A stable Engineered Regulatory T cell therapy platform with modular targeting for autoimmune and inflammatory disorders

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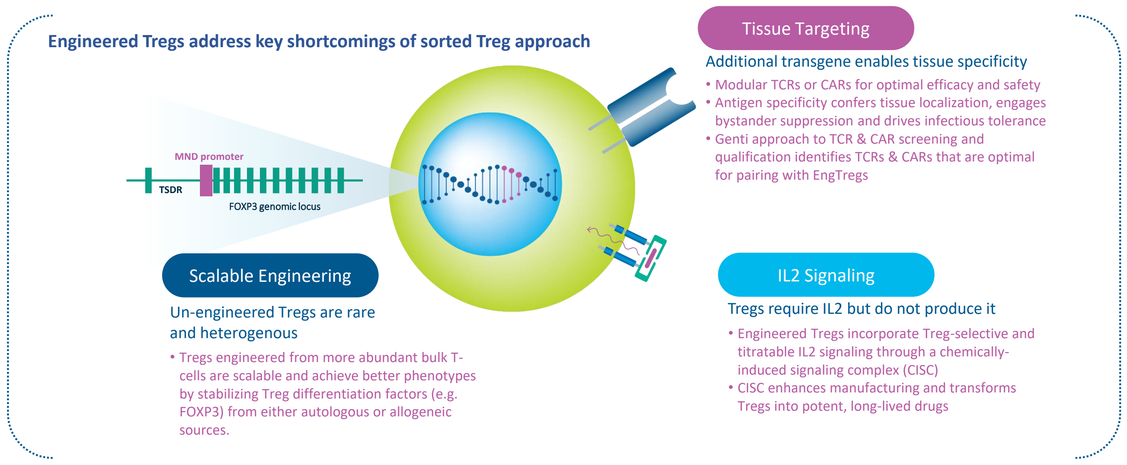
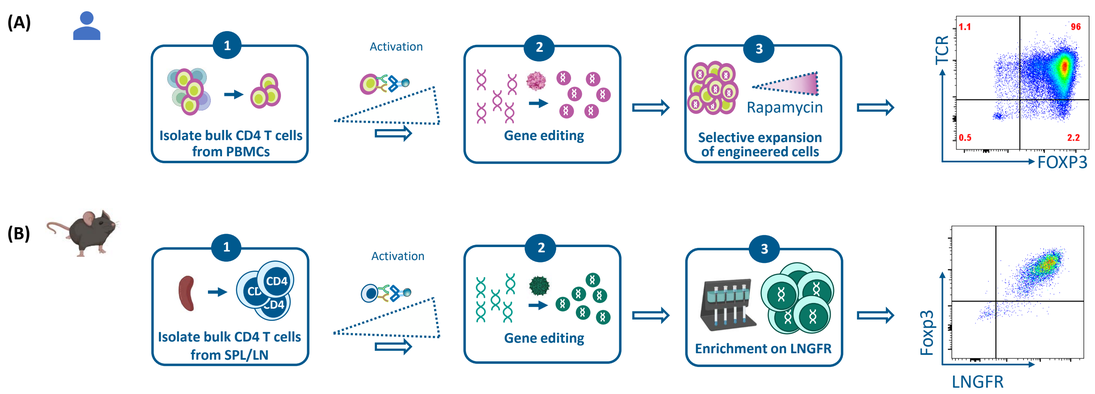
Premise



Limitations of expanding sorted natural regulatory T cell. Natural Tregs are a rare subset within the PBMCs of most individuals. While the expansion of these rare cells in culture following cell sorting has been a common approach over the past decade, several well documented, key inherent limitations involving stability of natural Tregs have come to light (Zhou X., *Nat. Immunol.*, 2009; Junius S., *Sci. Immunol.*, 2020).

Engineered Treg generation



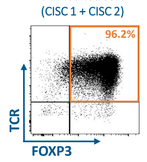
Engineered Treg production processes. A) Human or (B) murine CD4+ T cells are isolated (Panel 1) and activated via α-CD3/α-CD28 microbeads. Cells are genetically modified using CRISPR-Cas9 mediated knock-in of transgenes delivered by AAV vectors (Panel 2). For human EngTregs, CISC receptor expression enables selective expansion of edited cells, while mouse surrogate EngTregs rely on enrichment by the extracellular LNGFR tag (Panel 3).

