Development of GNTI-122, a novel engineered regulatory T cell therapeutic, for future application in type 1 diabetes

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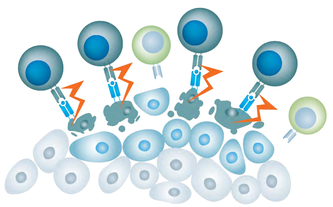
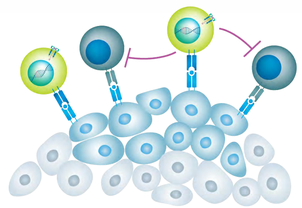
OVERVIEW | Establish Proof-of-Concept to Support the Development of GNTI-122 Engineered Treg Therapy for the Treatment of Type 1 Diabetes

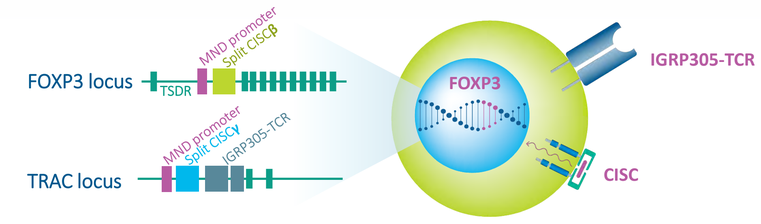
Type 1 diabetes is an autoimmune disease caused by T-lymphocyte-mediated destruction of insulin-producing beta cells that eventually leads to uncontrolled hyperglycemia and life-long dependence on daily insulin administration.



GNTI-122, a novel engineered T regulatory cell in development for the treatment of recently diagnosed T1D, 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.

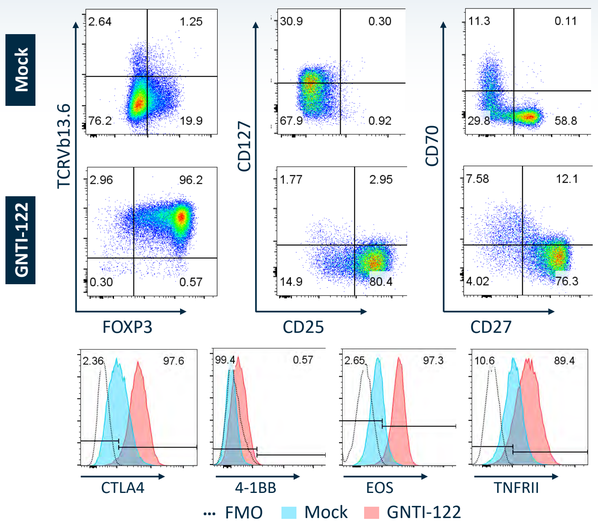


GNTI-122 is engineered from autologous CD4 T cells with an RNA-guided nuclease gene editing to knock-in an MND promoter into the FOXP3 gene to stabilize its expression, a pancreatic islet antigen-specific TCR into the TRAC locus, and a rapamycin-mediated IL-2 signaling complex (CISC).



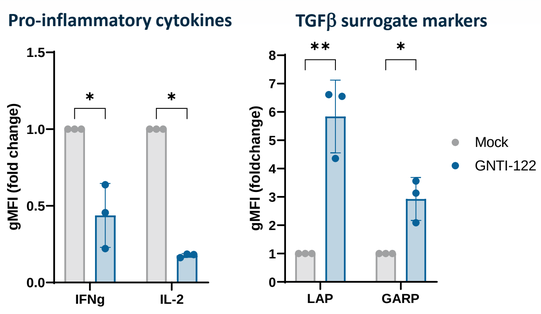
RESULTS | GNTI-122 Cells Have a Treg Phenotype and Suppress Islet Antigen-Reactive T Effector Cells from Donors with T1D

