Natural Killer and γδ T Cells Derived from Engineered Induced Pluripotent Stem Cells Have Potent Preclinical Activity to Treat B cell-mediated Autoimmune Diseases

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Context:

- Century Therapeutics is developing IPSC-derived cell therapies for the treatment of B-cell mediated autoimmune diseases
- B-lymphocyte antigen CD19 specific chimeric antigen receptor (CAR) expressing T-cell therapy has been established as an effective treatment for CD19+ malignancies. Recently these therapies are being evaluated as treatments for subsets of autoimmune diseases thought to be mediated by B cells producing auto-reactive antibodies.
- Gamma delta (γδ) T cells and natural killer (NK) cells exhibit the potency and cytolytic features of conventional alpha beta (αβ) CAR-T cells with a lower expansion capacity and persistence upon antigen engagement, potentially providing tighter control over B cell depletion and mitigating the risk of prolonged B cell aplasia.

Objectives:

- Determine the *in vitro* and *in vivo* B-cell killing efficiency of IPSC-derived γδ iT-Cells and iNK-Cells against acute lymphoblastic leukemia NALM-6 cells.
- Assess the IPSC-derived cell therapies in a PBMC-humanized NOD SCID Gamma (NSG) mouse model.

Approach and Methodology:

- iPSCs derived by re-programming peripheral γδ T cells (TiPSC) and peripheral blood mononuclear cells (PiPSC) were engineered using MAD7, a Class 2 Type V Crispr-Cas9 nuclease, to express a CD19-targeting (FMC63) CAR, membrane-bound IL-15/IL15Ra, and Allo-Evasion™ edits, which includes expression of Human Leukocyte Antigen alpha-chain E (HLA-E) and G (HLA-G), as well as knock-out of B2M and CIITA to abrogate Class I and II HLA surface expression.
- Engineered TiPSC and PiPSC were then differentiated into hematopoietic progenitor cells (HPC), and further differentiated into γδ T cells and NK cells respectively.
- PBMCs were isolated from leukopaks obtained from normal donors. From these, B cells were isolated for in vitro assays. Normal T cells were transduced for CD19 CAR expression, as a positive control in experiments.
- IPSC-derived γδ T and NK (iT and iNK, respectively) killing efficiency of B cells and acute lymphoblastic leukemia NALM-6 cells were tested in an in vitro single round killing assay. Effector cells and target cells were plated at multiple effector-to-target (E:T) cell ratios, and incubated for 24 hours, at which point cells were stained and analyzed by flow cytometry for target cell cytolysis.
- A PBMC-humanized NOD SCID Gamma (NSG) mouse model was used to test the B cell killing capacity of iT and iNK. Mice were intravenously engrafted with PBMCs, and 14 days later received a single intravenous injection of iT, iNK, or primary CAR-T. One week later, mice were humanely euthanized, and blood and tissues collected for Fluorescence-Activated Cell Sorting (FACS).

Conclusions:

- IPSC-derived γδ T and NK cells were generated using a proprietary differentiation process yielding >90% pure CD19 CAR+ γδ T and NK cells. Cells were confirmed by FACS to be positive for IL-15/IL15Ra, HLA-E and HLA-G transgenes, and negative for B2M and CIITA.
- In vitro, both iT and iNK had near equivalent potency in killing of B cells and NALM-6 target cells. Significant killing of B cells and NALM-6 cells was observed with both iT and iNK.
- Mouse blood and tissues were evaluated by FACS and IHC for presence of human B cells following CD19 CAR effector cell treatment. Compared to untreated humanized mice, B cell depletion was observed in blood and tissues of iT and iNK treated mice, at levels equivalent to or greater than that observed in primary CD19 CAR-T treated mice. In blood and bone marrow, significant and near complete B cell depletion was observed with iT and iNK treatment. Splenic B cell depletion was incomplete but nearly equivalent across all treatments including CAR-T.