Highly scalable manufacturing results in stable Tregs without the need for complex sorting

Dual edit strategy yields pure EngTreg product

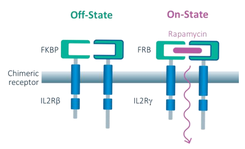
Edit 1: FOXP3 Locus
- ✓ Drive endogenous FOXP3
- ✓ CISC subunit 1
- ✓ Hypo-immune components

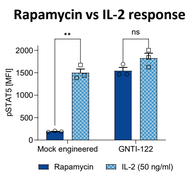
Dual edited cells (CISC 1 + CISC 2)

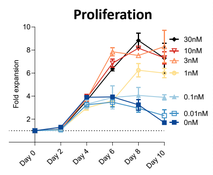


Edit 2: TRAC Locus
- ✓ KO endogenous TCR
- ✓ CISC subunit 2
- ✓ Added transgene (ST2, TCR, CAR)

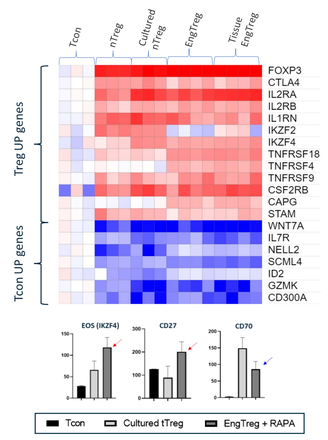
CISC imparts a selective, tunable IL-2 like signal

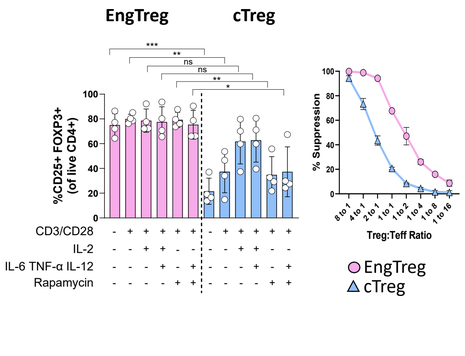




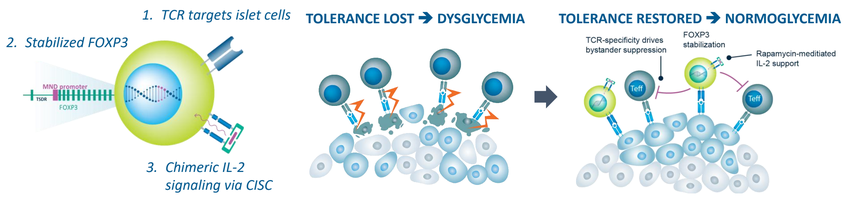


Engineered Tregs maintain higher stability and suppressive capacity compared to cultured nTregs (cTregs)

