Poster #1106

CAR19 EngTregs offer broad capacity to modulate immune tolerance and improve safety in B and T cell mediated autoimmune disorders

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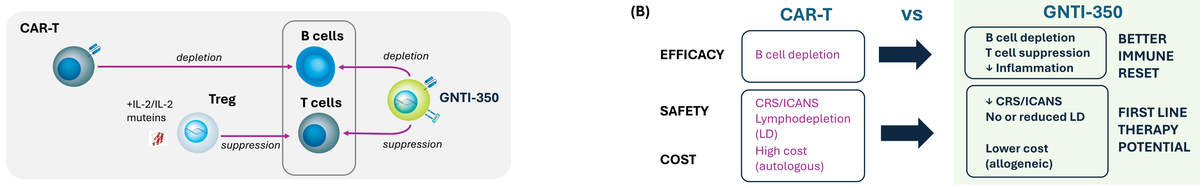
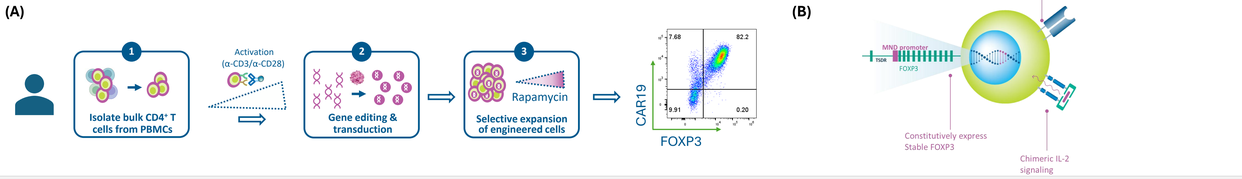
Premise of GNTI-350: *Impacts to B cells & T cells with a better safety profile than CAR-T*



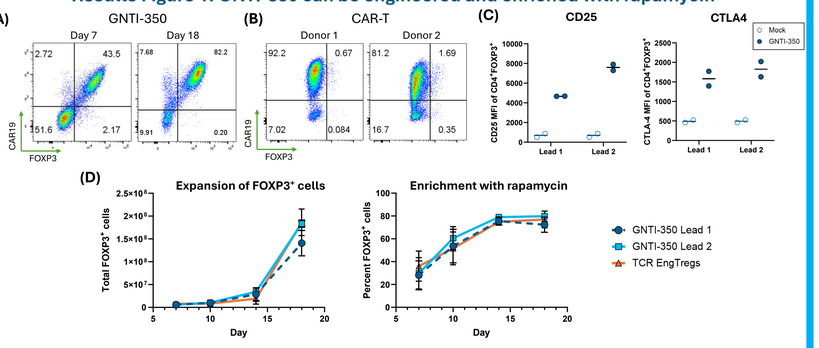
Overview of GNTI-350 as a therapeutic approach to B cell mediated autoimmune diseases. (A) GNTI-350 is an engineered, allogeneic, CAR19 T-regulatory cell therapy designed to deplete B cells and suppress T cells, which may offer a better immune reset than CAR-T cell therapies. (B) This product may be administered with reduced or eliminated lymphodepletion due to CISC-mediated IL-2 signaling to support engraftment. GNTI-350 therapy could be more efficacious, safe, and provide a lower cost of goods (CoGs) than traditional CAR-T and non-engineered Treg cell therapies. CRS: cytokine release syndrome. ICANS: Immune effector cell-associated neurotoxicity syndrome

