



Effective Engineering of CD8+ T Cells from Autoimmune Disease Patients Utilizing a CD8-Targeted Lipid Nanoparticle Encapsulating an Anti-CD19 CAR mRNA (CPTX2309)

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Disclosures

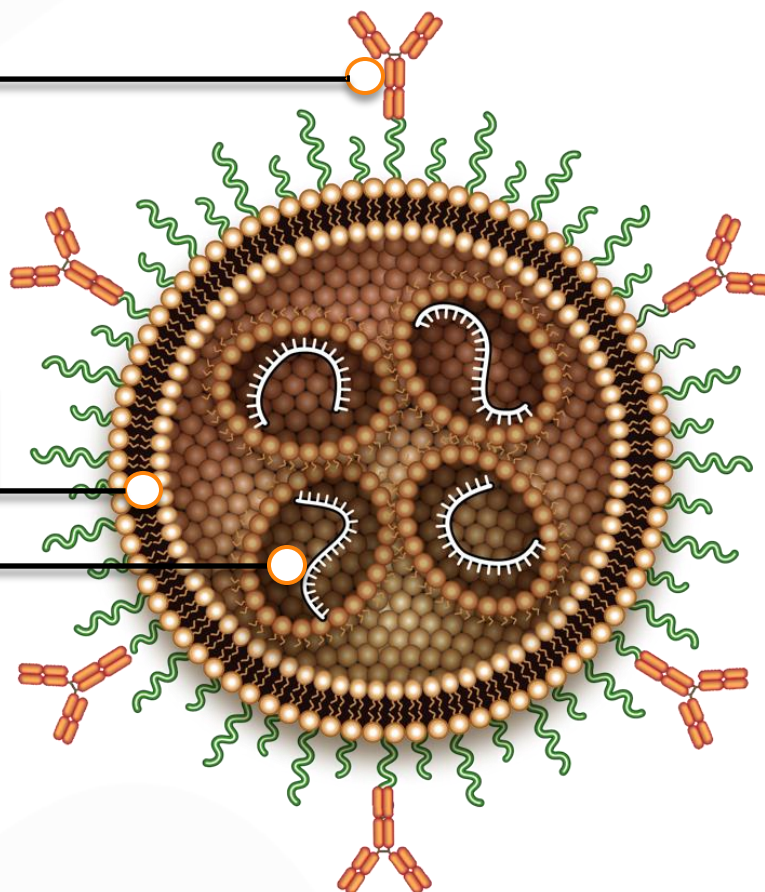
Currently serve as the Co-Founder and VP of Research at Capstan Therapeutics, Inc. and hold equity in the company

