gentibio

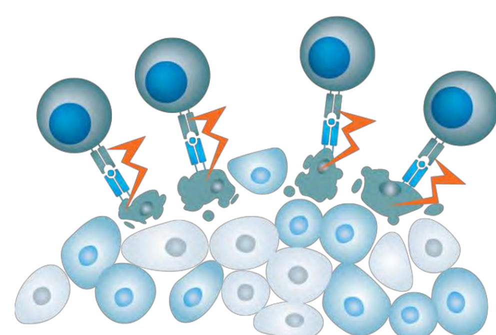
Development and characterization of GNTI-122, an autologous engineered human regulatory T cell therapy for type 1 diabetes

Jennifer Y Yam, Priya Saikumar-Lakshmi, Ashley E Landuyt, Gene I Uenishi, Tingxi Guo, Tiffany F Chen, Marko Repic, Thomas J Wickham, Karen T Mueller

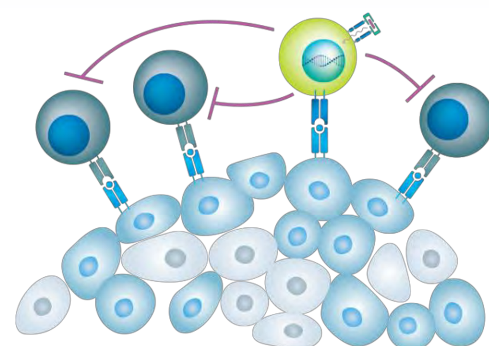
GentiBio, Inc., Cambridge, MA, USA

OVERVIEW

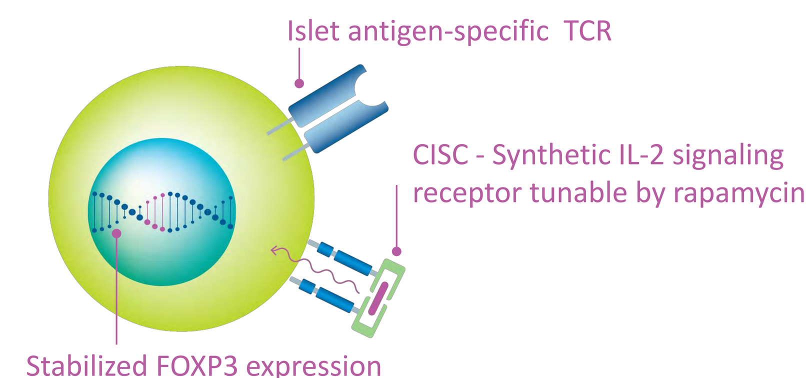
Type 1 diabetes is an autoimmune disease caused by T-lymphocyte-mediated destruction of insulin-producing beta cells that eventually leads to uncontrolled hyperglycemia and life-long dependence on daily insulin administration.



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.

