Characterization and efficacy of engineered regulatory T-cell therapy, GNTI-122, for treating Type 1 Diabetes

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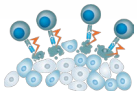
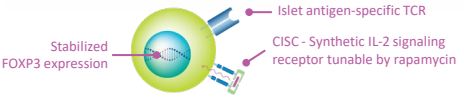
IDF 2022 LISBON LI2022-0687

OVERVIEW

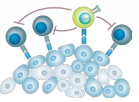
Type 1 diabetes (T1D) results from effector T cell (Teff) destruction of pancreatic islet cells. Sorted T regulatory cell (Treg) therapy addresses this pathology but is limited by heterogeneity and potential instability of Forkhead box protein P3 (FOXP3) expression, lack of tissue specificity and Treg-specific IL-2 support.



GNTI-122, a novel engineered autologous cell therapy in development for the treatment of T1D, is a genome-engineered human Treg (EngTreg) with stable FOXP3, an islet-specific T cell receptor (TCR), and a chemically induced signaling complex (CISC) that promotes selective IL-2 signal with rapamycin, restoring the Treg/Teff balance.

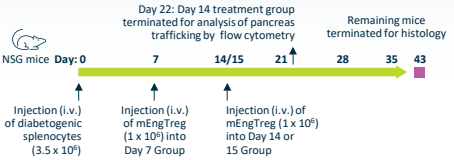


GNTI-122 is designed to protect islet cells from damage by homing to the pancreas and draining lymph nodes and suppressing pathogenic effector T cells via the mechanisms of bystander suppression and infectious tolerance.

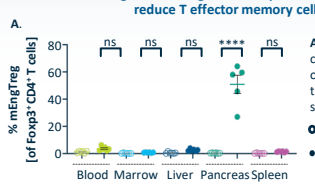


RESULTS | Murine single-islet antigen-specific engineered Tregs suppressed disease in a T1D mouse model and showed high trafficking to the pancreas

Experimental design



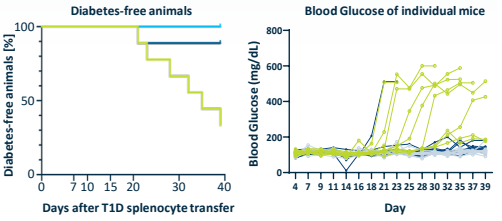
Engineered Tregs home to pancreas and reduce T effector memory cells



A. Graphed flow cytometry analysis of BDC2.5 mEngTreg trafficking to selected organs.

- Splenocytes only
- Splenocytes + mEngTregs

Islet-specific mouse engineered Treg cells (mEngTregs) prevent onset of diabetes in an adoptive transfer disease model.

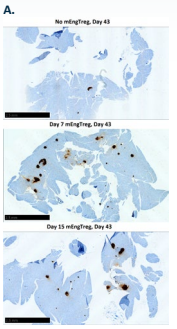


Diabetes-free survival in recipient NSG mice following injection of splenocytes from diabetic NOD mice.

Glucose measurements in recipient NSG mice following injection of splenocytes from diabetic NOD mice.

Similar studies supporting this model of Type 1 Diabetes were previously published[1].

Engineered Tregs reduce pancreatic inflammation and preserve beta cells

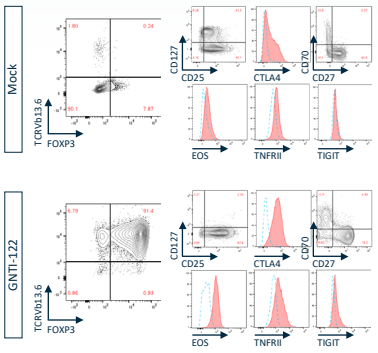


Insulin

Histological analysis of pancreata at study termination on Day 43. **A.** Immunohistochemistry stains for Insulin. **B.** Quantification of beta cell mass by insulin staining of pancreata. Approx. 20 pancreatic islets quantified per mouse.

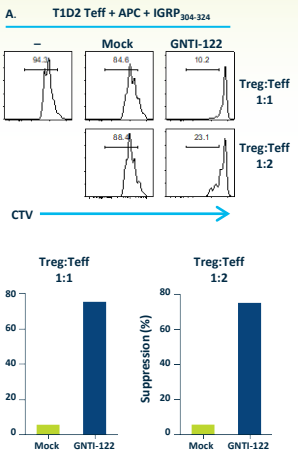
RESULTS | GNTI-122 Cells display a Treg phenotype with direct and bystander suppression of T1D patient leukocyte samples

