Characterization of CNTY-101, an Allogeneic Anti-CD19 iPSC-Derived NK Product, for the Treatment of B Cell-Driven Autoimmune Diseases

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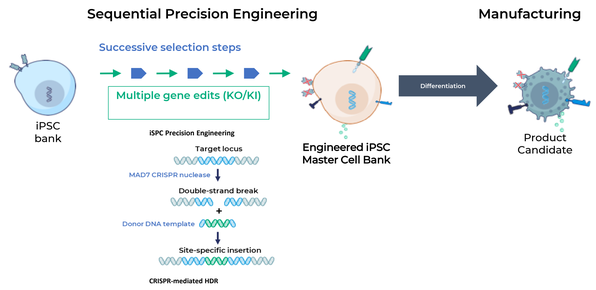
Background

Achieving long-term remission in B cell-driven autoimmune diseases (AID), including Systemic Lupus Erythematosus (SLE), remains a substantial unmet need. Recently, numerous autologous anti-CD19 CAR T therapies have shown promise in treating patients affected by SLE by depleting autoreactive B cells. Allogeneic induced pluripotent stem cell (iPSC)-derived cells present a unique opportunity to offer a clonal, fully characterized, off-the-shelf cell therapy product. We describe a cell therapy product, CNTY-101, with multiple precision gene edits, featuring antigen-specific killing of CD19+ B cells, homeostatic cytokine support for enhanced persistence, edits to prevent rejection by the patients' immune system, and a safety switch.

Methods

CNTY-101 is differentiated next-generation CD19-targeted induced pluripotent stem cell (iPSC)-derived iNK cell therapy with six (6) precision gene edits including Century's Allo-Evasion™ technology. CNTY-101 has been engineered to express a CD19 chimeric antigen receptor (CAR) for re-directed tumor killing, and secretable interleukin 15 (IL-15) for enhanced persistence. The expression of HLA class I (HLA-I) and II (HLA-II) was disrupted through genetic ablation of beta 2 microglobulin (β2M) and the class II transactivator (CIITA), respectively, allowing evasion of the patient's adaptive immune system, while expression of human leukocyte antigen (HLA)-E was engineered to prevent NK cell-mediated rejection. Lastly, a safety switch consisting of a short epidermal growth factor receptor (sEGFR) variant containing the Cetuximab binding epitope was included to allow for elimination of the cells if necessary. *In vitro* functional assays were performed to demonstrate the functionality of each edit.



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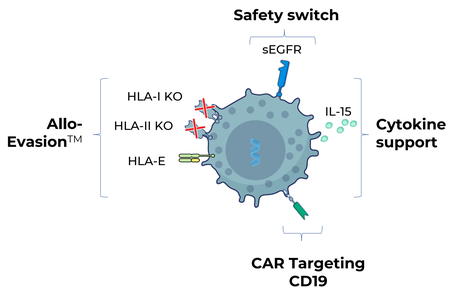

Figure 1: CNTY-101 Effectively Depletes B Cells With Greater Potency When Compared to Autologous Primary CAR-T Cells

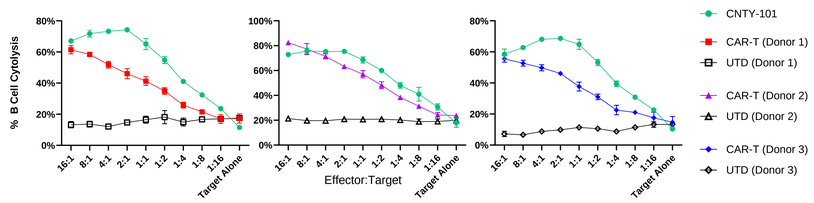

Fig 1. To assess the cytolytic activity against B cells, isolated B cells from healthy donor peripheral blood mononuclear cells (PBMCs) were subject to co-culture with CNTY-101, CD19-targeting PBMC-derived CAR-T cells obtained from an autologous donor, or untransduced PBMC-derived T cells from an autologous donor. B cell cytolysis was quantified by measuring the percentage of dead cells within the Cell-traced B 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.