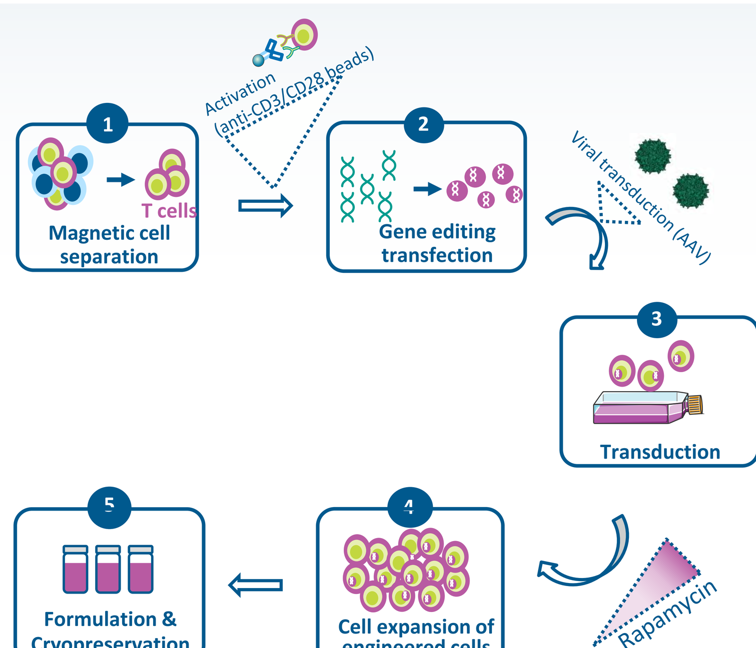


GNTI-122 is engineered from autologous CD4 T cells with CRISPR-Cas9 gene editing to knock-in an MND promoter into the FOXP3 gene to stabilize its expression, a pancreatic islet antigen-specific TCR into the TRAC locus, and a rapamycin-activated, synthetic IL-2 signaling receptor (CISC).



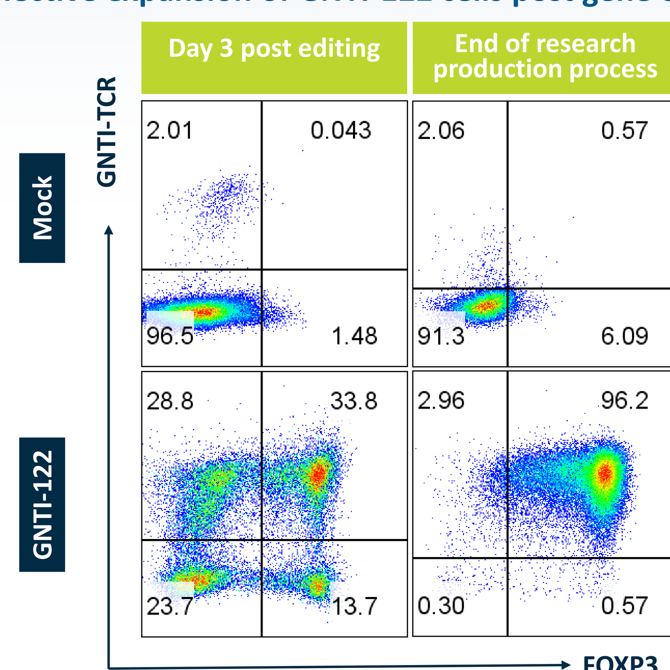
The objective of this study was to establish the proof-of-concept to support the development of GNTI-122 engineered Treg therapy for the treatment of type 1 diabetes.

RESULTS | CISC Enables Enrichment of GNTI-122 Cells Post Gene Editing and Provides Tunable IL-2-like signaling



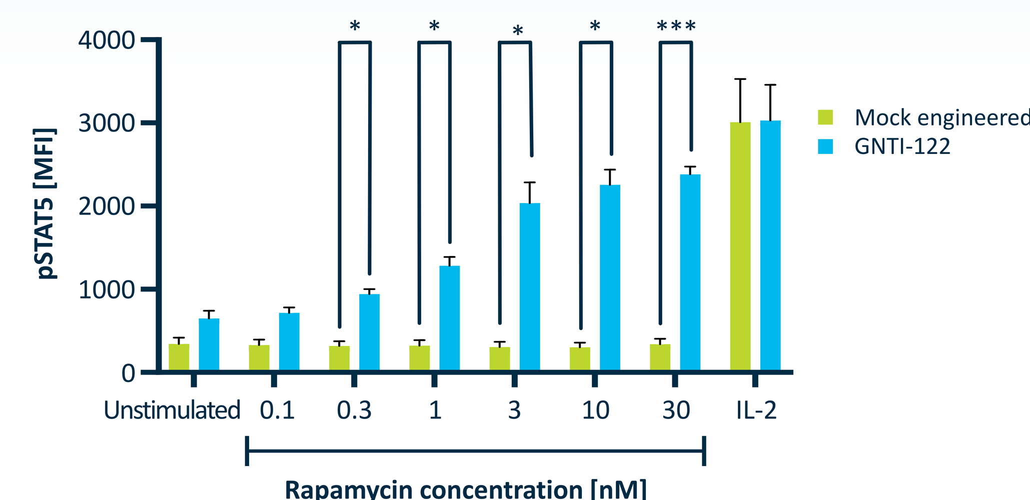
The process for generation of research grade GNTI-122 autologous engineered Tregs. $CD4^+$ cells were isolated via magnetic enrichment from PBMCs. The cells were then genetically modified using CRISPR-Cas9 to knock-in transgenes delivered by AAV vectors. CISC receptor enables selective expansion and enrichment of engineered Tregs in the presence of rapamycin. The expanded cells were cryopreserved and used for further analyses and studies.