Lead Program CPTX2309 for Autoimmune Disorders











TARGETING BINDER
Humanized Anti-CD8 Ab

PROPRIETARY LNP
Capstan CTL-2.5

mRNA
Proprietary Capstan mRNA
encoding an anti-CD19 CAR

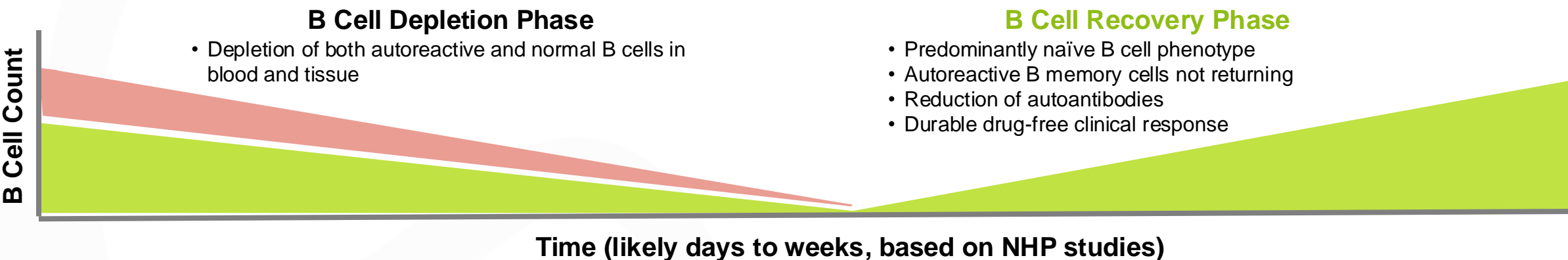
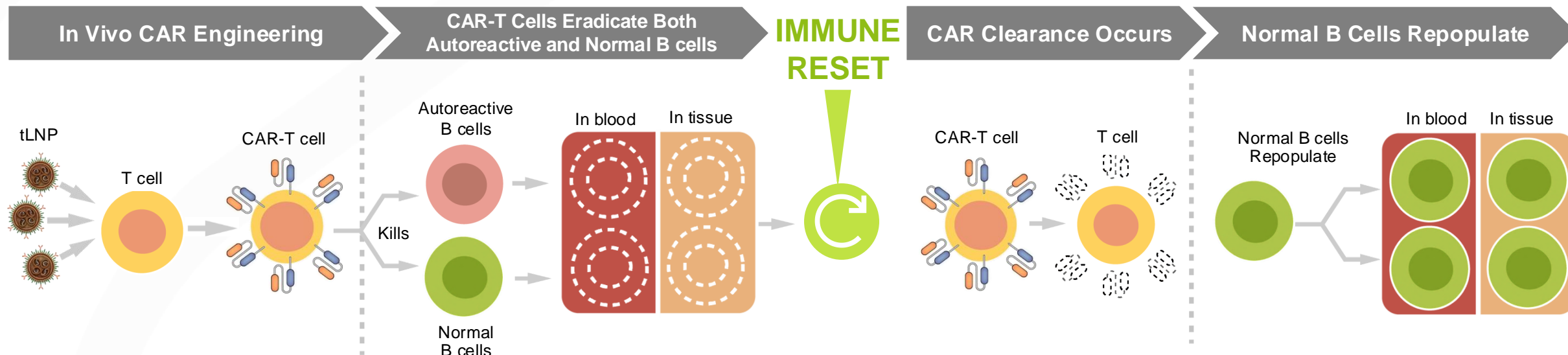


TARGET PRODUCT PROFILE:

Criteria for success in autoimmune disorders	CPTX2309
 REDOSABLE	
 OFF-THE-SHELF	
 TRANSIENT AND RAPID	
 DEEP B CELL DEPLETION	
 NO LYMPHO-DEPLETION	

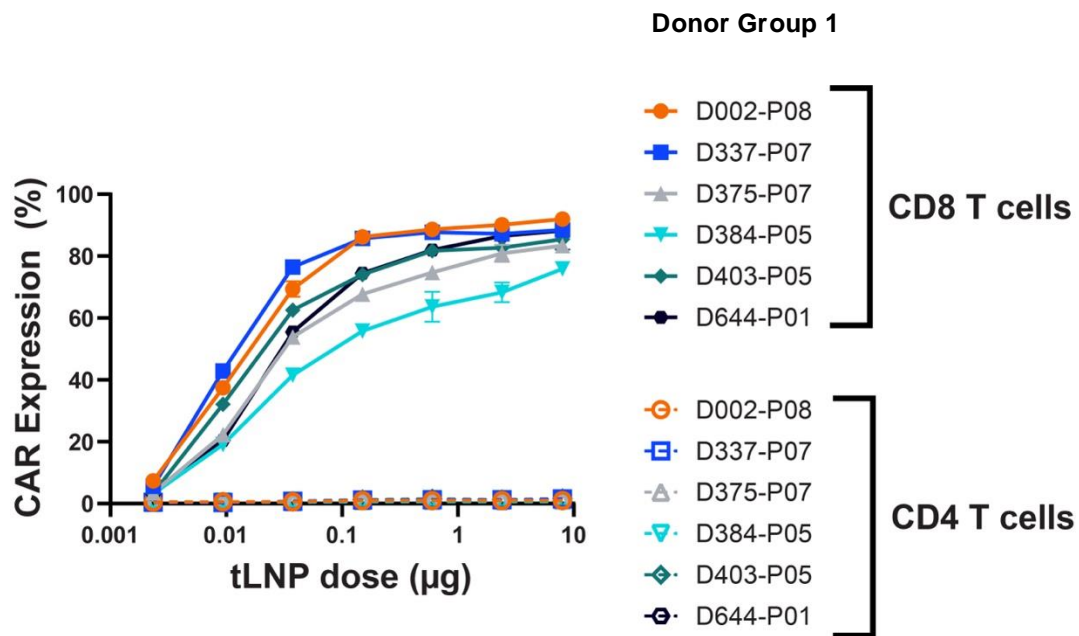
Note: To analyze B cell depletion in NHP, a CD8-directed tLNP containing a murine **anti-CD20 CAR** payload was used as a **CPTX2309 surrogate (CPTX2309-S)** due to the lack of CD19 cross-reactivity between human and NHP.