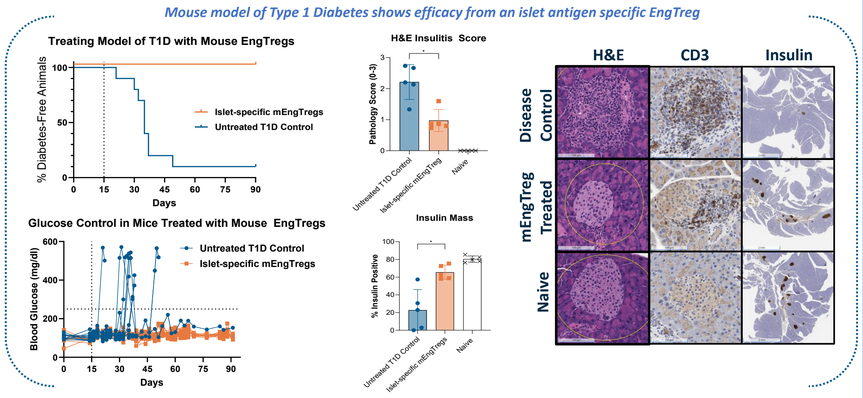




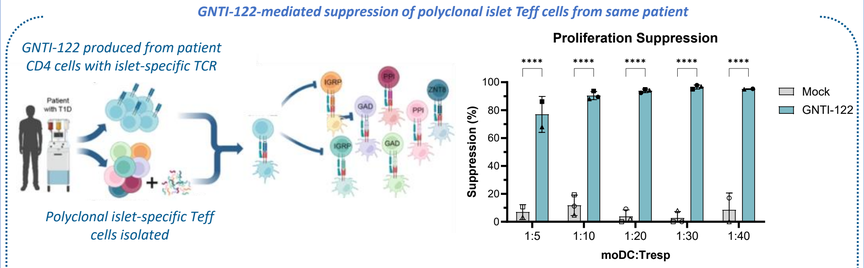
GNTI-122 suppresses pathogenic islet-specific Teffs that cause T1D



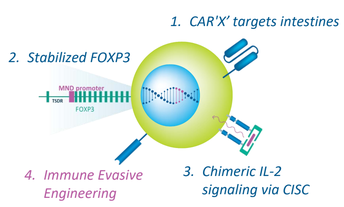
Mouse model of Type 1 Diabetes shows efficacy from an islet antigen specific EngTreg



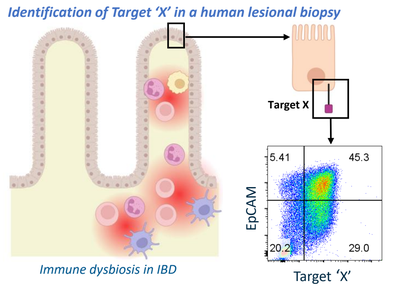
GNTI-122-mediated suppression of polyclonal islet Teff cells from same patient



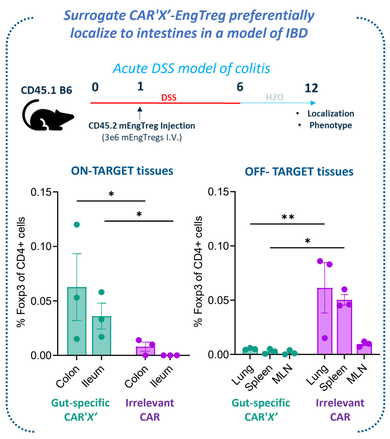
GNTI-932 Hypo-immune (off-the-shelf) allogeneic EngTreg for IBD



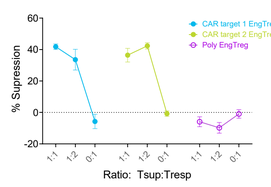
Identification of Target 'X' in a human lesional biopsy



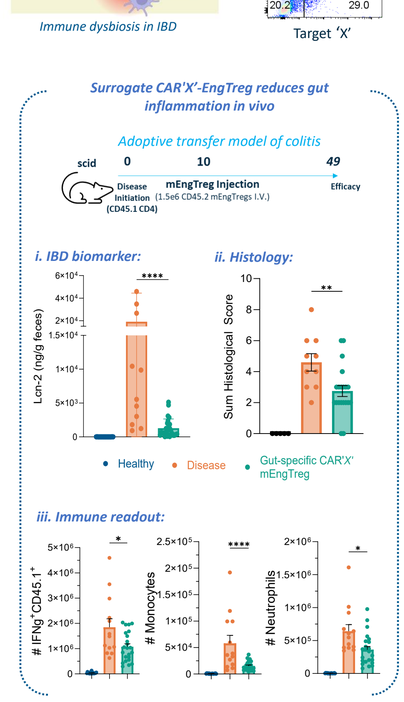
Surrogate CAR'X'-EngTreg preferentially localize to intestines in a model of IBD