Selective expansion of GNTI-122 cells post gene editing



Frequency of GNTI-122 cells by flow cytometry. FACS analysis of GNTI-122 cells and mock engineered cells 3 days after editing (left) and at the time of cryopreservation (right).

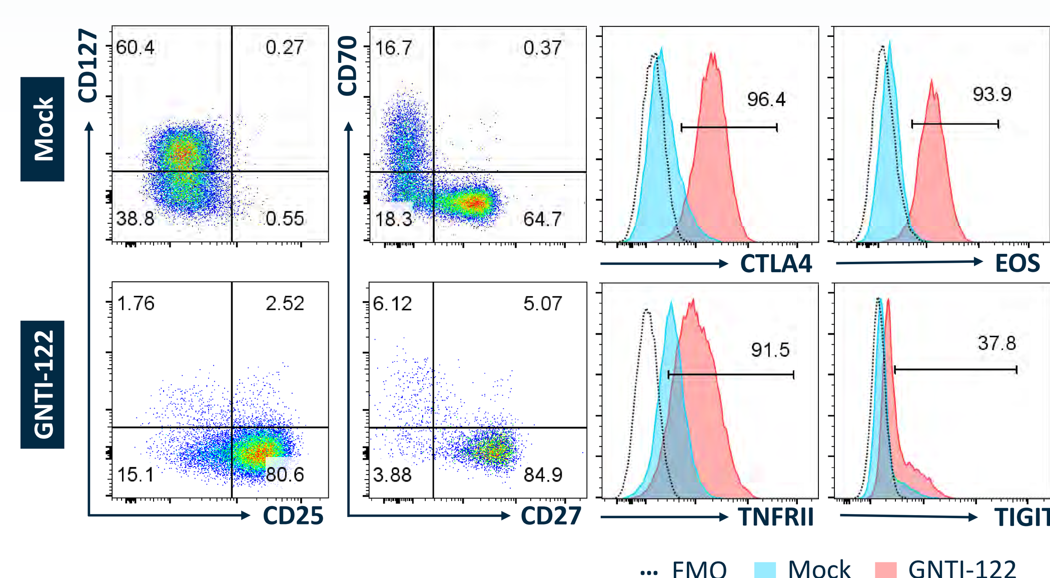
CISC delivers tunable IL-2-like signal to GNTI-122 cells



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 dose of rapamycin. Repeated measures ANOVA cell type, dose and interaction, $p<0.0001$, Sidak's multiple comparison tests at each dose (*$p<0.05$,***$p<0.001$).The errors bars represent mean +/- SEM, N=3 donors. The cells are gated on live $CD3^+$ $CD4^+$ population of both mock-engineered and GNTI-122 cells.