Figure 2: CNTY-101 Effectively Depletes B Cells from Systemic Lupus Erythematosus (SLE) Patient PBMCs

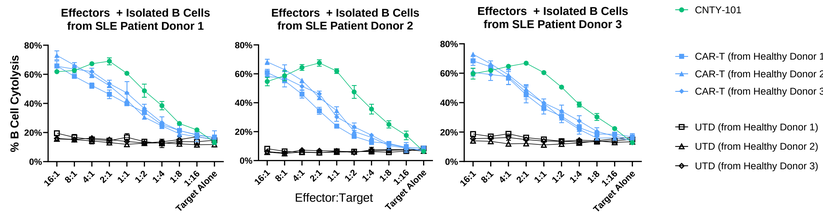

Fig 2. To assess the cytolytic activity against B cells derived from Systemic Lupus Erythematosus (SLE) patients, isolated B cells from SLE patient peripheral blood mononuclear cells (PBMCs) were subjected to co-culture with CNTY-101, CD19-targeting PBMC-derived CAR-T cells, or untransduced PBMC-derived T cells. B cell cytolysis was quantified by measuring the percentage of dead cells within the Cell-traced B 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.

Figure 3: CNTY-101 Effectively Depletes Immortalized CD19+ Cells After Single or Multiple Rounds of Target Addition

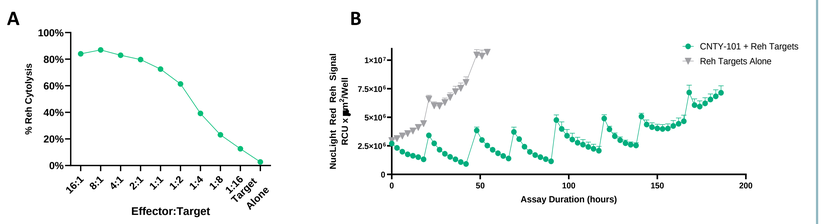

Fig 3A. CNTY-101 was co-cultured with Reh target cells, an immortalized cell line expressing CD19. B cell cytolysis was assessed by quantifying the percentage of dead cells within the Cell-traced B cell population for each Effector (E) to Target (T) ratio after 24 hours of co-culture. Each data point represents the average of three replicates, with error bars indicating the standard deviation of the mean. Increasing values on the Y-axis indicate greater cell cytotoxicity.

Fig 3B. NucLight Red-expressing Reh cells were co-cultured with CNTY-101 or cultured alone. Every 24 hours, fresh Reh cells were added back into the assay at the same target seeding density. NucLight target cell growth was quantified using Incucyte S3 Live Cell Imager. Each data point is the average of 3 replicates NucLight Red signal at a given assay timepoint ± standard deviation to the mean.

Figure 4: CNTY-101 Secretes Less Inflammatory Cytokine in an *in vitro* assay When Compared to Autologous Primary CAR-T Cells

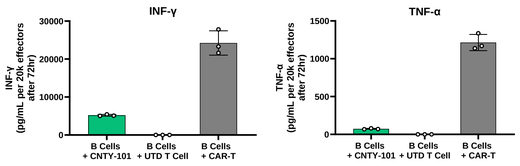

Fig 4. Isolated B cells from healthy donors were co-cultured with CNTY-101 (green), untransduced T cells from an autologous donor (black) or CAR-T cells from an autologous donor (grey). Supernatant was harvested 72 hours after co-culture setup and INF-γ (right) and TNF-α (left) levels were measured using Meso Scale Discovery multiplex ELISA plates. Figures repent cytokine detection at an E:T of 1:1 (20k effectors, 20k B cells) where columns represent the average of 3 replicates and error bars represent standard error of the mean.

Figure 5: IL-15 Transgene Enhances Persistence of CNTY-101 and is Further Enhanced with the addition of Exogenous IL-2

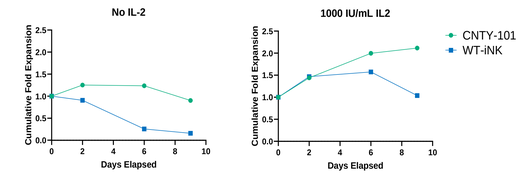

Fig 5. CNTY-101 or wild-type (WT) iNK cells were cultured in a 9-day persistence assay without exogenous IL-2 support (left) or with 1000 IU/mL IL-2 (right). Cumulative fold expansion was calculated based on the initial seeding number of iNK cells at start of the assay.

