Poster #0981

GNTI-350: A CAR-Treg Therapy Offering Durable Immune Reset with Improved Safety for B Cell–Driven Autoimmune Diseases

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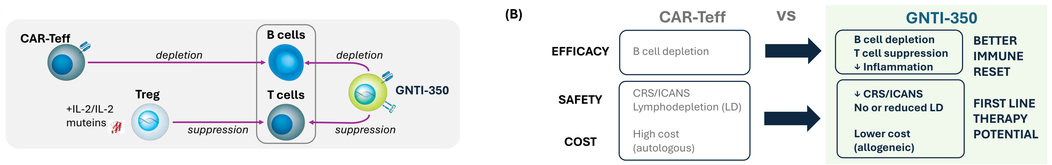
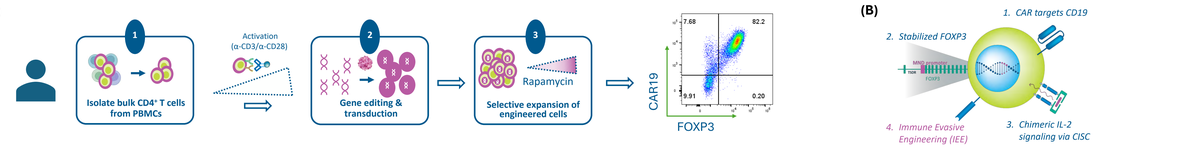
Premise of GNTI-350: *Impacts to B cells & T cells with better efficacy and safety profile than CAR-Teff*



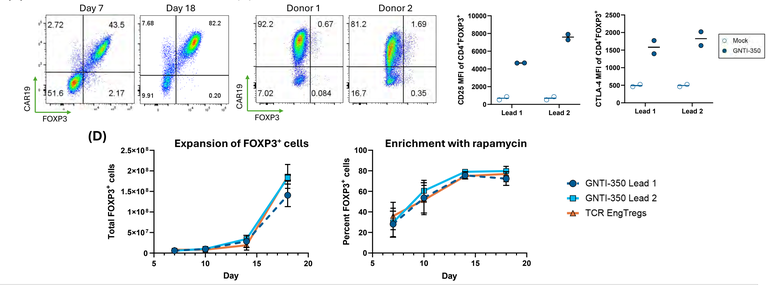
Overview of GNTI-350 as a therapeutic approach to B cell mediated autoimmune diseases. (A) GNTI-350 is an engineered, allogeneic, CAR19 T-regulatory cell therapy designed to deplete B cells and suppress T cells, which may offer a better immune reset than CAR-Teff cell therapies. (B) This product may 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-Teff and non-engineered Treg cell therapies. CRS: cytokine release syndrome. ICANS: Immune effector cell-associated neurotoxicity syndrome

Generation of GNTI-350: *CAR19 EngTregs*



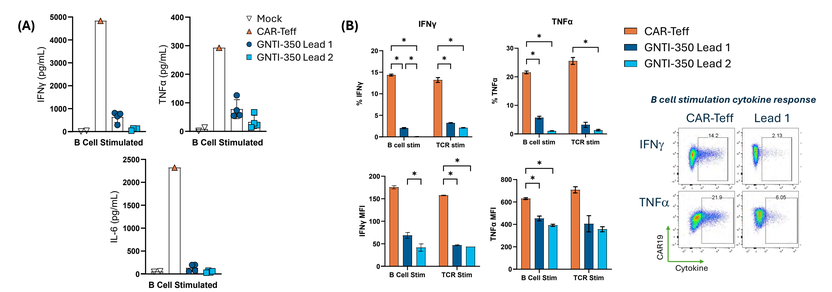
Engineered Treg production processes.[1,2] (A) For human Engineered Tregs (EngTregs), CD4+ T cells were isolated via magnetic enrichment from PBMCs and activated via α-CD3/α-CD28 microbead stimulation. Cells were then genetically modified using either 1) lentiviral transduction to deliver CAR19 and/or 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 the envisioned design for GNT-350 cell product with gene edits: FOXP3 is constitutively expressed to maintain a stable Treg phenotype, CISC provides IL-2 support to further maintain stable, long-lived cells, anti-CD19 CAR provides targeting toward B cells, and Immune Evasive Engineering (IEE) enables improved persistence of the allogeneic cell product.