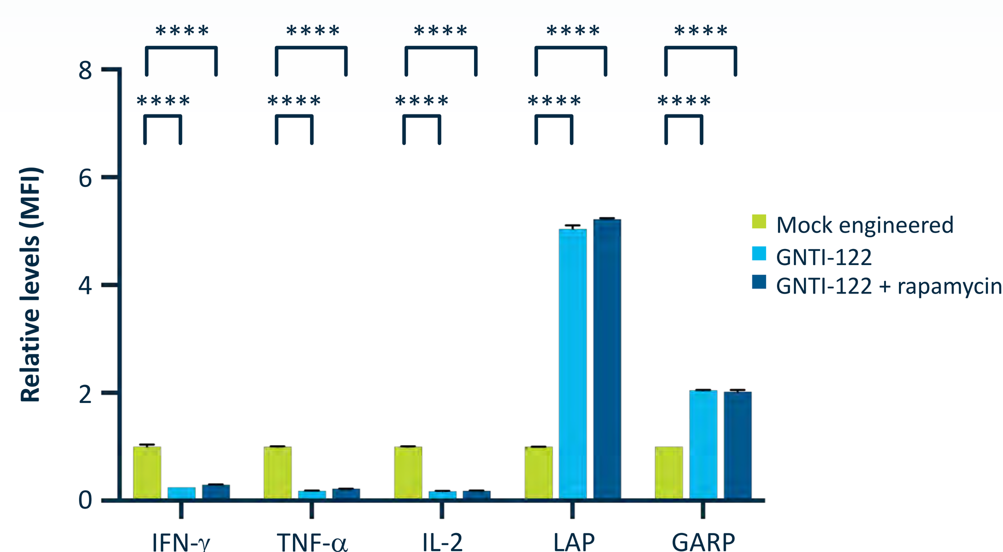
RESULTS | GNTI-122 Cells Have a Treg Phenotype and Function

GNTI-122 cells express Treg associated markers



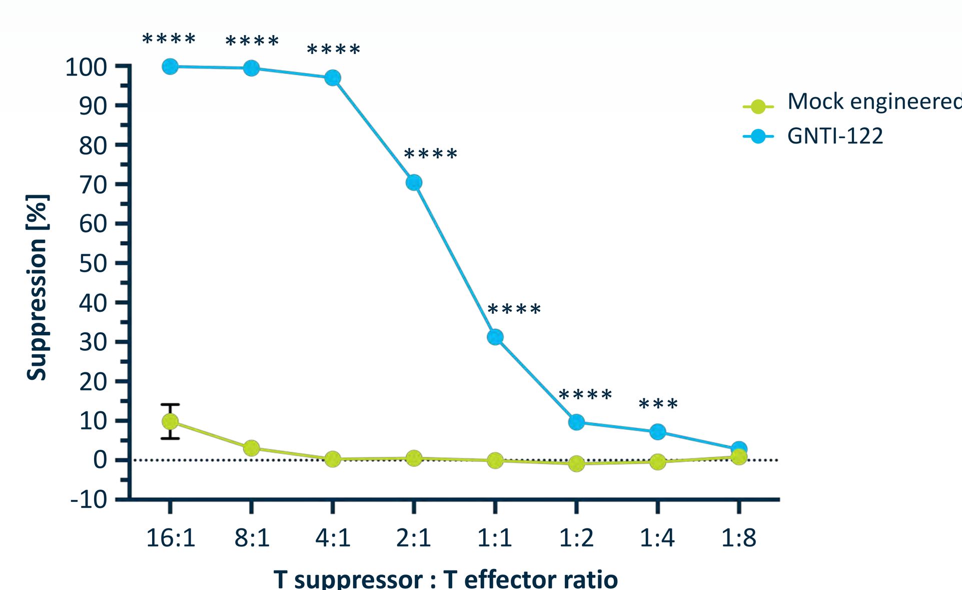
GNTI-122 cells and their corresponding mock controls generated in parallel were stained after a 3-day rest in culture post-thaw. Mock cells were gated on CD4 positive cells, and GNTI-122 cells were gated on islet-specific TCR^+FOXP3^+ cells. Representative donor data shown, reproduced across 6 independent donors.