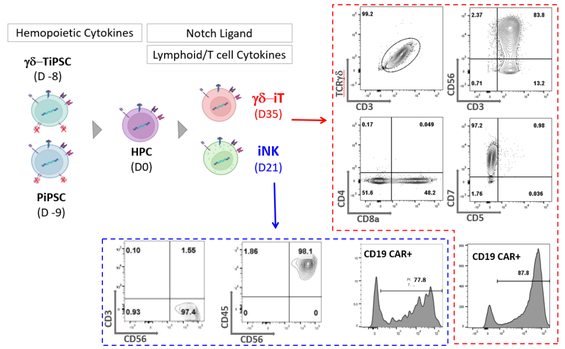
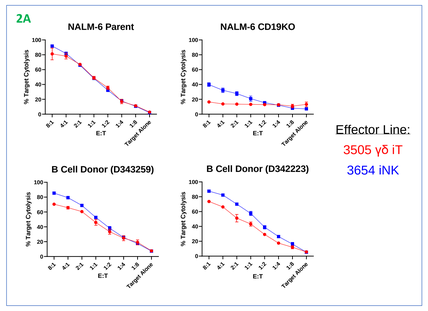


Figure 1. Generation of IPSC-derived γδ iT-cells and iNK cells: The HPC aggregation-mediated differentiation process yields a pure CD34+/CD43+ population that is subsequently differentiated through either a T cell lineage (γδ–iT) or natural killer cell lineage (iNK). D35 γδ–iT cells are characterized by high CD3+/TCRgd+/ CD56+/CD7+ surface expression, while D21 iNKs are characterized by high CD45+/CD56+/CD3- expression. For both lines, high surface level CD19-CAR expression is maintained (>75%).



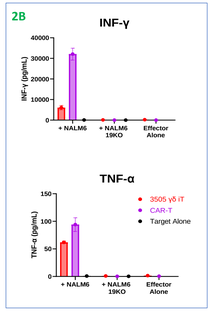


Figure 2. Depletion of CD19+ NALM-6 cells and isolated B cells from healthy donors using IPSC-derived γδ iT Cells and iNK Cells: **2A.** iNK or γδ iT cells were co-cultured with CD19-expressing cell line NALM-6, NALM-6 with CD19 knock-out, or isolated B cells from healthy donor peripheral blood mononuclear cells (PBMCs). Target cell cytolysis was quantified by measuring the percentage of dead cells within the Cell-traced Target cell population for each Effector (E) to Target (T) ratio after 24 hours of co-culture. Each data point represents the average of three replicates, with error bars indicating the standard deviation of the mean. Increasing values on the Y-axis correspond to higher levels of B cell cytolysis.
2B. γδ iT or PBMC-derived CAR-T cell were co-cultured with CD19-expressing NALM-6 cells, NALM-6 CD19KO, or cells cultured alone. Supernatant was harvested 72 hours and INF-γ (top) and TNF-α (bottom) levels were measured using Meso Scale Discovery multiplex ELISA plates. Figures represent cytokine detection at an E:T of 1:1 (20k effectors, 20k targets), columns show the average of 3 replicates and error bars represent standard error of the mean.

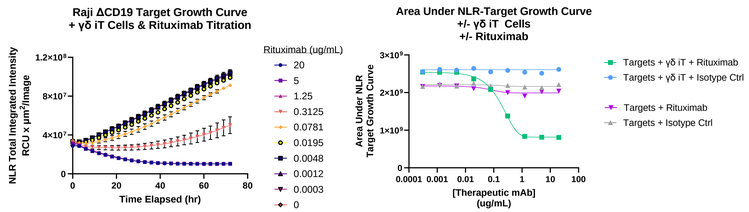
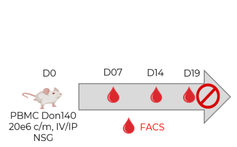


Figure 3. Demonstrating Antibody-Dependent Cellular Cytotoxicity Function using γδ iT: γδ iT cells were co-cultured with NucLight Red (NLR)-expressing CD19 Knockout Raji cells to eliminate CAR-mediated cytolytic activity. The treatments included either Rituximab or a host-matched isotype control. (Left) The target NLR signal was measured over time using an Incucyte S3 instrument, during co-culture with γδ iT cells and a titration of Rituximab. (Right) The area under the curve (AUC) for NLR target growth, with and without γδ iT cells, demonstrates the γδ iT cell-specific ADCC function when cultured with Rituximab compared to the host-matched isotype control.