Generation of GNTI-350: *CAR19 EngTregs*



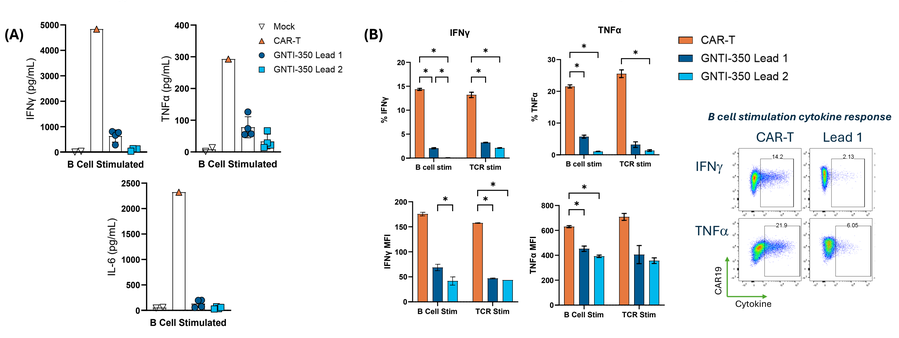
Engineered Treg production processes.[1,2] (A) For human Engineered Tregs (EngTregs), CD4+ T cells were isolated via magnetic enrichment from PBMCs and activated via α-CD3/α-CD28 microbead stimulation. Cells were then genetically modified using either 1) lentiviral transduction to deliver CAR19 and/or 2) CRISPR-Cas9 mediated knock-in of transgenes delivered by AAV vectors. CISC (chemically induced signaling complex) receptor expression enables selective expansion of the intended, edited cell population. (B) Schematic of a GNT-350 cell with gene edits: FOXP3 is constitutively expressed to maintain a stable T-reg phenotype, CISC provides IL-2 support to further maintain stable, long-lived cells, and anti-CD19 CAR provides targeting toward B cells.

Results Figure 1: GNTI-350 can be engineered and enriched with rapamycin



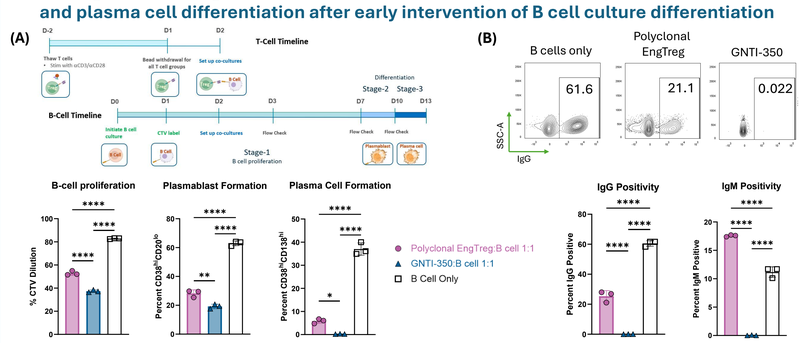
GNTI-350 cell production metrics. (A) Flow cytometry of the GNTI-350 cell product post cell editing through enrichment. Cells were edited to overexpress FOXP3 and CAR19. (B) Generation of CAR-T effectors introduced by transduction of CAR19 LVV. (C) GNTI-350 express Treg markers. Data shows 2 donors. (D) Research grade cell metrics including expansion and enrichment are graphed over time. Cell production is comparable to TCR EngTreg production, indicating a robust, reproducible CAR19 EngTreg production process. Data graphed are three donors + SD.

Results Figure 2: GNTI-350 express lower levels of CRS-associated cytokines compared to CAR-T



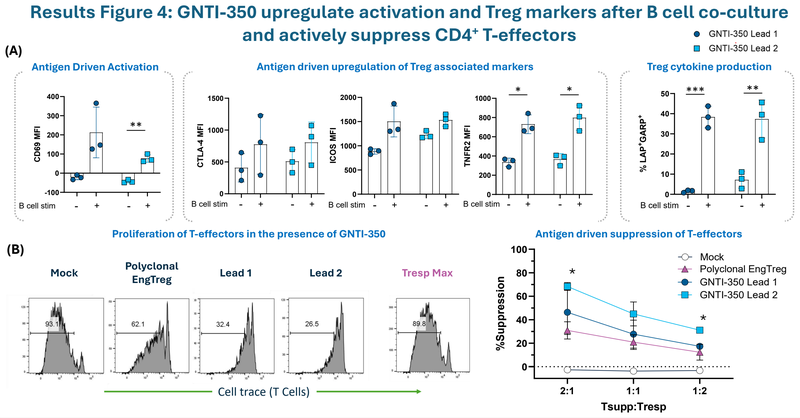
GNTI-350 therapy has a lower risk of CRS compared to CAR-T cell therapy. (A) Cytokine bead array of primary B cells cocultured with GNTI-350 cell products or CAR-T and mock T cell comparators. Supernatants were harvested 24hrs post co-culture. Individual points represent 4 different GNTI-350 donors (two donors for mock, one donor for CAR-T). (B) Intracellular cytokine staining of GNTI-350 or CAR-T comparators after coculture with B cells or anti-CD3/anti-CD28 dynabeads. Graphs show the percentages (top) and median fluorescence intensity (MFI) (bottom) of IFNγ or TNFα secretion. Flow cytometry ICS data is representative of three donors. Statistics were determined by 2-way ANOVA, p*≤0.05