GNTI-122 cells from patients of T1D express Treg associated markers

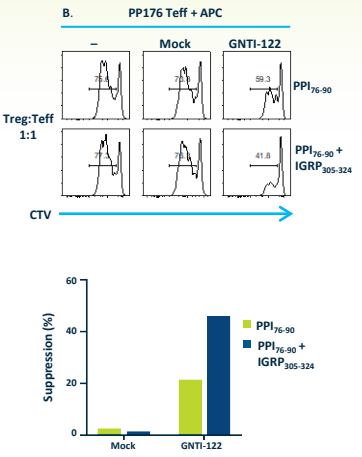


GNTI-122 cells express Treg markers (FOXP3⁺CD25⁺CD127⁻) & increase functional (CTLA-4, TIGIT) and stability (TNFRII, CD27⁺CD70⁻, EOS) markers. Mock cells are gated on CD4+ cells, and GNTI-122 cells are gated on TCR⁺FOXP3⁺ cells. Reproduced across 3 independent donors of patients with T1D

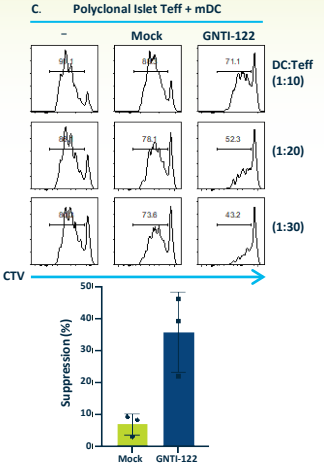
GNTI-122 cells inhibit the proliferation of Teff cells via direct suppression



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



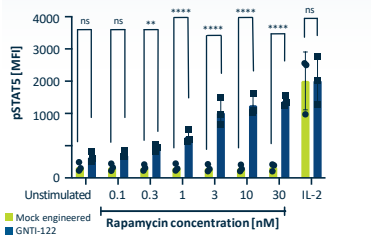
GNTI-122 cells inhibit the proliferation of polyclonal islet antigen-specific Teffs



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.

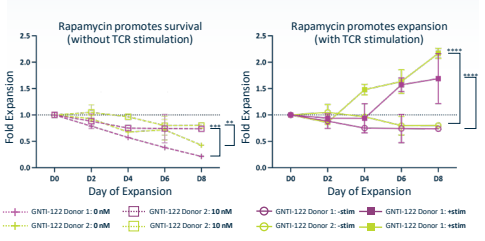
RESULTS | Rapamycin induced dose dependent IL-2 signaling via CISC and expanded GNTI-122

CISC delivers tunable IL-2-like signal to GNTI-122 cells



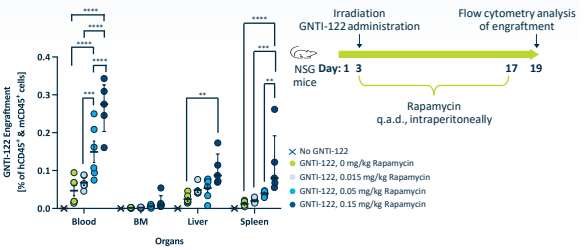
Phosphorylated STAT5 (pSTAT5) MFI show dose-specific response with rapamycin in GNTI-122 cells in culture. Quantification of mean fluorescence intensity (MFI) of pSTAT5 at each concentration of rapamycin. Repeated measures ANOVA with Sidak's multiple comparison tests at each dose (**p<0.01, ****p<0.0001).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

GNTI-122 expands in the presence of rapamycin and TCR-stimulation in vitro



GNTI-122 expands in the presence of rapamycin and TCR stimulation in vitro. GNTI-122 cells cultured for 8 days ±TCR stimulation via anti-CD3/CD28 beads, and with 0 or 10 nM rapamycin. Without TCR stimulation, Rapamycin and CISC stimulation maintained GNTI-122 survival. With rapamycin, TCR stimulation promoted GNTI-122 expansion. 2-way ANOVA with Tukey's multiple comparison test, significance displayed for paired conditions at day 8 (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001)

GNTI-122 expands in response to rapamycin dose in vivo

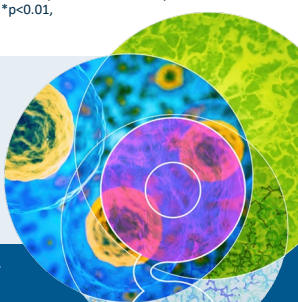


GNTI-122 expands in response to rapamycin dose in vivo. GNTI-122 cells were injected into irradiated NSG mice at a dose of $5x10^6$ cells/mouse, and were administered with Rapamycin every other day intraperitoneally for 17 days post cell injection. On day 19, mice were sacrificed and engraftment levels 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

- Single islet-antigen-specific engineered Tregs control islet destruction, preserve function, and prevent diabetes progression
- GNTI-122 engineered from donor patients of T1D exhibit Treg phenotype, cytokine expression, and direct and polyclonal suppression of islet-specific Teffs
- CISC provides an IL-2-like signal and specifically expands GNTI-122 in response to rapamycin concentration in vitro and in vivo
- GNTI-122 overcomes the key limitations of sorted Treg cell therapy, demonstrating direct and bystander suppression in vitro and efficacy in vivo, 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.

REFERENCES: 1. S.J. Yang, et al. Pancreatic islet-specific engineered Tregs exhibit robust antigen-specific and bystander immune suppression in type 1 diabetes models. *Science Translational Medicine*. **14**. (2022).