Figure 6: Ablation of HLA-I expression protects CNTY-101 from CD8 T-cell mediated elimination

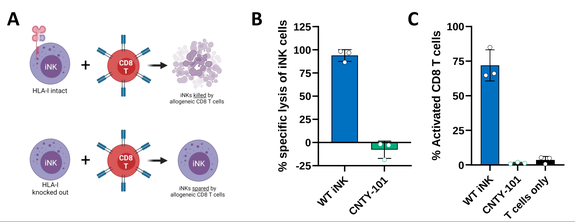

Fig 6A. Schematic illustration depicting Allo-evasion from Cytotoxic T Lymphocytes (CTLs) through HLA-I ablation.

Fig 6B. Specific lysis of iNK cells that were unedited (blue) or CNTY-101 (green) after co-culture with isolated CTLs from three individual PBMC donors with mismatched HLA, co-cultured at a 1:5 iNK:T cell ratio. Each data point represents an individual CTL donor, with standard deviation indicated.

Fig 6C. CD25 expression on CD8+ cells when co-cultured with wild-type iNK cells (blue), CNTY-101 (green), or cultured alone (black). Each data point represents an individual CTL donor, with standard deviation indicated.

Figure 7: Engineered HLA-E in CNTY-101 Protects β2M KO iNK Cells from NK Cell-Mediated Killing

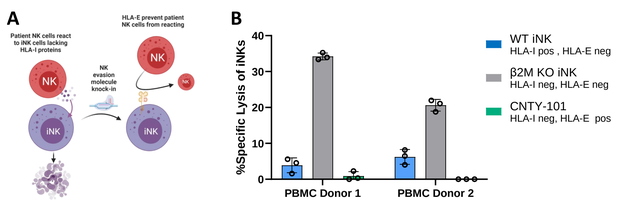

Fig 7A. Schematic illustration depicting Allo-evasion from NK cells through the insertion of HLA-E into β2m KO Cells.

Fig 7B. Two donor-mismatched PBMCs were co-cultured with wild-type iNK cells (blue), iNKs lacking β2m (grey bars), or CNTY-101, lacking β2m but with the inclusion of HLA-E (green), at a 25:1 Effector (E) to Target (T) ratio in the presence of 10 ng/mL IL-15 for 72 hours. Specific lysis was determined at the 72-hour timepoint by viability dye using flow cytometry. Each data point represents the mean of triplicate wells ± standard deviation to the mean.

Fig 8: *In Vitro* Depletion of CNTY-101 using PBMC NK Cells through Cetuximab-Mediated ADCC

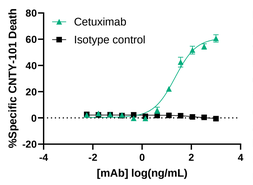

Fig 8. Cryopreserved PBMCs were thawed and rested in media containing IL-2. PBMC cells were then co-cultured with CNTY-101 at a 25:1 Effector (E) to Target (T) ratio and with various doses of Cetuximab (green) or human IgG1 isotype control (black). Percent specific cell lysis of CNTY-101 is plotted based on the dose of antibody, harvested at a 24-hour timepoint. Each data point represents the mean of triplicate wells ± standard deviation to the mean.

Summary

- We demonstrate CNTY-101's ability to induce CD19-specific cytolysis of both CD19+ tumor cells and isolated B cells from multiple healthy and SLE patient donors.
- When compared with peripheral blood mononuclear cell (PBMC) derived CAR-T cells, CNTY-101 exhibits greater potency while observing less detectable inflammatory cytokine secretion after B cell depletion.
- The inclusion of the homeostatic cytokine transgene allows for the persistence of CNTY-101 which is enhanced with the addition of exogenous IL-2.
- The inclusion of Allo-Evasion™ edits (β2M and CIITA knockouts and HLA-E knock-in) allows for evasion from recipient PBMCs.
- sEGFR is an elimination tag that can be engaged using anti-EGFR antibodies to deplete CNTY-101.