CAR'X'-dependent suppression of T-eff proliferation in vitro

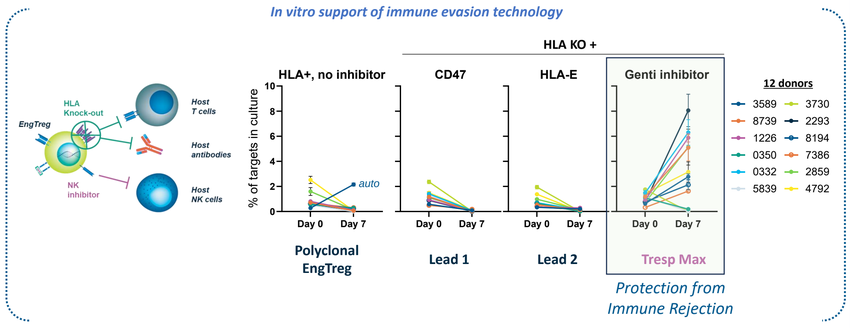


Surrogate CAR'X'-EngTreg reduces gut inflammation in vivo



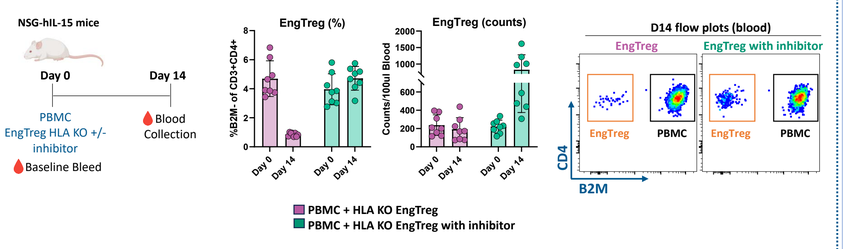
Best in class proprietary Hypo-immune Engineering enables allogeneic persistence

In vitro support of immune evasion technology

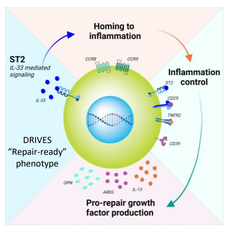


Protection from Immune Rejection

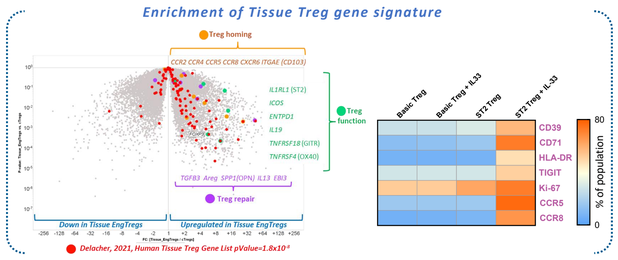
In vivo proof of concept showing IEE driven protection of EngTregs in the NSG-hIL15 model with PBMC transfer



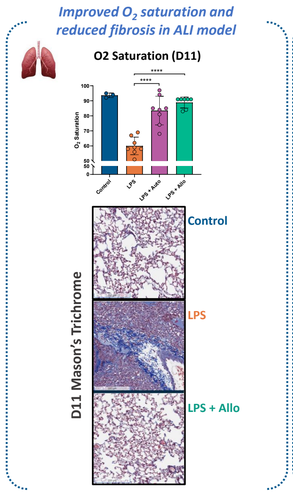
GNTI-823 curtails acute inflammation and supports tissue repair



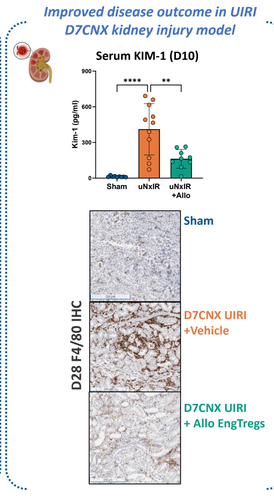
Enrichment of Tissue Treg gene signature



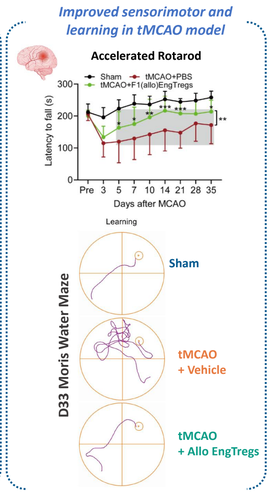
Improved O_2 saturation and reduced fibrosis in ALI model



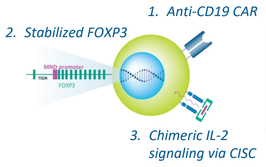
Improved disease outcome in UIRI D7CNX kidney injury model