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



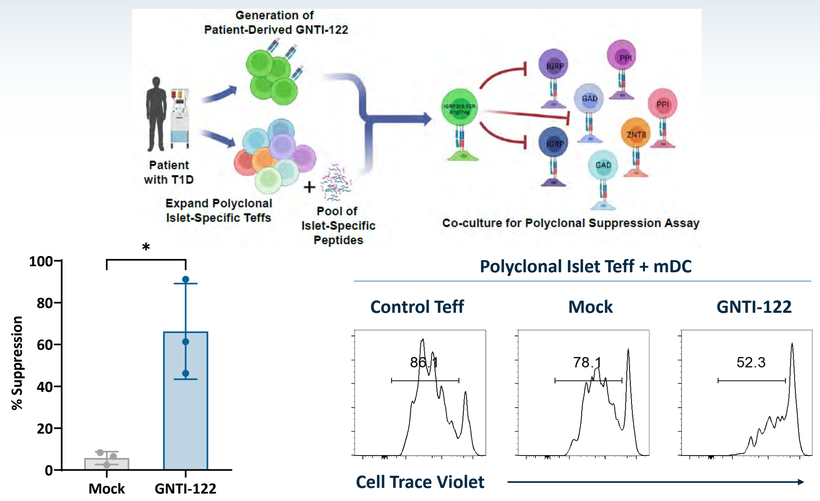
GNTI-122 cells express Treg markers (FOXP3$^+$CD25$^+$CD127$^-$) and increase functional (CTLA-4, 4-1BB) and stability (TNFRII, CD27$^+$CD70$^-$, EOS) markers. Mock cells are gated on CD4+ cells, and GNTI-122 cells are gated on TCR$^+$FOXP3$^+$ cells. Representative donor data shown. Reproduced in 3 independent donors with T1D.

GNTI-122 cells express TGF-β associated markers and not pro-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. Data from 3 donors with T1D. One-sample t-test (* p,0.05; ** p<0.01).

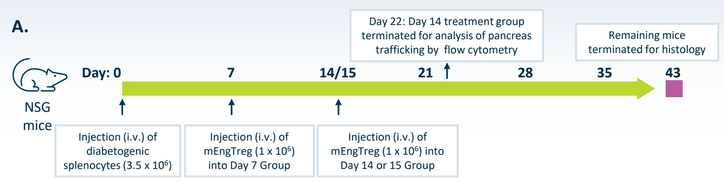
GNTI-122 cells inhibit proliferation of patient-derived polyclonal islet antigen-reactive Teffs

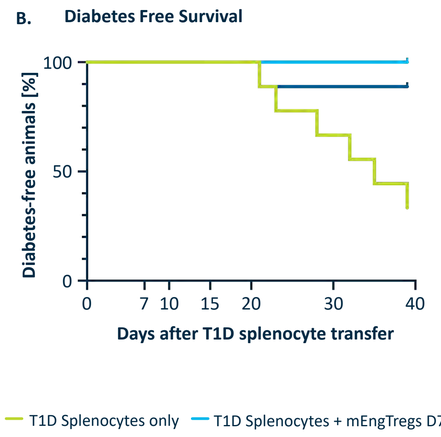


GNTI-122 cells are cocultured with autologous Teffs expanded from patient donors with T1D, and monocyte-derived dendritic cells (DC) as antigen-presenting cells. The Teffs specific to 9 different cognate peptides were isolated and DCs were loaded with their cognate peptides. Suppression was calculated 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. Welch t-test (* p<0.05)

RESULTS | Mouse Engineered Tregs Protect Pancreatic Beta Cells in an Adoptive Transfer Model of Type 1 Diabetes