#PBMC's / 1e5 live cells on day of implant

Cell	Average
T	1.49E+04
NK	1.24E+04
NK-T	0.32E+04
B	0.75E+04
CD14+	2.87E+04

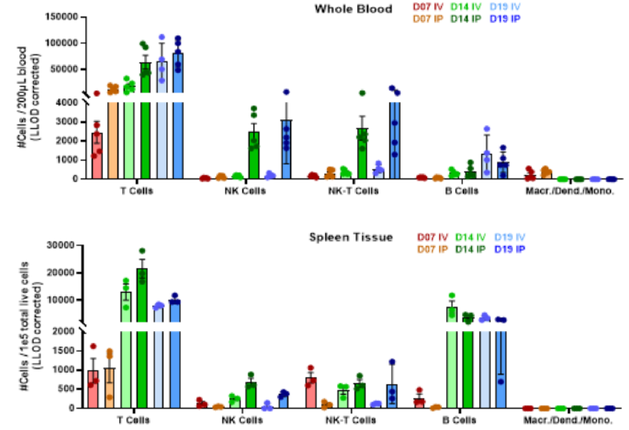
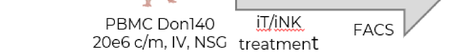


Figure 4. Engraftment of PBMC Dosed Systemic or Intraperitoneal in NSG Mice: To assess engraftment timeline of donor-derived PBMC populations, NSG animals were humanized with 20e6 cells/mouse PBMC Donor140 cells via IV or IP route. Whole blood and spleen tissue was collected for FACS evaluation on D7, D14 and D19 post humanization. PBMC levels are comparable between D14 and D19 post humanization. B cell levels are comparable for IV and IP implanted routes.



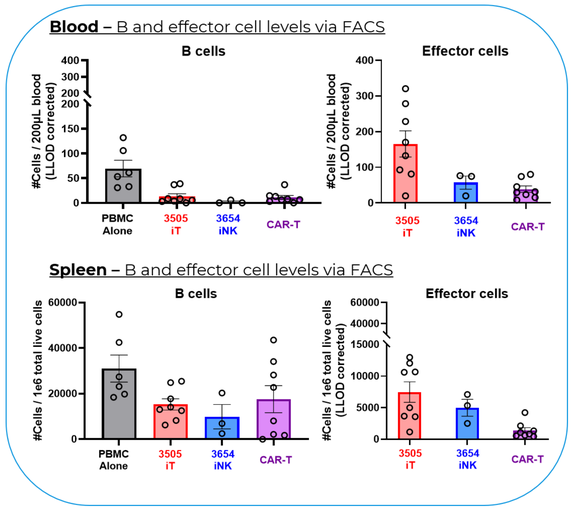


Figure 5. B Cell Depletion of Humanized Mice using IPSC-derived γδ iT-Celss and iNK cells: To evaluate the B cell killing capacity of CD19 CAR iNK and iT cells in vivo, a study was designed by humanizing NSG mice IV with 20e6 cells/mouse PBMC Donor 140 cells for 14 days. Whole blood and spleen were collected for FACS evaluation on D6 post treatment. γδ IT cells (p=0.0084), iNK cells (p=0.004) and pCAR-T cells (p=0.0083) significantly deplete B cells found in whole blood. B cells are not significantly depleted in the spleen via FACS analysis.