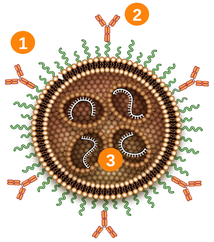
A Novel Product Candidate (CPTX2309) for In Vivo mRNA Engineering of Anti-CD19 CAR T Cells Utilizing Novel CD8-Targeted Lipid Nanoparticles

Theresa L. Hunter[1], Ferran Soldevila[1], Yan Zhang[1], Brittany Ross[1], Daiki Matsuda[1], Yanjie (Belle) Bao[1], John J. Li[1], Michelle Nguyen[1], Matthew Butcher[1], Michael Pica[1], Claudia Fernandez[1], James Vestal[1], Goutam Mondal[1], Yi Kuo[1], Jeffrey Chen[1], Josephine Nguyen[1], Young Yoon Choi[1], Diana Galvan[1], Duy P. Nguyen[1], Donald Jhung[1], Stuart A. Sievers[1], Steven P. Tanis[1], Cory Bentley[1], Michael Rosenzweig[1], Priya Karmali[1], Adrian I. Bot[1], Haig Aghajanian[1], Gregor B. Adams[1]

[1]Capstan Therapeutics, Inc.; San Diego, California, USA.

BACKGROUND

Autologous chimeric antigen receptor (CAR) T cell therapies have revolutionized cancer treatment and are now demonstrating promising activity in a number of autoimmune diseases (AID). Challenges in cell manufacturing, scaling, and the need for inpatient treatment highlight the necessity for an off-the-shelf solution for AID, devoid of lymphodepletion conditioning and viral vectors. Inspired by the success of CAR products and of mRNA lipid nanoparticles (LNPs) in COVID vaccines, Capstan is developing CPTX2309, a product of our CellSeeker™ tLNP platform, to deliver a sequence-enhanced mRNA payload encoding an anti-CD19 CAR to preferentially reprogram CD8-expressing T cells *in vivo* for treating AID.



CPTX2309 IS MADE UP OF THREE COMPONENTS:

1. LNP DELIVERY VEHICLE

Capstan's proprietary lipid nanoparticle is a non-viral dose-tunable system designed for increased tolerability and biodegradability to allow for repeat *in vivo* dosing.

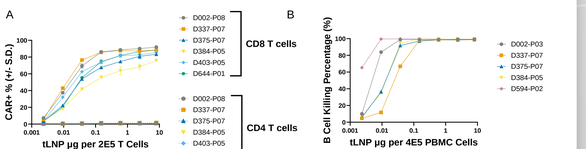
2. CD8 TARGETING BINDERS

Humanized CD8 antibody conjugated onto the nanoparticle surface, creating targeted lipid nanoparticles (tLNPs) to enable engineering of CD8 T cells and for preferential delivery to specific cells of disease-specific payloads.

3. ENHANCED ANTI-CD19 CAR mRNA

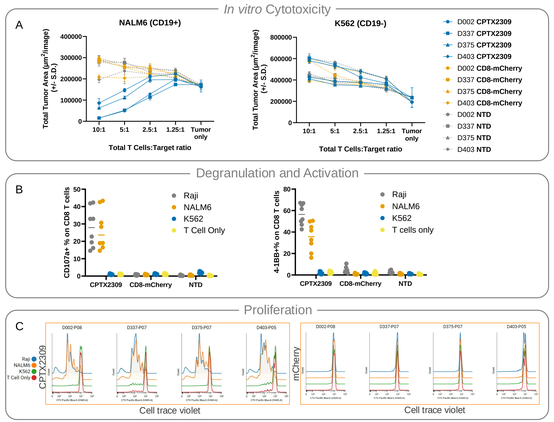
mRNA with improved performance encoding a fully human CAR recognizing CD19.

FIGURE 1 | DOSE DEPENDENT ENGINEERING OF NON-ACTIVATED HUMAN CD8 T CELLS USING CPTX2309



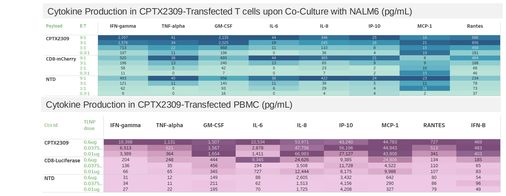
A) Percent CAR expression 24 hours after *in vitro* transfection of non-activated human T cells with CPTX2309. B) Killing of autologous B cells 72 hours after *in vitro* transfection of non-activated human T cells with CPTX2309. Killing measured by flow cytometry and normalized to non-transfected control. Each color represents a separate human donor.