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



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

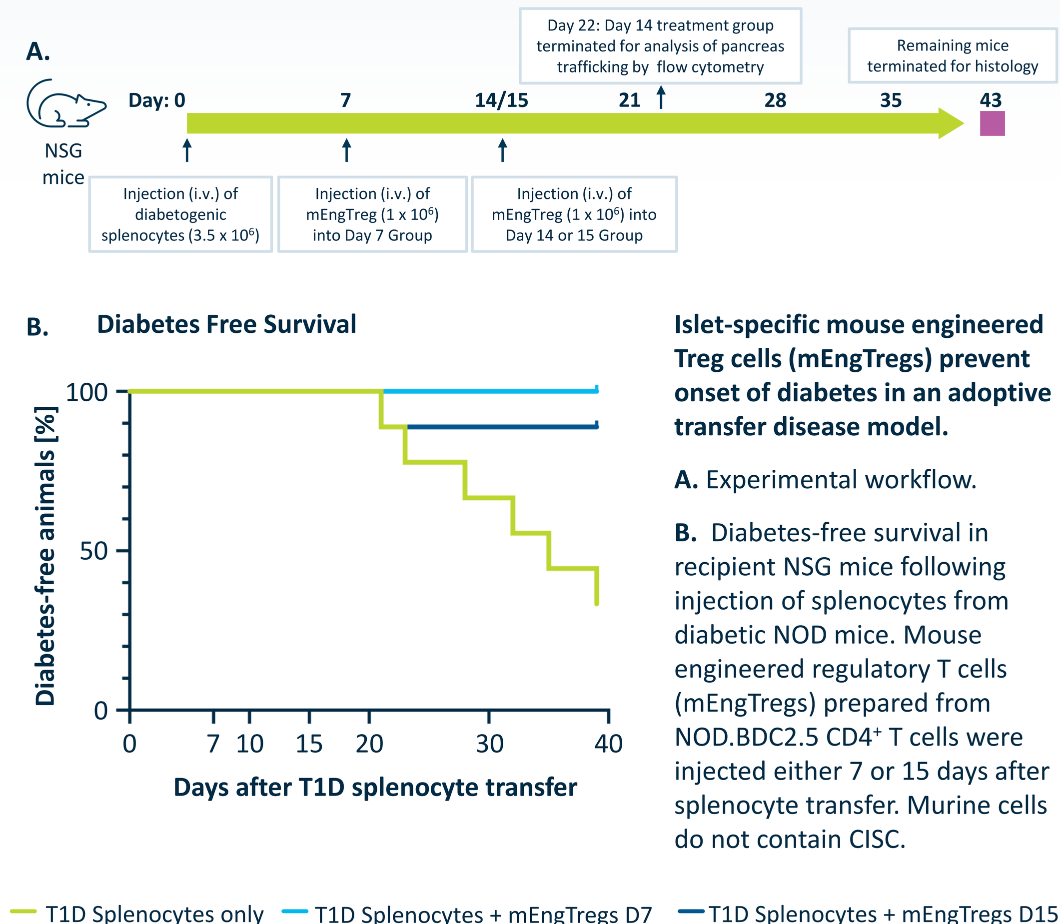
GNTI-122 cells inhibit the proliferation of islet-TCR^+FOXP3^- T effector cells



Mock cells or GNTI-122 were cultured with autologous islet-specific TCR^+FOXP3^- T effectors and stimulated with monocyte-derived dendritic cells loaded with cognate peptide. Suppression indicates inhibition of Teff as determined by flow cytometry. 2-way ANOVA. Representative donor data shown, reproduced across 3 independent donors.

RESULTS | Efficacy of Mouse Engineered Treg Therapy in an Adoptive Transfer Type 1 Diabetes Model

