Development of Multi-Engineered iPSC-Derived CAR-NK Cells for the Treatment of B-Cell Malignancies

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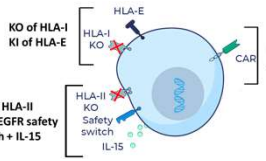
Introduction

- Induced-pluripotent stem cells (iPSCs) can be differentiated into various somatic cells, including different immune cell types. We have engineered iPSC-derived NK cells (iNK cells) with multiple features to generate novel cancer cell therapies.
- iPSCs have unlimited replication capacity, which significantly simplifies cell engineering because multiple gene edits can be incorporated without driving cells to exhaustion. Once the gene editing process is completed, we single-cell clone the edited cells and do in depth genetic characterization to select clones with the intended gene edits and no off-target insertions or deletions.
- Following the genetic characterization, selected clones are differentiated into NK cells and tested in vitro and in vivo to identify the final clinical candidate. The use of a single-cell iPSC clone enables the generation of a master cell bank producing a highly uniform cell product that can be made available off-the-shelf at any clinical site.
- CNTY-101 is an iPSC-derived CAR-NK clinical candidate for the treatment of B-cell malignancies. It incorporates six gene edits designed to improve persistence and functionality as well as safety. These modifications include edits to reduce graft rejection due to alloreactivity, the expression of a homeostatic cytokine (IL-15) to improve functionality and persistence, the introduction of a chimeric antigen receptor (CAR) targeting CD19 to mediate tumor cell engagement and killing, and a safety switch to eliminate the cells if ever necessary.
- To prevent rejection by the patient's CD8 T cells, the beta-2-microbulin (β2M) gene was disrupted with simultaneous insertion of a transgene encoding HLA-E. HLA-E was introduced to prevent NK cell cytotoxicity against the engineered cells, which lack HLA-I. For resistance to CD4 T cell-mediated allogenic rejection, the class II major histocompatibility complex transactivator (CIITA) gene was disrupted with simultaneous insertion of a transgene encoding a shortened version of EGFR and IL-15. EGFR is an elimination tag that can be engaged by clinically approved anti-EGFR antibodies such as cetuximab. The CAR construct targeting the CD19 antigen was inserted into the AAVS1 safe harbor locus.

CNTY-101: Allogeneic iPSC-Derived NK (iNK) Product Candidate For The Treatment Of B Cell Malignancies



CNTY-101 incorporates six gene edits into three engineering steps where we combined the knock-out (KO) β2m to prevent MHC Class I expression with the knock-in (KI) of HLA-E, the KO of CIITA to prevent MHC Class I expression with the KI of the EGFR safety switch and IL-15, and the KI of the CAR construct into AAVS1 locus.

Materials and Methods

iNK cells were derived from Induced Pluripotent Stem Cells (iPSC) using a cell-free system and a 30-day differentiation process where the cells were exposed to different cytokine cocktails and extracellular matrix components.

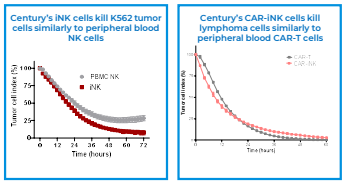
At the end of the differentiation process, the iNK cells were either immediately used (fresh cells) or cryopreserved for later use.

CNTY-101 was engineered using a Class 2 Type V CRISPR nuclease to mediate homology directed repair. The gene edits were done sequentially and at the end of the process, the edited iPSCs were single-cell cloned. Single-cell clones were individually characterized using genetic and functional assays to select the final CNTY-101 development candidate.

Acknowledgements

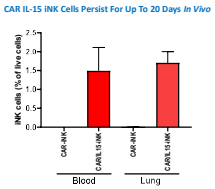
We want to acknowledge Lucas Thompson for his significant contributions to this poster

iNK Cells Kill Tumor Cells Through the Engagement of Innate Receptors And CARs



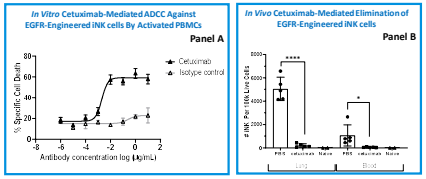
iNK cells and PBMC NK cells were incubated with K562 tumor cells labelled with Nuclight Red (NLR) (**Left panel**). CD19-CAR iNK cells and peripheral blood CD19-CAR T cells were incubated with Raji tumor cells labelled with NLR (**Right panel**). Tumor cell index measures the density of tumor cells in the wells. Cultures were imaged every 3 hours on the Incucyte live cell imager.

iNK Cells Engineered With IL-15 Have Increased Persistence *In Vivo*



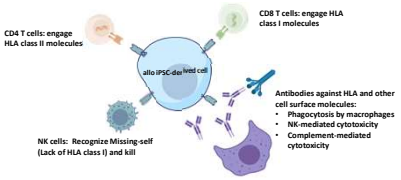
CAR-iNK cells engineered with or without secretable IL-15 were administered as a single dose (10^6 cells/mouse) and 20 days post-injection, the animals were sacrificed to investigate the persistence of the iNK cells. iNK cells were detected by FACS analysis.

iNK Cells Engineered With A Shortened Version Of EGFR Are Eliminated By Cetuximab Both *In Vitro* And *In Vivo*

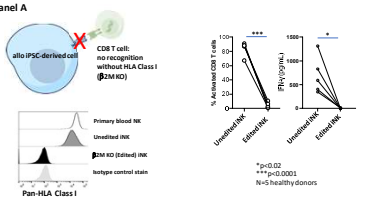


Panel A: IL-2 activated PBMC were co-cultured with EGFR-engineered iNK cells at a 25:1 E:T ratio in the presence of Cetuximab or human IgG1 isotype control for 16 hours. Cell viability was assessed by FACS analysis.
Panel B: $15x10^6$ cryo-preserved iNK cells were administered IV on Day 1. On Days 2 and 3, PBS or 40 mg/kg Cetuximab were administered IP. On Day 5, mice were humanely euthanized and sampled for FACS analysis.