Capstan Aims to **Reset the Immune System** of Patients with Autoimmune Disease with in vivo CAR Therapy that is Transient, Tunable, and Scalable



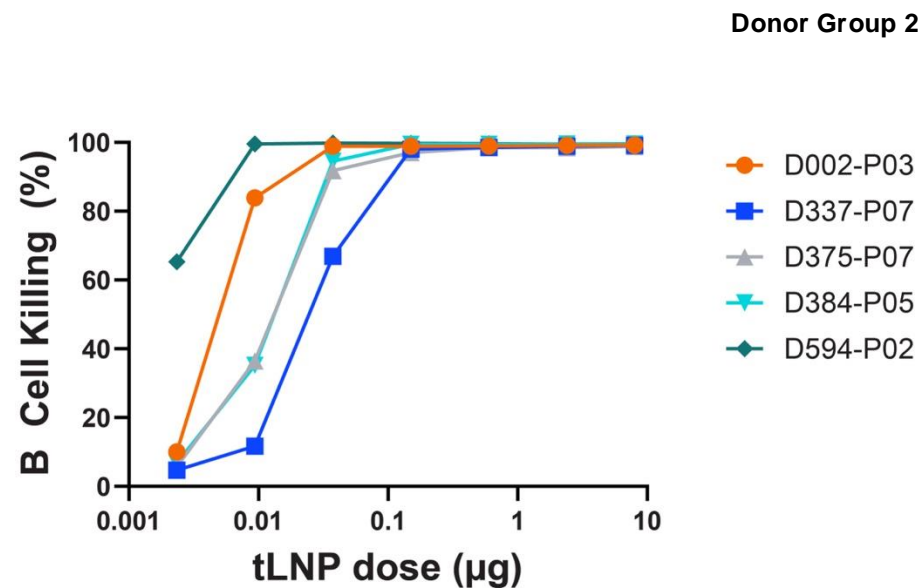
CPTX2309 Efficiently Produces Functional CAR-T Cells In Vitro Across Multiple Healthy Donors

DOSE DEPENDENT CAR EXPRESSION IN HUMAN CD8 T CELLS IN VITRO



T cells (2×10^5) isolated from healthy donor peripheral blood mononuclear cells (PBMC) were transfected with CPTX2309 at escalating doses in vitro for 1 hour followed by measurement of CAR expression at 24 hours. Each shape represents a distinct donor.

