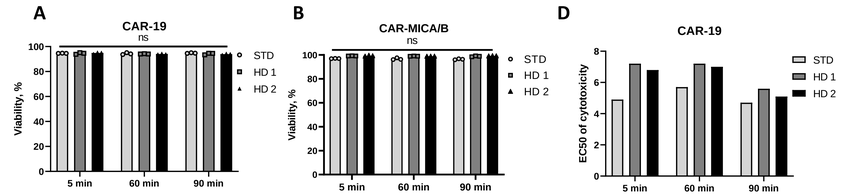


Figure 3. Cytotoxic efficiency of HD-fill cell products.

A. CD19 specific cytotoxicity was detected by a flow cytometry-based killing assay using CAR-19 iNK and NALM-6 cells in a series of E:T ratios. **B**. MICA/B specific cytotoxicity was determined by an image-based killing assay using CAR-MICA/B iNK and CaSki cells in a series of E:T ratios. **C.** CAR-MICA/B iNK cells from HD-fill cryopreservation showed a comparative ADCC efficacy across various E:T ratios. **D.** CAR-MICA/B iNK cells from HD-fill cryopreservation retain potent cytotoxicity in a serial restimulation assay against tumor targets (E:T = 10:1).

In-use Stability of High-Density Cryopreserved iNK Cells is Comparable to Control



C

		CAR-19			CAR-MICA/B		
	Hold Time	STD	HD 1	HD 2	STD	HD 1	HD 2
CD45CD56% of viable	5 min	98.5	97.6	98.5	97.4	98.2	98.5
	90 min	97.2	96.1	97.6	95.8	97.1	97.8
CD16% of	5 min	99.7	99.9	99.8	99.8	100.0	99.9
CD45CD56	90 min	99.3	99.8	99.7	99.7	99.9	99.9
CAR% of	5 min	n/a	n/a	n/a	99.4	100.0	100.0
CD45CD56	90 min	n/a	n/a	n/a	99.2	100.0	100.0

Figure 4. HD-fill iNK cells are stable at room temperature for 90 minutes.

A, B. Cell viability of HD-fill CAR-19 and CAR-MICA/B iNK cell products is comparable to the STD condition after hold times of 5, 60, and 90 minutes at room temperature. Viability variation is analyzed by two-way ANOVA. **C.** CAR-19 and CAR-MICA/B iNK cells retain expressions of NK identity and transgenes after holding at room temperature up to 90 minutes. **D, E.** Potency of HD-fill iNK cell products is analyzed by the flow cytometry-based killing assay. Both NK products show consistent CAR-mediate cell killing.

High-Density Cryopreserved iNK Cells are Stable in Long-Term Storage at -80ºC and <-150ºC to Support Expanded Patient Access

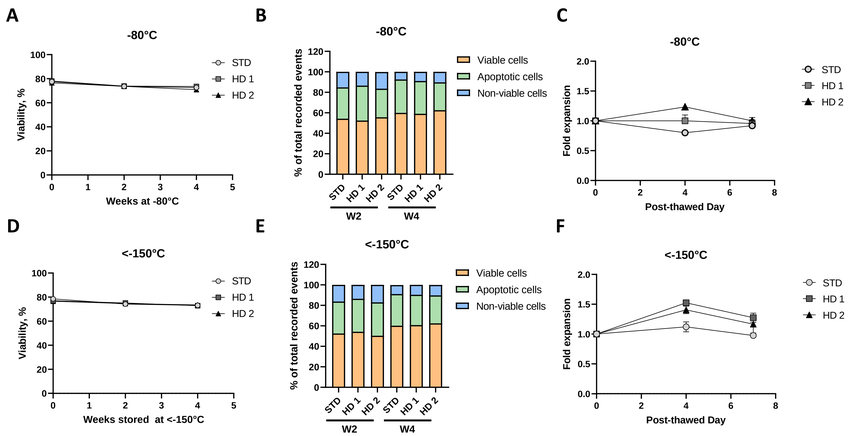


Figure 5. Cell health and product composition remain stable at -80°C storage for at least 1 month.

Cell health evaluated by **A, D.** Cell viability, **B, E.** apoptotic fraction immediate post-thaw, and **C, F.** cell proliferation from CAR-19 iNK stored in -80°C (A-C) are comparable to iNK cells stored at the vapor phase liquid nitrogen (<-150°C, D-F). Week 4 cells post-cryo proliferation is showed in C and F.

CONCLUSION

We demonstrate the successful generation of HD-fill CAR-iNK cells and stable storage at -80ºC and <-150ºC without interfering with drug product integrity, identity, and function, with preclinical data demonstrating consistent viability, recovery, and potency between control arm and selected test configurations. HD-fill for off-the-shelf immunotherapies will further enable flexibility to clinicians and patients in the out-patient setting for the treatment of hematologic and solid tumors.