Experimental design and efficacy



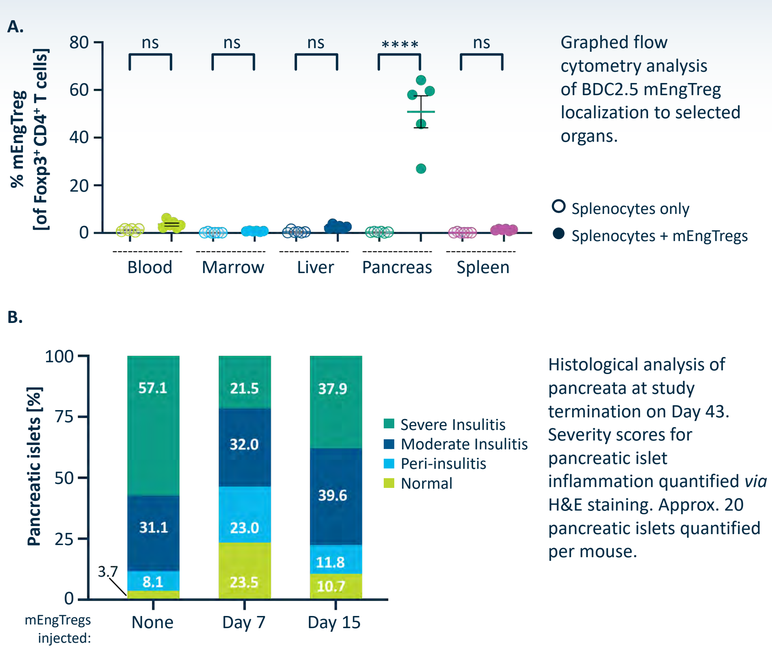


Islet-specific mouse engineered Treg cells (mEngTregs) prevent onset of diabetes in an adoptive transfer disease model.

A. Experimental workflow.

B. Diabetes-free survival in recipient NSG mice following injection of splenocytes from diabetic NOD mice. Mouse engineered regulatory T cells (mEngTregs) prepared from NOD.BDC2.5 CD4$^+$ T cells were injected either 7 or 15 days after splenocyte transfer.

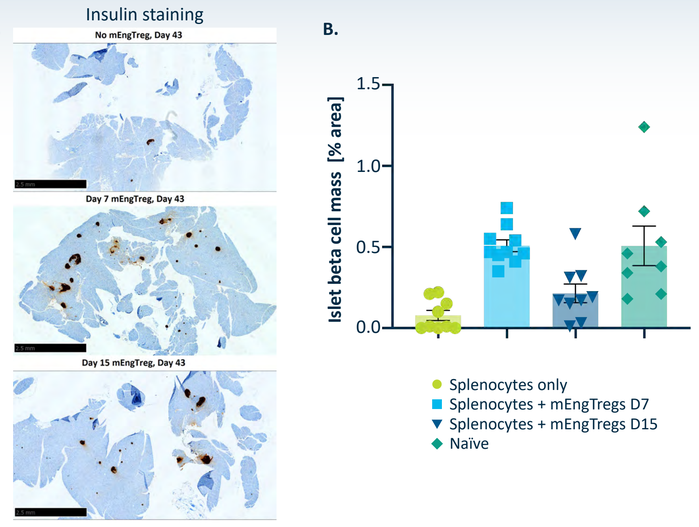
Engineered Tregs localize to pancreas and reduce islet inflammation



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

Histological analysis of pancreata at study termination on Day 43. Severity scores for pancreatic islet inflammation quantified *via* H&E staining. Approx. 20 pancreatic islets quantified per mouse.

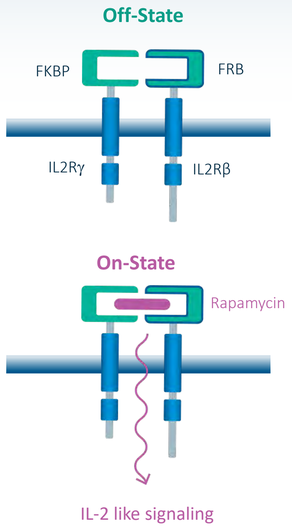
Engineered Tregs preserve pancreatic beta cells

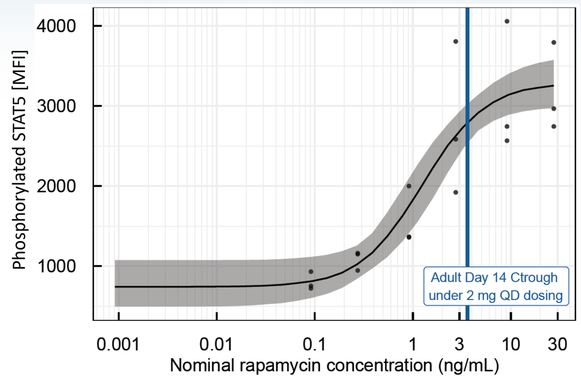


Histological analysis of pancreata at study termination on Day 43. **A.** Representative micrographs of insulin staining in pancreas. Scale bar: 2.5 mm. **B.** Quantification of beta cell mass by insulin staining of pancreata. Approx. 20 pancreatic islets quantified per mouse.