B CELL KILLING IN VITRO

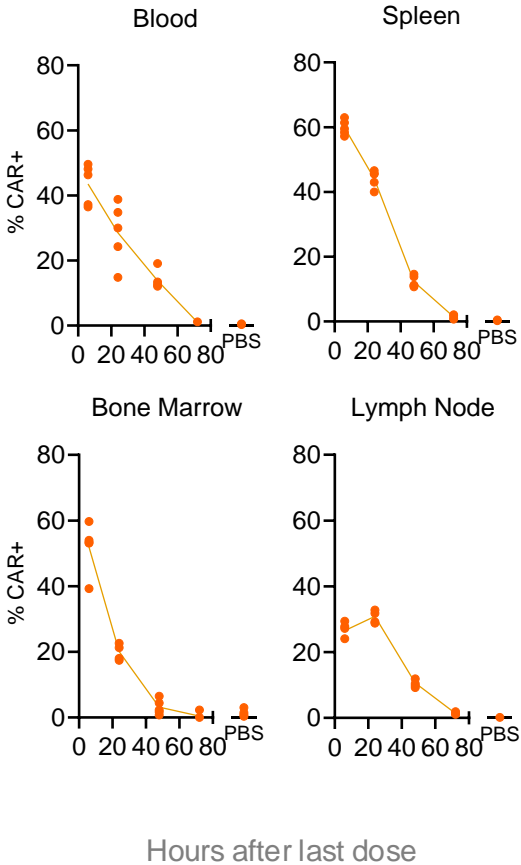


Primary human PBMCs (4×10^5) from healthy donors comprising both T and B cells, were transfected with CPTX2309 at escalating doses in vitro for 1 hour followed by measurement of B cell killing at 72 hours. B cell killing % is normalized to the non transfected control. Each shape represents a distinct donor.

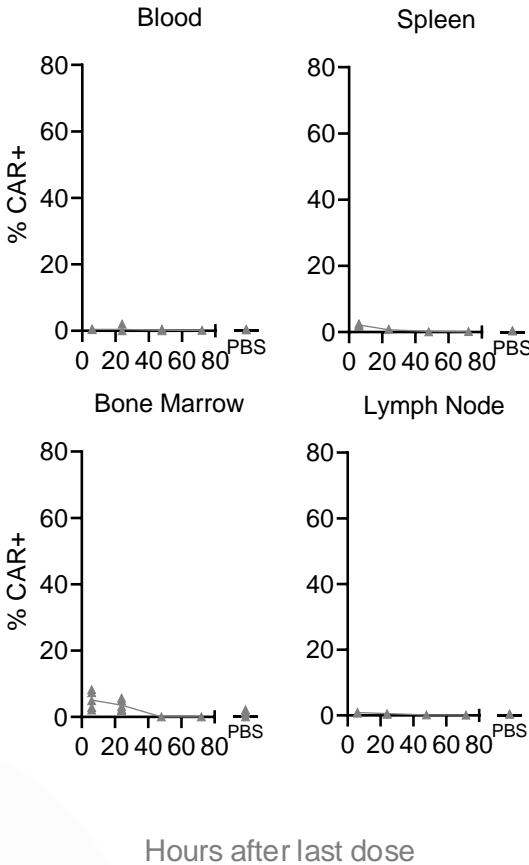
Rapid and Preferential In Vivo Engineering of CAR-T Cells in Blood and Tissue