Results Figure 3: Autologous GNTI-350 decreases B cell proliferation, plasmablast formation, and plasma cell differentiation after early intervention of B cell culture differentiation



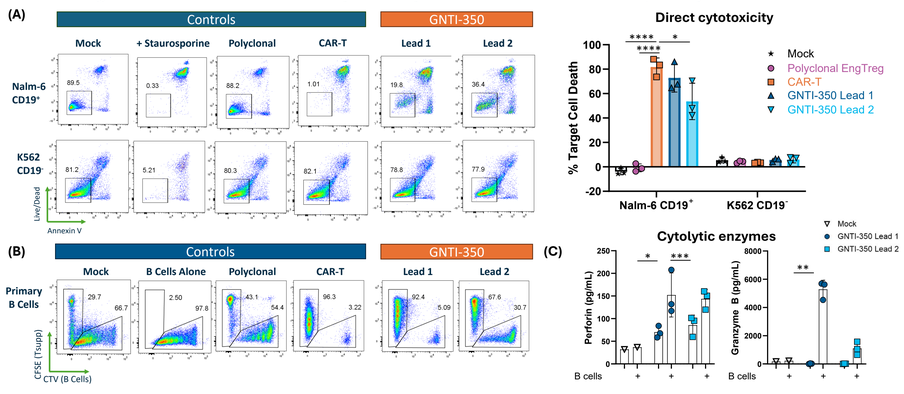
GNTI-350 prevents B cell differentiation. (A) GNTI-350 cell products cocultured with primary B cells[3,4] prevent B cells from differentiating into plasmablasts and plasma cells as compared to polyclonal EngTreg controls. (B) GNTI-350 cocultured with primary B cells abrogates secretion of IgM and total IgG from B cells. Representative flow cytometry from one donor shown (top). Graphs show technical replicates and are representative of 3 donors (bottom). All statistics were determined by one-way ANOVA. p*≤0.05, p**≤0.01, p****≤0.0001

Results Figure 4: GNTI-350 upregulate activation and Treg markers after B cell co-culture and actively suppress CD4+ T-effectors



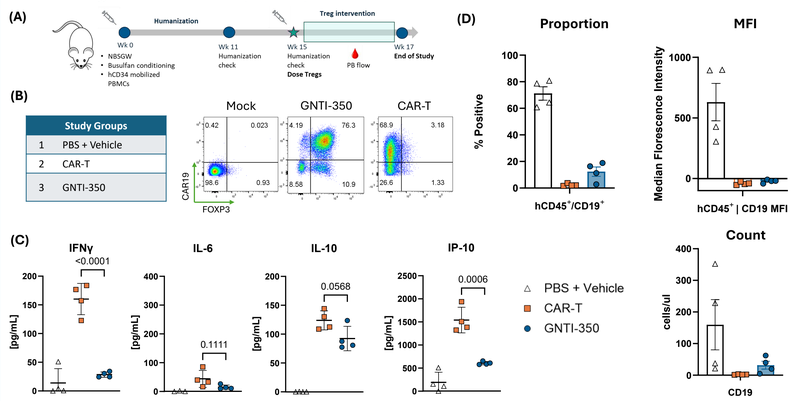
GNTI-350 activation and suppression. (A) Primary B cells and GNTI-350 EngTregs were cocultured for 24hrs. Activation marker and Treg marker upregulation was analyzed by flow cytometry. Statistics were performed using t-tests *p≤ 0.05, **p≤ 0.01, ***p≤ 0.001 (B) GNTI-350 cells suppress CD4+ T-resp after activation with B cells. Primary B cells and GNTI-350 cells were cocultured, and T-resp stimulated with αCD3/αCD28 dynabeads (1:10 cell:bead) for 16hrs. T-resp were then washed, beads removed and added to the B cells + EngTreg culture. 4d later, the level of proliferation (representative flow, 2:1 Treg:Tresp) was determined using dilution of cell-trace dye. Suppression was calculated by: ((Tresp Max – Sample Tresp Proliferation)/Tresp Max)*100. 3 donors compiled. Statistics = one-way ANOVA *p ≤ 0.05.

