GNTI-122 is a Dual-Engineered Regulatory T Cell Therapy Product for Type 1 Diabetes with Enhanced Stability, Tissue Specificity, and Tunable IL-2 Signaling

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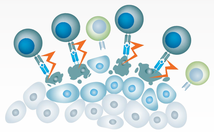
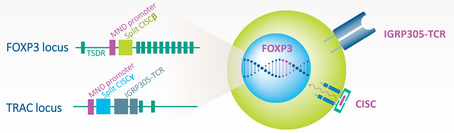
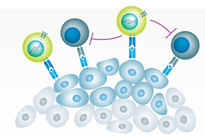
OVERVIEW



Type 1 diabetes (T1D) is an autoimmune disease caused by autoreactive T lymphocyte-mediated destruction of insulin-producing beta cells that leads to uncontrolled hyperglycemia and life-long dependence on daily insulin administration.

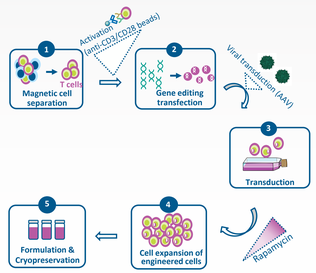


GNTI-122 is engineered from autologous CD4 T cells using HDR-mediated gene editing facilitated by RNA-guided nucleases to knock-in the following: An MND promoter into the FOXP3 gene to stabilize its expression; IGRP305-TCR, a pancreatic islet antigen-specific TCR, into the TRAC locus; and a chemically inducible signaling receptor (CISC).



GNTI-122 is designed to protect islet cells from damage by homing to the pancreas and draining lymph nodes and suppressing pathogenic effector T cells via bystander suppression and infectious tolerance. The addition of CISC enables GNTI-122 to expand in the IL-2 scarce environment of a diabetic pancreas.

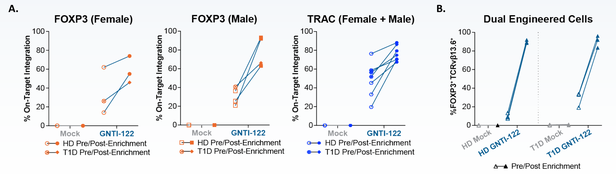
GENERATION OF GNTI-122



$CD4^+$ T cells were isolated via magnetic enrichment from PBMCs. The cells were then genetically modified using CRISPR-Cas9 to knock-in transgenes delivered by AAV vectors. CISC receptor enables selective expansion and enrichment of engineered Tregs in the presence of rapamycin. The expanded cells were cryopreserved at >80% purity.

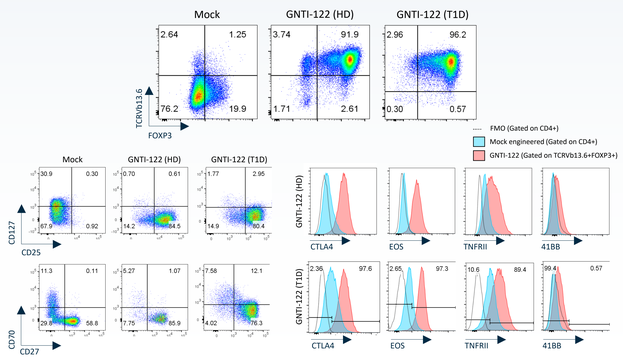
GNTI-122 ENGINEERING IMPARTS TREG PHENOTYPE AND FUNCTION IN HEALTHY DONOR (HD) & T1D PATIENT CELLS