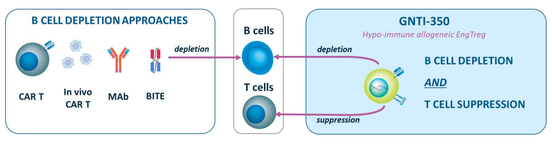


Improved sensorimotor and learning in tMCAO model

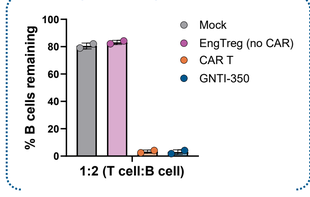


GNTI-350 shows improved immune reset and safety versus CAR T

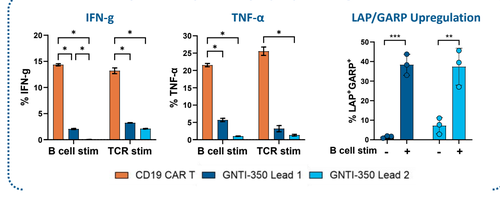




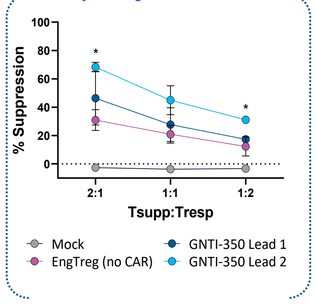
B cell depletion comparable to CAR T cells



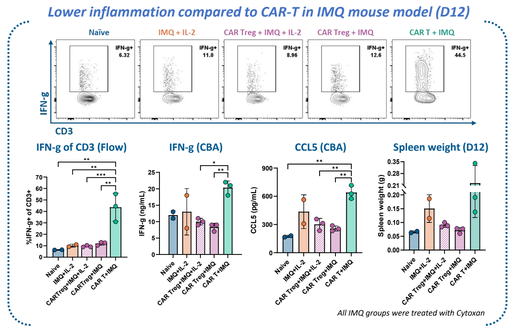
Favorable cytokine expression profile following B cell co-culture



Enhanced T cell suppression following B cell co-culture



Lower inflammation compared to CAR-T in IMQ mouse model (D12)



All IMQ groups were treated with Cytoxan

CONCLUSIONS

- GentiBio's Engineered Treg platform overcomes scaling and stability limitations of Treg therapeutics by starting with more abundant T cell sources and enriching FOXP3+ edited cells with an engineered IL-2 signaling receptor.
- Engineered Tregs express higher levels of Core Treg genes such as FOXP3, IL2RA and CTLA4 as well as FOXP3 Synergy genes, such as IRF4 and EOS, compared to cTregs at both the bulk and single cell RNA transcript levels.
- GentiBio's Hypo-immune Engineering enables allogeneic Treg products with long persistence, low COGs, and the potential for repeat dosing.
- GNTI-122 suppresses pathogenic T1D-driving islet specific Teffs; murine surrogate EngTregs preserve islets and prevent diabetes onset.
- GNTI-823 Engineered Tissue Tregs express high levels of tolerogenic and repair mediators; murine surrogate EngTregs are efficacious in models of acute lung injury, kidney injury, and ischemic stroke.
- GNTI-932 selectively binds to a gut epithelial target, showing enhanced homing and efficacy in the context of IBD pathology.
- GNTI-350 depletes B cells, induces comprehensive immune reset, and displays a better safety profile compared to CD19 CAR T cells.

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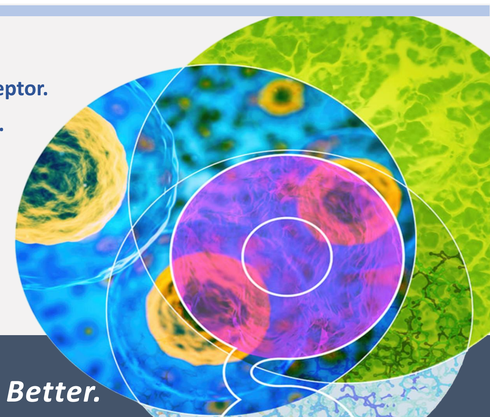
*, **, ***, **** = p-value < 0.01, 0.005, 0.001 and 0.0001 respectively.



We make Tregs. *Better.*