Results Figure 5: GNTI-350 demonstrates cytotoxicity toward CD19+ cells lines and primary B cells similar to CAR-T



GNTI-350 EngTregs can be cytotoxic toward target cells. (A) GNTI-350 EngTregs and CD19^{high} NALM6 or CD19^{negative} K562 cells were cocultured for 48hrs. Cell death was assessed by live/dead and annexin V staining on labeled target cells. Staurosporine is a control for apoptotic cell death. Statistics were performed using one-way ANOVA *p≤ 0.05, ****p≤ 0.0001. Cell death is calculated as 100% - live (Annexin V/Live Dead negative) (B) B cells[3,4] were cultured with GNTI-350 EngTregs for 96hrs and CTV dilution determined by flow cytometry. Data is representative of 2 healthy donors. (C) CBA on supernatants collected at 24hrs post coculture noted in (B). GNTI-350 EngTregs secrete more perforin and granzyme B after stimulation with primary B cells, potentially indicating a cytotoxic suppressive mechanism. Statistics were performed using a paired multiple t- test *p≤ 0.05, **p≤ 0.01, ***p≤ 0.001.

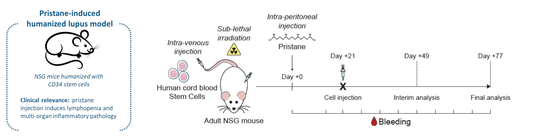
Results Figure 6: GNTI-350 depletes splenic B cells similar to CAR-T cells in a humanized mouse model



GNTI-350 depletes B cells *in vivo*. (A) An *in vivo* study in a CD34+ mobilized PBMC mouse model was performed to determine the persistence of GNTI-350 EngTregs, impact to peripheral and splenic B cells, and level of cytokine secretion from CAR-T or GNTI-350 EngTregs. (B) Study design and cell input as measured by flow cytometry. (C) CBA on serum collected from mice treated with CAR-T or GNTI-350 EngTregs at endpoint. (D) Splenic B cell populations were analyzed by flow cytometry. Statistics were determined by unpaired t-test with p-values noted.

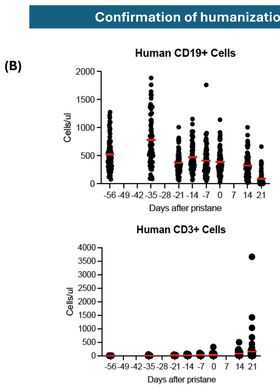
Results Figure 7: GNTI-350 cells elicit a superior safety profile compared to CAR-T effectors in a humanized inflammatory murine model

Study Design

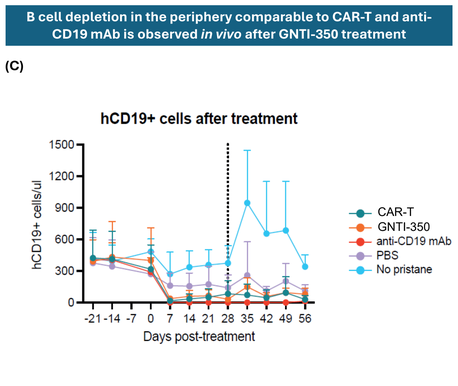


Group	Pristane	N	Rationale
No pristane		6	Healthy control
PBS	+	10	Disease control
CAR-T	+	10	B cell depletion effect
GNTI-350	+	10	Efficacy on inflammatory disease
Ineblizumab (anti-CD19 mAb)	+	10	Standard of care control for B cell depletion