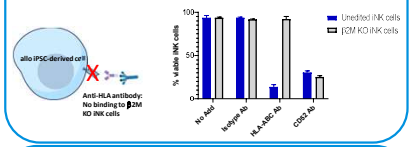
Allo-Evasion Gene Edits Are Designed To Avoid Rejection By The Host Immune System



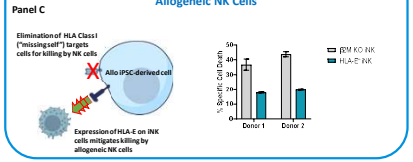
Allo-Reactive T cells Do Not Recognize β2M KO iNK Cells



β2M KO iNK Are Resistant Complement-Dependent Cytotoxicity Mediated By Anti-HLA Antibodies

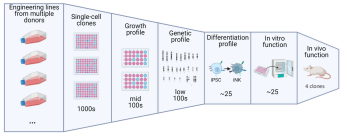


iNK Cells Engineered With HLA-E Are Less Susceptible To Killing By Allogeneic NK Cells



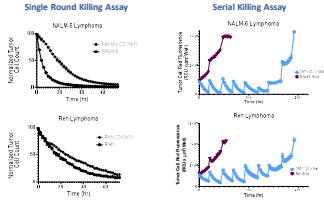
Panel A: Unedited and β2M KO iNK cells were exposed to allogeneic T cells. T cell reactivity was evaluated by analyzing the % of $CD25^+$ activated T cells and the levels of IFN-g.
Panel B: Unedited and β2M KO iNK cells were incubated with a pan-HLA antibody and exposed to rabbit serum complement for 1 hour. Cell viability was determined by Trypan Blue staining.
Panel C: β2M KO iNK cells engineered or not with HLA-E were incubated with allogeneic PBMCs at a 25:1 E:T ratio for 72 hours. Cell viability was assessed by FACS analysis.

CNTY-101 Discovery Pipeline Funnel



To identify the CNTY-101 development candidate, we engineered iPSC lines from five different donors. After characterization of the bulk cells, we generated single-cell clones. The cells were genetically characterized to select clones that incorporated all gene edits and no off-target effects. Twenty-five clones were tested in vitro before selecting four clones to test in vivo for final candidate selection.

CNTY-101 *In Vitro* Cytotoxicity Against Lymphoma Cell Lines

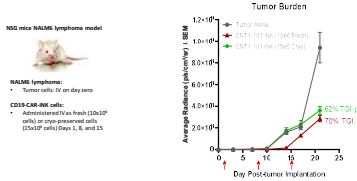


CNTY-101 iNK cells were incubated with NALM-6 and Reh lymphoma cells labelled with NLR

Left panel: the cytotoxicity assay was run for 72 hours at an E:T ratio of 10:1.

Right panel: serial killing assay with CNTY-101. NALM-6 or Reh lymphoma cells were cultured alone or in the presence of CNTY-101 iNK cells. At the beginning, 100K CNTY-101 iNK cells were co-cultured with 20K NALM-6 or Reh cells. At every 24 hours and for a total of 9 days, fresh tumor cells were added to the cultures.

CNTY-101 Has Robust Anti-Tumor Activity Against Lymphoma Xenograft



1x10^5 luciferase-labeled NALM-6 lymphoma cells were administered IV on day zero. CNTY-101 iNK cells were given IV as fresh (10x10^6 cells) or cryo-preserved cells (15x10^6 cells) on Days 1, 8, and 15. Mice were imaged every 3-4 days using the IVIS SpectrumCT imager.

Summary

- We have engineered iPSC-derived NK cells with multiple features to develop innovative anti-cancer cell therapies. CNTY-101 is an iPSC-derived CD19 CAR-NK clinical candidate for the treatment of B-cell malignancies. It incorporates six gene edits designed to improve persistence, functionality, and safety
- CAR-iNK cells kill tumor targets through both innate receptors and engineered CARs. Their persistence and functionality are improved by the expression of IL-15. Edits to knock-out MHC Class I and II expression prevent recognition by allogeneic T cells and expression of HLA-E mitigates killing by allogeneic NK cells
- To improve safety, the iNK cells were engineered with a shortened version of EGFR. Proof-of-concept studies have demonstrated that the INK cells can be quickly eliminated by the administration of Cetuximab, a clinically approved antibody against EGFR
- CNTY-101 iNK cells have strong antitumor activity against human lymphoma cell lines. In vitro, CNTY-101 eliminates lymphoma cell lines through multiple rounds of killing. In vivo, CNTY-101 mediates significant tumor growth inhibition after administration of fresh or cryopreserved cells