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



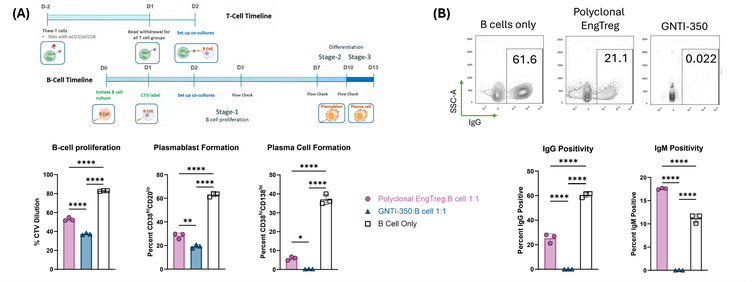
GNTI-350 cell production metrics. (A) Flow cytometry of the GNTI-350 cell product post-editing and enrichment. Cells were edited to overexpress FOXP3 and CAR19. (B) Generation of CAR-Teff effectors introduced by transduction of CAR19 LVV. (C) GNTI-350 express Treg markers. Data shows 2 donors. (D) Research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

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



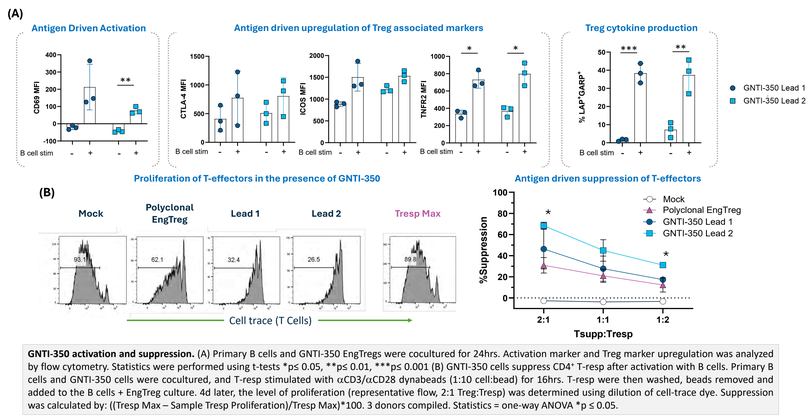
GNTI-350 therapy has a lower risk of CRS compared to CAR-Teff cell therapy. (A) Cytokine bead array of supernatants from 24hr cocultures of primary B cells with GNTI-350 cell products or CAR-Teff and mock T cell controls. Individual points represent 4 different GNTI-350 donors (two donors for mock, one donor for CAR-Teff). (B) Intracellular cytokine staining of GNTI-350 or CAR-Teff control 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

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



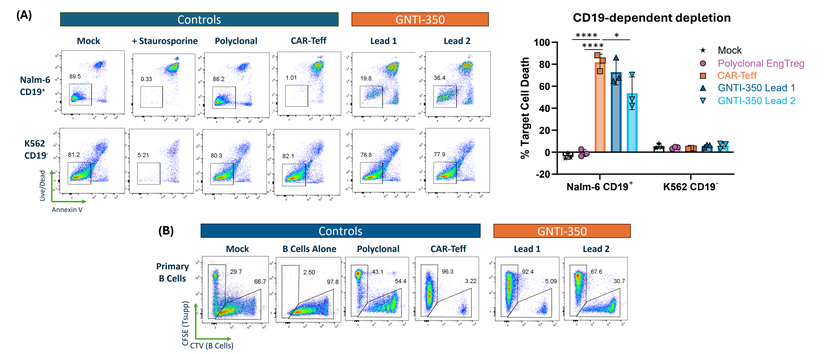
GNTI-350 prevents B cell differentiation. (A) GNTI-350 cell products cocultured with primary B cells[3,4] prevent B cells from differentiating into plasmablasts and plasma cells as compared to polyclonal EngTreg controls. (B) GNTI-350 cocultured with primary B cells abrogates secretion of IgM and total 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

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



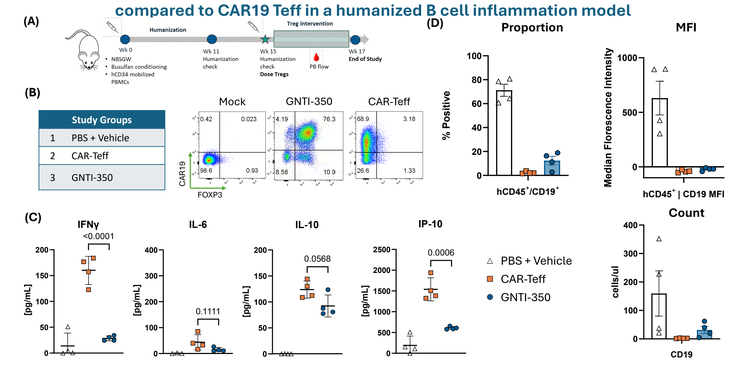
GNTI-350 activation and suppression. (A) Primary B cells and GNTI-350 EngTregs were cocultured for 24hrs. Activation marker and Treg marker upregulation was analyzed by flow cytometry. Statistics were performed using t-tests *p≤ 0.05, **p≤ 0.01, ***p≤ 0.001 (B) GNTI-350 cells suppress CD4+ T-resp after 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.