Rapamycin-mediated enrichment increases frequency of dual engineered cells



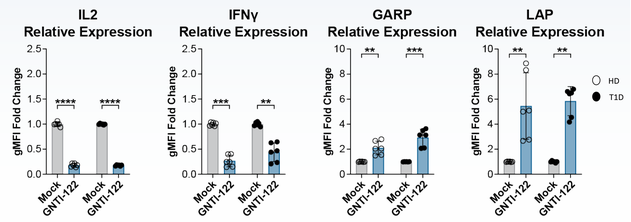
Analysis of rapamycin-mediated enrichment of dual engineered cells. The frequency of cells demonstrating expression of both FOXP3 and IGRP305-TCR significantly increases in the presence of rapamycin due to CISC activation. There are no significant differences in dual engineering frequencies or enrichment efficiency between GNTI-122 generated from healthy donors (HD) or donors with T1D. **A.** dPCR quantification of the frequency of engineered alleles **B.** Frequency of FOXP3 and TCRvβ13.6 (the TCRβ V-gene of the IGRP305-TCR) measured at single-cell resolution by flow cytometry, gated on live CD4+.

GNTI-122 cells, from both HD & T1D donors, express Treg associated markers



GNTI-122 cells express Treg markers (FOXP3$^+$CD25$^+$CD127$^-$) & increase functional (CTLA-4, 41BB) and stability (TNFRII, CD27$^+$CD70$^-$, EOS) markers. Mock cells are gated on CD4+ cells, and GNTI-122 cells are gated on TCR$^+$FOXP3$^+$ cells. Reproduced across 3 independent donors of patients with T1D.

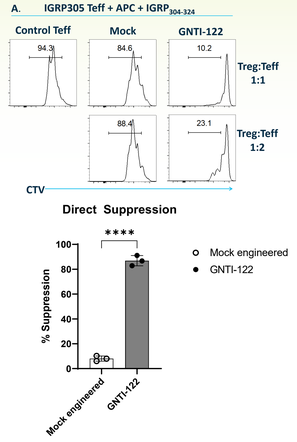
GNTI-122 cells express TGF-β associated markers and not inflammatory cytokines



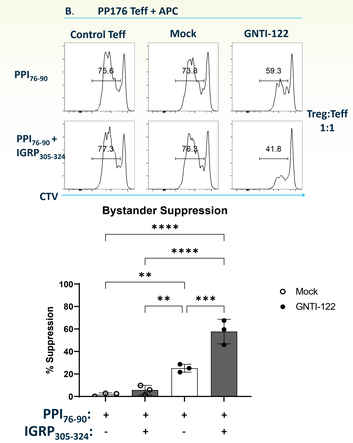
Cells were stimulated with PMA, ionomycin or with anti-CD3/CD28 beads and blocked with monensin before staining for the indicated cytokines. The relative geometric mean fluorescence intensity (gMFI) levels were normalized to mock cells. 2-way ANOVA. Representative donor data shown, reproduced across 6 independent donors.

GNTI-122 REDUCES PROLIFERATION OF PATHOGENIC TEFFS VIA ANTIGEN-SPECIFIC STIMULATION

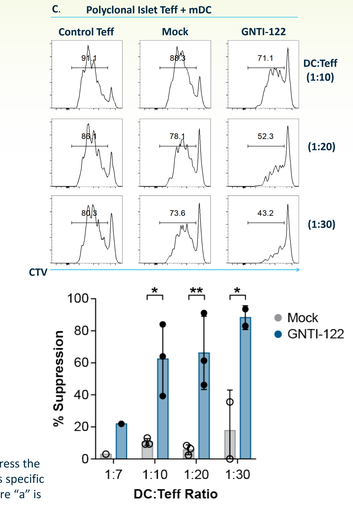
GNTI-122 cells inhibit the proliferation of Teff cells via direct suppression



GNTI-122 cells inhibit the proliferation of Teff via bystander suppression



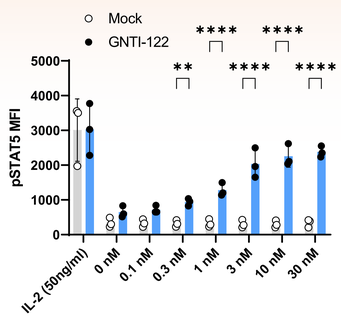
GNTI-122 cells inhibit the proliferation of polyclonal islet antigen-specific Teffs