RESULTS | CISC Activation with Rapamycin Emulates IL-2 Signaling and Promotes GNTI-122 Expansion in the Absence of IL-2

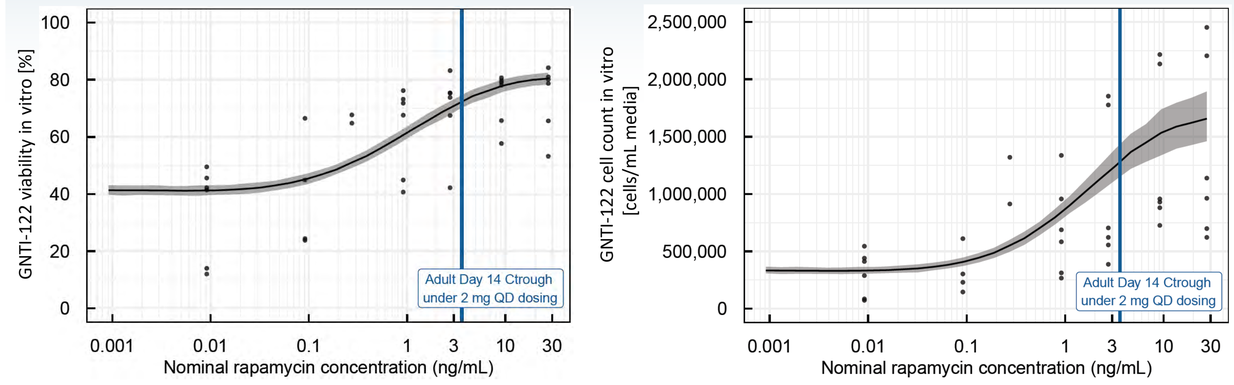
CISC delivers tunable IL-2-like signal to GNTI-122 cells





Phosphorylated STAT5 (pSTAT5) MFI show rapamycin concentration-dependent CISC activation in GNTI-122 cells in vitro. A. Schematic depiction of CISC. **B.** GNTI-122 cells were exposed to rapamycin and mean fluorescence intensity (MFI) of pSTAT5 at each concentration of rapamycin was quantified (N=3 donors). Simulation of rapamycin concentration-response relationship (Emax model) of in vitro CISC activation alongside rapamycin predicted trough exposures in adult humans at the 2 mg rapamycin dose. Median and 90% CI were derived from 500 population mean parameters sampled from the uncertainty distribution of the parameter estimates.

CISC promotes survival and expansion of GNTI-122 cells in vitro

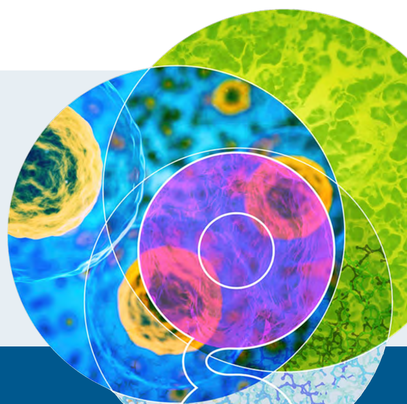


GNTI-122 expands in vitro in the presence of TCR stimulation at clinically achievable rapamycin exposures. GNTI-122 cells were cultured for 10 days in the presence TCR stimulation via anti-CD3/CD28 beads, and increasing concentrations of rapamycin (in the absence of exogenously added IL-2). Cell viability and proliferation were analyzed at the end of the culture on Day 10. The relationships between rapamycin concentrations, and GNTI-122 viability (**A**) and expansion (**B**) on Day 10 of culture were characterized via a non-linear mixed effect (NLME) model trained on longitudinal in vitro GNTI-122 cell count and viability data. Rapamycin predicted trough exposures in adult humans at the 2 mg rapamycin dose and median and 90% CI derived from 500 population mean parameters sampled from the uncertainty distribution of the parameter estimates are shown.

CONCLUSIONS

- GNTI-122 engineered from donors with T1D display Treg phenotype, cytokine profile, and polyclonal suppression of islet antigen-specific Teff cells
- Mouse engineered islet-specific Treg cells localize to pancreas to suppress inflammation, preserve pancreatic islets and prevent diabetes
- CISC provides on-demand IL-2-like signaling controllable by subtherapeutic rapamycin exposure levels to promote GNTI-122 viability and expansion in the absence of exogenous IL-2
- GNTI-122 represents a novel therapeutic modality with potential to restore immune tolerance in T1D

We make Tregs. *Better.*



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