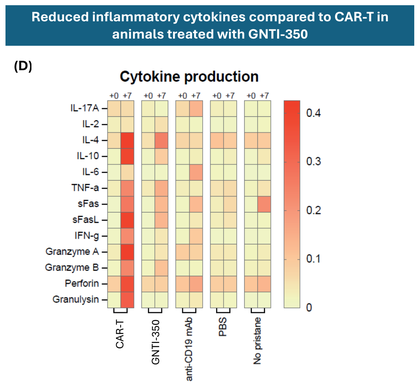
Confirmation of humanization



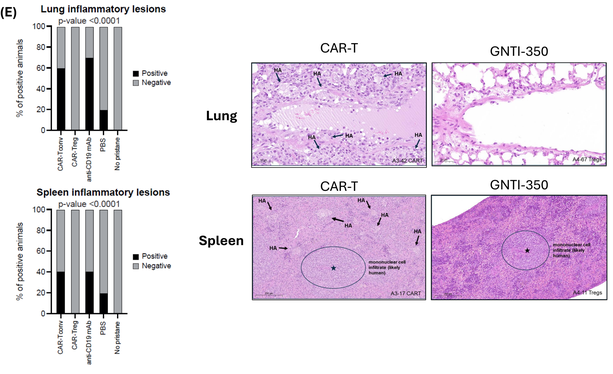
B cell depletion in the periphery comparable to CAR-T and anti-CD19 mAb is observed *in vivo* after GNTI-350 treatment



Reduced inflammatory cytokines compared to CAR-T in animals treated with GNTI-350



GNTI-350 show superior protection of multiple organs against inflammatory damage compared to CAR-T and CD19 mAb

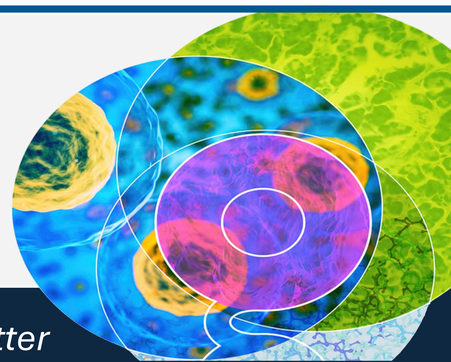


GNTI-350 is safer than CAR-T comparators *in vivo*. (A) Schematic and study design of a cord blood humanized mouse model treated with pristane to induce an inflammatory, SLE-like, disease. (B) Humanization of the mice was monitored by evaluating total CD45+cells, B cell levels (CD19+), and the appearance of T cells. GNTI-350 Tregs were not dosed until T cells appeared in the animals on Day 21 (bottom). (C) Peripheral blood flow cytometry was collected throughout the study before and after GNTI-350 dosing. hCD19+ B cells were depleted after treatment except in the no disease control group . (D) Analysis by CBA of serum collected from treated animals shows reduced cytokines and cytolytic enzymes compared to CAR-T cell treatment consistent with other GNTI-350 *in vitro* assessments. (E) 8 weeks post cell transfer, necropsy was performed and tissues taken for histology. GNTI-350 treatment resulted in improved inflammatory lesions in the spleen and lung, while CAR-T effector and anti-CD19 mAb treated animals showed marked increases in inflammatory lesions. HA = histiocytic aggregates

CONCLUSIONS

1. GNTI-350 can be engineered with stable FOXP3, IL-2 support through CISC, and CD19 targeting, and demonstrates a T-reg phenotype by surface markers and function.
2. GNTI-350 is suppressive against T cells as well as plasmablast and plasma cell formation.
3. GNTI-350 demonstrates a cytotoxic mechanism against NALM6 and primary B cells, which suggests a CAR-T like B cell depletion without cytokine release syndrome side effects.
   - Cytotoxic mechanism appears to be CAR19 (humanized) and target (CD19) specific as opposed to all CAR-EngTreg products.
4. Demonstrated lower proinflammatory and cytotoxic cytokines from GNTI-350 compared to CAR-T comparators indicates a better safety profile.
5. B cell depletion from GNTI-350 treatment *in vivo* is comparable to CAR-T cell treatment, but GNTI-350 results in lower inflammatory cytokine secretion and demonstrates protection against organ damage compared to CAR-T and anti-CD19 mAb. These data suggest that GNTI-350 is a safer, and potentially superior, cell therapy.

References:
1. Cook et al, "A chemically inducible IL-2 receptor signaling complex allows for effective in vitro and in vivo selection of engineered CD4+ T cells", Molecular Therapy. August 2023
2. Uenishi et al, "GNTI-122: an autologous antigen-specific engineered Treg cell therapy for type 1 diabetes". JCI Insight. February 2024
3. Cheng et al, "Ex vivo engineered human plasma cells exhibit robust protein secretion and long-term engraftment in vivo." Nature Communications. October 2021
4. Hung et al, "Engineering protein-secreting plasma cells by homology directed repair in primary human B cells." Molecular Therapy. August 2017



We make Tregs. *Better*