GNTI-122 cells are cocultured with autologous Teffs from patient donors with T1D, and monocyte-derived dendritic cells as antigen-presenting cells (APCs). **A**. The Teffs express the same TCR, and APCs were loaded with their cognate peptide. **B.** The Teffs express a different TCR, and the APCs are loaded with corresponding cognate peptide. **C.** The Teffs specific to 9 different cognate peptides were isolated and APCs were loaded with their cognate peptides. Suppression was calculated as follows: % suppression = ((a-b)/a)x100, where "a" is the percentage of Teff proliferation in the absence of Tregs and "b" is the percentage of Teff proliferation in the presence of Tregs.

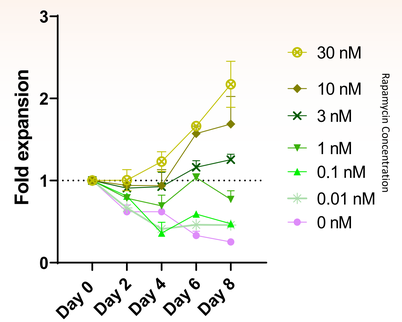
RAPAMYCIN MEDIATES IL-2 LIKE SIGNAL THROUGH CISC ACTIVATION IN VITRO AND IN VIVO

Rapamycin promotes cell proliferation by mediating STAT5 phosphorylation



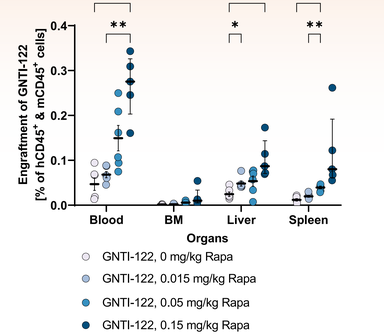
Phosphorylated STAT5 (pSTAT5) MFI show dose-specific response with rapamycin in GNTI-122 cells in culture. Quantification of mean fluorescence intensity (MFI) of pSTAT5 at each concentration of rapamycin. Repeated measures ANOVA with Sidak's multiple comparison tests at each dose (**p<0.01, ****p<0.0001). The errors bars represent mean +/- SEM, N=3 donors.

GNTI-122 expands in a rapamycin concentration-dependent manner



CISC supports GNTI-122 expansion in a rapamycin dose-dependant manner. Fold expansion of representative donor across 7 concentrations of rapamycin over 8 days. All samples are TCR stimulated. Data represents the mean +/- SEM of duplicates.

Rapamycin improves engraftment of GNTI-122 in vivo



GNTI-122 engrafts in response to rapamycin dose in vivo. GNTI-122 cells were injected into irradiated NSG mice at a dose of $5x10^6$ cells/mouse, and were administered with rapamycin every other day intraperitoneally for 17 days post cell injection. On day 19, mice were sacrificed and engraftment levels were checked in each specified organ. 2-way ANOVA with Tukey's multiple comparison test, significance displayed (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001)

CONCLUSIONS

- GNTI-122 engineered from CD4 T cells, from healthy donors and T1D patient donors, exhibit stable Treg phenotype and cytokine expression.
- The pancreatic islet antigen-specific TCR of GNTI-122 enables targeted direct Teff suppression in addition to bystander and polyclonal Teff suppression.
- CISC provides an IL-2-like signal and specifically expands GNTI-122 in response to rapamycin in vitro and in vivo
- GNTI-122 overcomes the key limitations of sorted Treg cell therapy supporting further evaluation of GNTI-122 in clinical trials

We make Tregs. *Better.*

ACKNOWLEDGEMENT: The laboratory of Dr. David Rawlings at Seattle Children's Hospital pioneered the gene editing approach to produce engineered Tregs.