FIGURE 2 | CPTX2309 ENGINEERED NON-ACTIVATED CD8 T CELLS DEMONSTRATE ANTIGEN-SPECIFIC FUNCTION



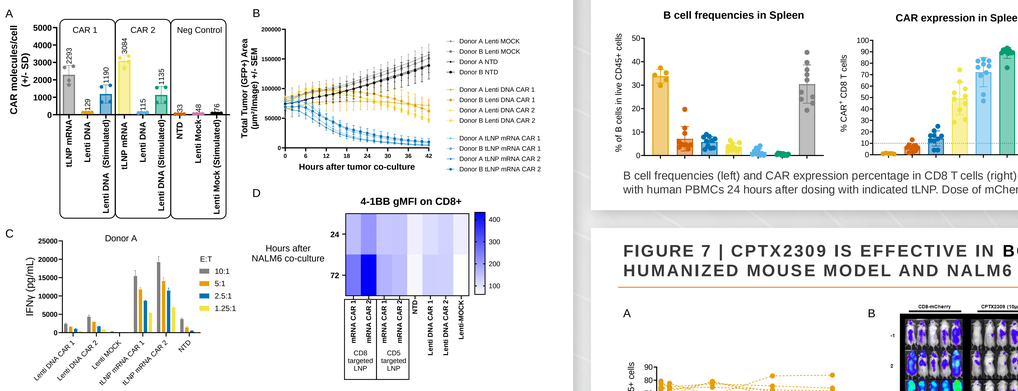
Non-activated T cells were transfected with CPTX2309 or mCherry control. A) Cytotoxicity against CD19+ (Nalm6, left) and CD19- (K562, right) target cells. NTD = non-transfected control. B) Degranulation measured by surface CD107a (left) and 4-1BB expression (right) after co-culture with CD19+ (Raji, Nalm6) or CD19- (K562) tumor cells. C) Proliferation of CPTX2309 (left) or mCherry (right) transfected T cells after co-culture with indicated tumor cells. Data is showing CD8 T cell proliferation with no proliferation observed in CD4 T cells (data not shown).

FIGURE 3 | CPTX2309 ENGINEERED CAR T CELLS ARE POLYFUNCTIONAL



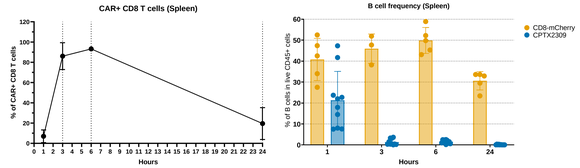
Secreted cytokines were measured after co-culture of human T cells transfected with CPTX2309 or control with CD19 expressing NALM6 tumor cells at four total T cells : target (E:T) ratios (top) or cytokine production in PBMC upon transfection with CPTX2309 (which transfects CD8 T cells to mediate B cell killing) or control at 3 tLNP doses (bottom). NTD: Non-transfected control.

FIGURE 4 | RAPID TARGET CELL CLEARANCE USING tLNP mRNA CAR-ENGINEERED CELLS *IN VITRO*



A) CAR molecules per cell quantified using PE Quantibrite beads (BD) on T cells transfected with tLNP containing mRNA CAR payload, T cells transduced with DNA lentiCAR, or negative controls. mRNA CAR expression was detected at 24 hours after transfection and Lenti DNA CAR expression was measured post cell thaw with (stimulated) or without dynabeads stimulation for 3 days. B) GFP-expressing tumor (target) cell killing by tLNP-transfected or lentiCAR-transduced T cells as measured by Incucyte imaging of GFP area. C) IFNγ produced by tLNP-transfected or lentiCAR-transduced T cells after co-culture with CD19+ tumor cells (representative data from one donor). A-B-C: CD5 targeted LNP was used to deliver mRNA CAR payload into both CD4 and CD8 T cells as lenti DNA CAR are CAR+ in both CD4 and CD8 cells. D) 4-1BB expression measured on tLNP-transfected or lentiCAR-transduced T cells at 24 and 72 hours after co-culture with CD19+ NALM6 cells. mRNA CAR and lenti DNA CAR have the same CAR amino acid sequence. CAR2 is the same CAR sequence in CPTX2309. NTD: non-transfected control.

FIGURE 5 | RAPID B-CELL TARGET CLEARANCE USING CPTX2309 *IN VIVO* AFTER A SINGLE DOSE

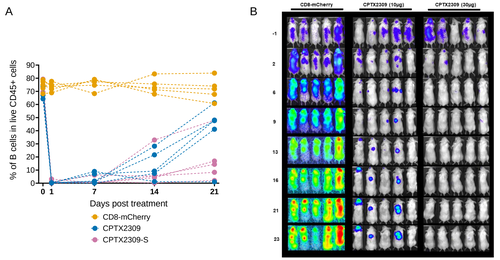


Frequencies of CAR expression (left) and B cells (right) in NSG mice engrafted with human PBMCs and treated with 30 µg of CPTX2309 or CD8-mCherry control.

FIGURE 6 | MINIMAL tLNP-CAR DOSE NEEDED TO ELIMINATE HUMAN PRIMARY B CELLS *IN VIVO*

B cell frequencies (left) and CAR expression percentage in CD8 T cells (right) in NCG mice engrafted with human PBMCs 24 hours after dosing with indicated tLNP. Dose of mCherry control was 7.5 µg.

FIGURE 7 | CPTX2309 IS EFFECTIVE IN **BOTH** CD34 HUMANIZED MOUSE MODEL AND NALM6 TUMOR MODEL



A) Time course of primary B cell percentage in NSG-CD34 mice dosed with 30 µg mCherry control tLNP, CPTX2309, or the same tLNP formulation containing an anti-CD20 mRNA CAR payload (CPTX2309-S). Mice were dosed every 3 days for 3 doses. B) NCG mice were engrafted with 1 x 10^6 human PBMCs on day -18 and inoculated with 5 x 10^5 Nalm6-luciferase tumor cells on day -8. Mice were bled and staged on day -1 based on tumor burden and human CD45+ cell engraftment. Mice were then dosed with indicated tLNP beginning on day 0, twice weekly for a total of 5 doses. Bioluminescence images at the indicated timepoint are shown.

CONCLUSION

CPTX2309 is a novel *in vivo* CAR therapy designed to bring together the unprecedented potency of CARs with the scalability and feasibility of the CellSeeker™ tLNP-mRNA platform. CPTX2309 rapidly, efficiently, and preferentially engineers anti-CD19 CAR CD8 T cells resulting in profound pharmacological activity both *in vitro* and *in vivo*, against primary or malignant human B cells. These data support the development of CPTX2309 for a broad range of B cell disorders, including autoimmune diseases, and point to the broad potential of this platform technology.