Experimental design and efficacy

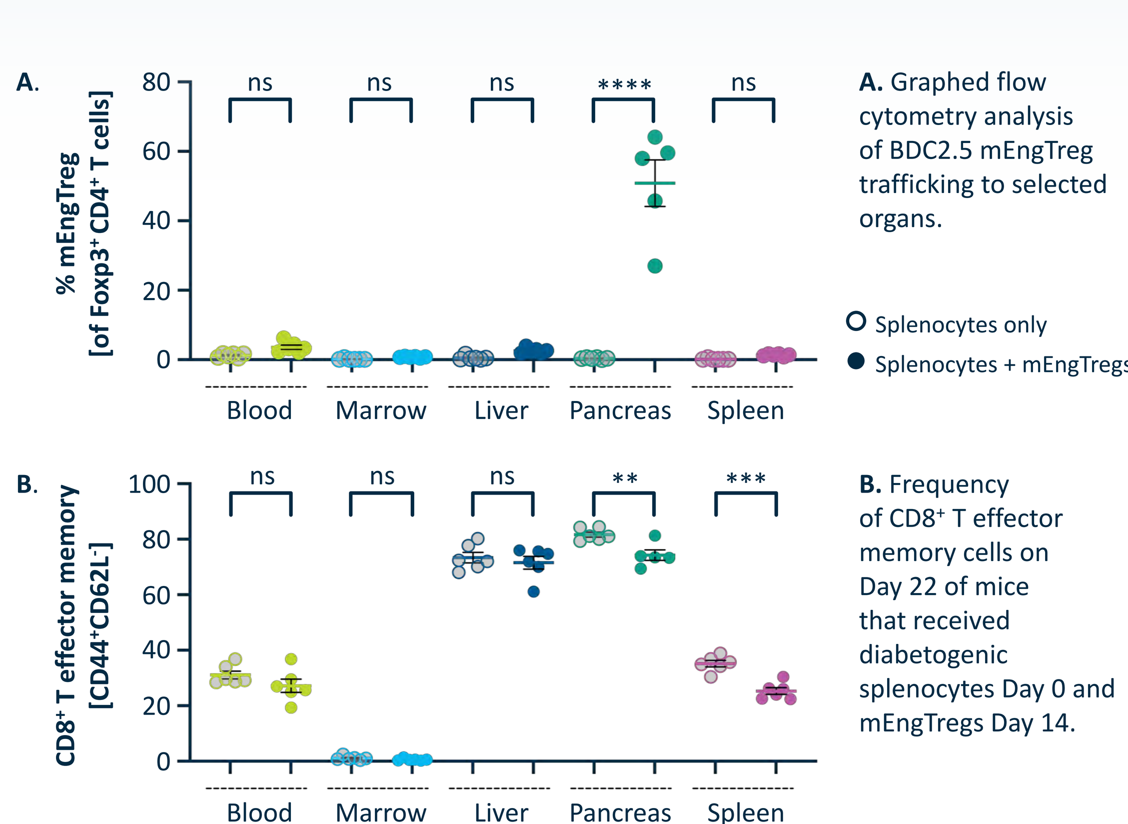


Islet-specific mouse engineered Treg cells (mEngTregs) prevent onset of diabetes in an adoptive transfer disease model.

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. Murine cells do not contain CISC.

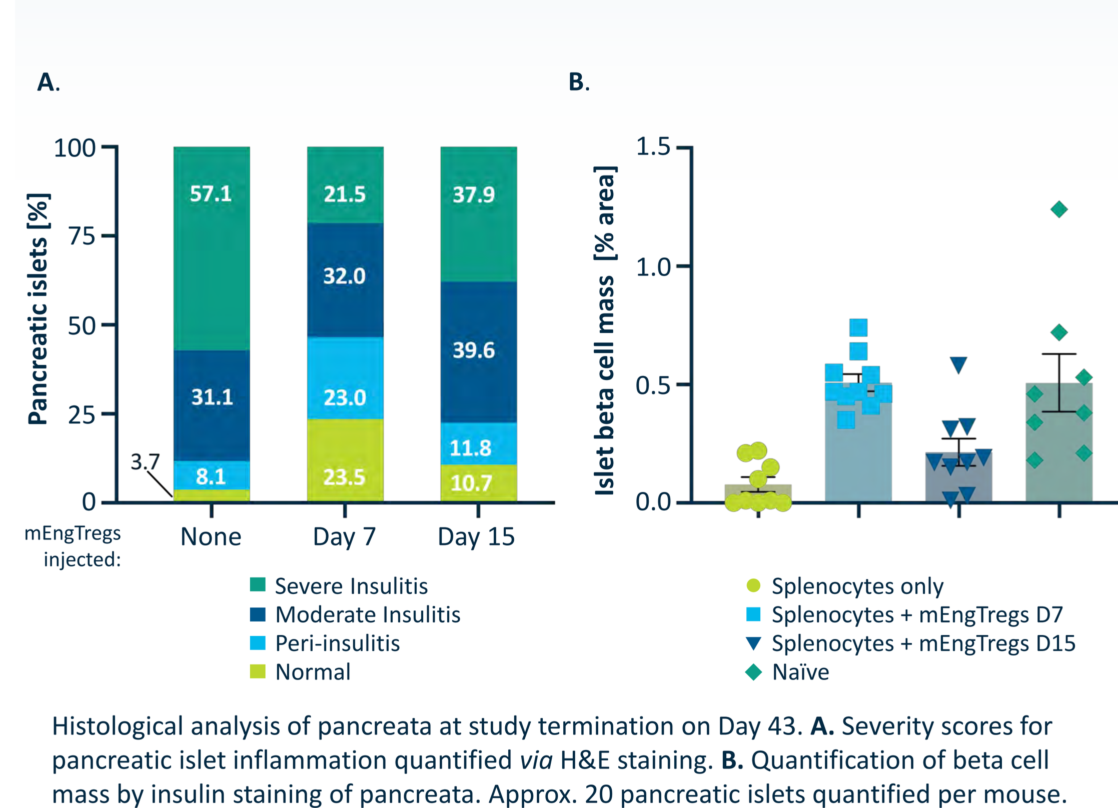
Engineered Tregs home to pancreas and reduce T effector memory cells