Figure 5: GNTI-350 depletes CD19+ cells lines and primary B cells



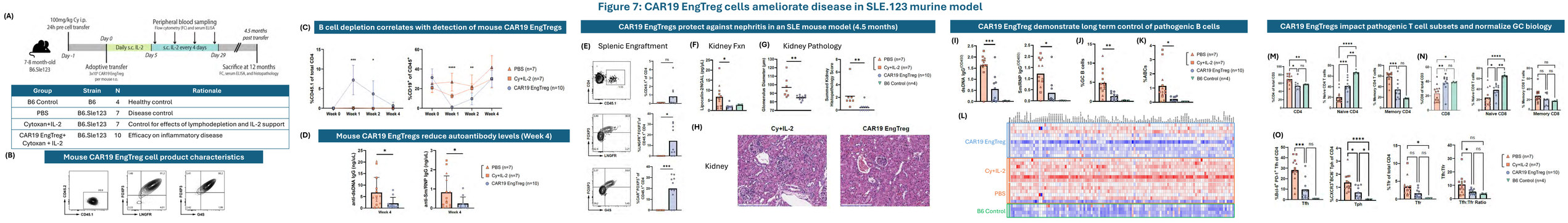
GNTI-350 EngTregs can deplete target cells. (A) GNTI-350 EngTregs and CD19^{high} NALM6 or CD19^{negative} K562 cells were cocultured for 48hrs. At a ratio of 1:1, cell death was assessed by live/dead and annexin V staining on labeled target cells. Staurosporine is a control for apoptotic cell death. 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.

Figure 6: GNTI-350 results in similar B cell depletion but lower inflammatory cytokine response compared to CAR19 Teff in a humanized B cell inflammation model



GNTI-350 depletes B cells *in vivo*. (A) An *in vivo* adoptive transfer study of GNTI-350 EngTregs was performed in a humanized mouse model (established by transplantation of human CD34+ HSPCs) to determine the persistence, impact to peripheral and splenic B cells, and level of cytokine secretion from CAR-Teff 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-Teff 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.

Figure 7: CAR19 EngTreg cells ameliorate disease in SLE.123 murine model



CAR19 EngTreg treatment ameliorates nephritis in an SLE mouse model by reducing autoantibodies and pathogenic B and T cells levels. (A) Schematic and study design of SLE.123 mouse model. A subset of disease control mice was preconditioned with cyclophosphamide (Cy) and received subcutaneous IL-2 as conditioning and cytokine support controls. **(B)** Representative flow plots of pre-transferred CAR19 EngTreg cells generated from B6.CD45.1+ donor CD4 T cells. **(C)** Left: Frequency of transferred CAR19 EngTreg in peripheral blood at 4 weeks post-transfer. Right: Frequency of CD19+ B cells of total CD45+ leukocytes over the same time course. Statistical significance: two-way ANOVA. **(D)** Levels of dsDNA (left) and Sm/RNP (right) autoantibodies in blood serum at 4 weeks post cell transfer. **(E)** Flow plots detecting presence of EngTregs in the spleen 4.5 months post cell transfer. **(F)** Levels of NGAL across treatment groups at endpoint. **(G)** Glomerular diameter measurements (left) and cumulative renal histopathology (right). **(H)** Representative images of scored kidney sections (200μm field). **(I)** Levels of dsDNA (left) and Sm/RNP (right) serum autoantibodies at study endpoint. **(J)** Levels of splenic germinal center B cells at study endpoint. **(K)** Levels of age-associated B cells at study endpoint. **(L)** Heatmap showing log2-normalized IgG signal intensities measured by autoantigen microarray. **(M)** CD4 T cell and **(N)** CD8 T cell subset compositions as determined by flow cytometry. Total (left), naïve (center), and memory (right). **(O)** Bar graph representations of endpoint levels of splenic T follicular helper cells (Tfh, left), splenic T peripheral helper cells (Tph, left center), splenic T follicular regulatory cells (Tfr), and the ratio of splenic Tfh:Tfr (right). Asterisks indicate statistical significance: p < 0.05 (*), p < 0.01 (**), p < 0.001 (***), p < 0.001 (****); error bars represent mean ± s.d. Statistical significance for all comparisons determined by two-way ANOVA (C), Welch's t-test (D-G), or one-way ANOVA (I-K, M-O).

CONCLUSIONS

1. GNTI-350 can be engineered with stable FOXP3, IL-2 support through CISC, CD19 targeting, and demonstrates a Treg phenotype by surface markers and function.
2. GNTI-350 demonstrates a targeted cytotoxic mechanism against CD19 expressing NALM6 and primary B cells, which suggests a CAR-Teff like B cell depletion without cytokine release syndrome side effects.
3. GNTI-350 suppresses T cells and curtails plasmablast and plasma cell differentiation as a means of B-cell specific suppression.
4. Lower proinflammatory cytokines compared to CAR-Teff comparators indicates a better safety profile.
5. In an SLE animal model, mouse CAR19 EngTreg treatment protected against nephritis and was associated with a long-term reduction in autoantibodies and autoreactive GC and ABC B cells. Impacts were observed beyond B cells in multiple immune cell populations involved in disease pathogenesis, including reductions in levels of Tfh and Tph cells, lower activated CD8+ T cells, and normalization of Tfh:Tfr ratio.

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

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