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

B. Frequency of $CD8^+$ T effector memory cells on Day 22 of mice that received diabetogenic splenocytes Day 0 and mEngTregs Day 14.

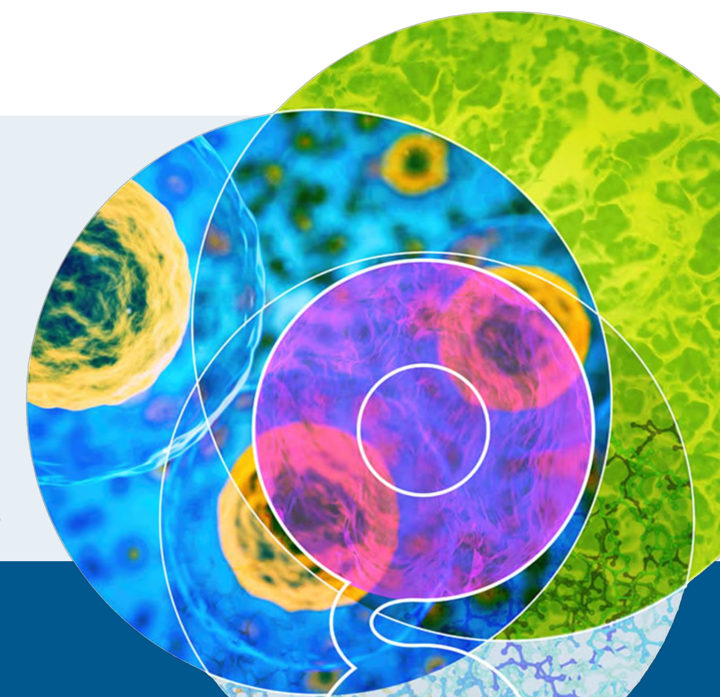
Engineered Tregs reduce pancreatic inflammation and preserve beta cells



Histological analysis of pancreata at study termination on Day 43. **A.** Severity scores for pancreatic islet inflammation quantified *via* H&E staining. **B.** Quantification of beta cell mass by insulin staining of pancreata. Approx. 20 pancreatic islets quantified per mouse.

CONCLUSIONS

- GentiBio's platform overcomes the scaling limitations of sorted patient Treg cells by starting with more abundant T cell sources and specifically enriching edited cells with an engineered IL-2 signaling receptor
- GNTI-122 engineered Treg cells display regulatory markers, cytokine profile, and exert suppressive function *in vitro*
- Mouse engineered islet-specific Treg cells suppress ongoing pancreas inflammation to preserve pancreatic islets and prevent diabetes



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. The research group of Dr. Jane Buckner at Benaroya Research Institute was integral in selecting the lead TCR.