Biodistribution Study in Mouse with CPTX2309

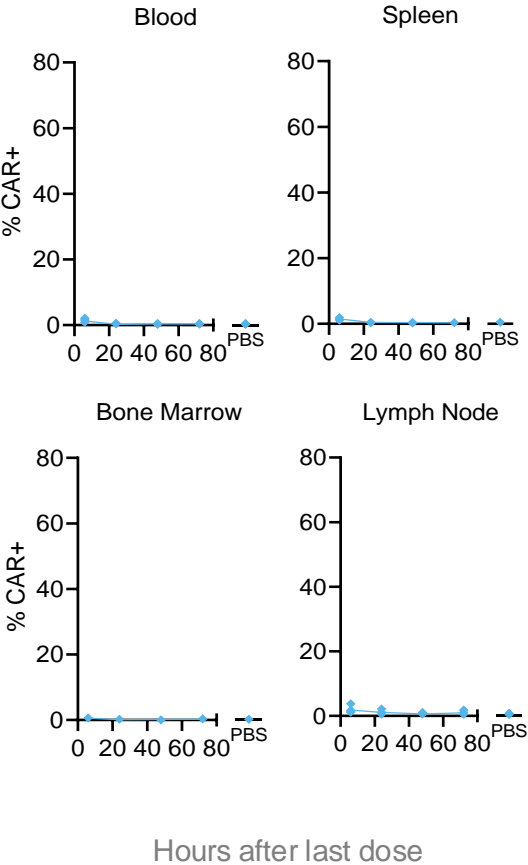
CYTOLYTIC LYMPHOCYTES*



CD4+ T CELLS



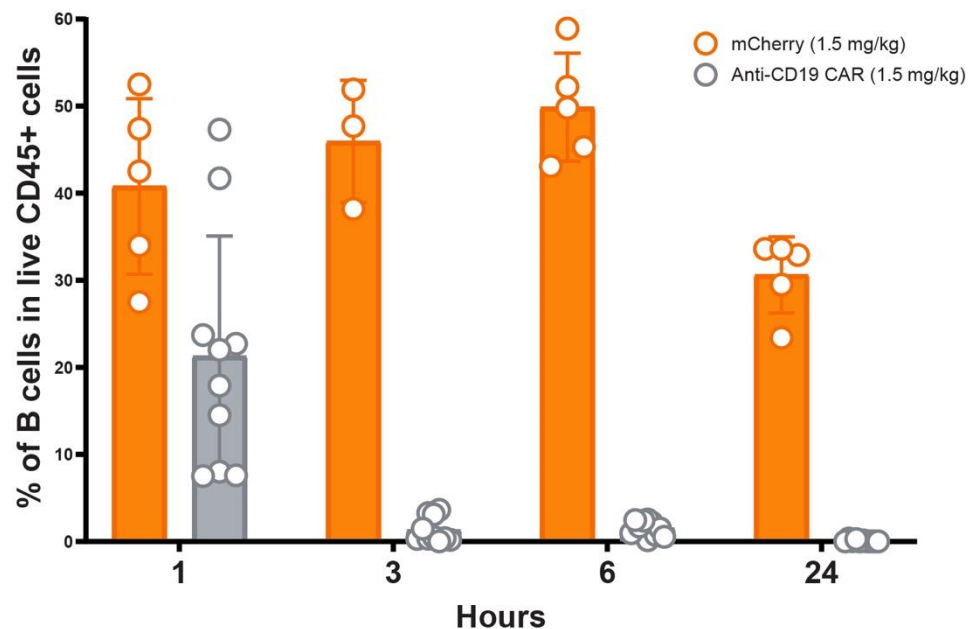
B CELLS



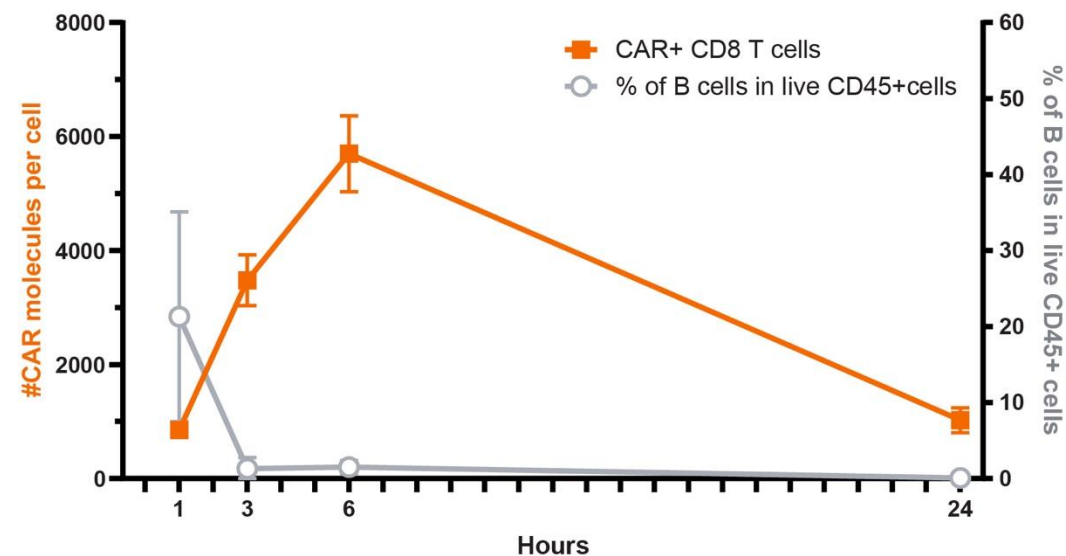
*CD4 negative T cells

CPTX2309 Administration to Humanized Mice Results in **Rapid and Complete Depletion** of Engrafted Human B Cells

RAPID B CELL DEPLETION IN SPLEEN IN HUMANIZED MICE



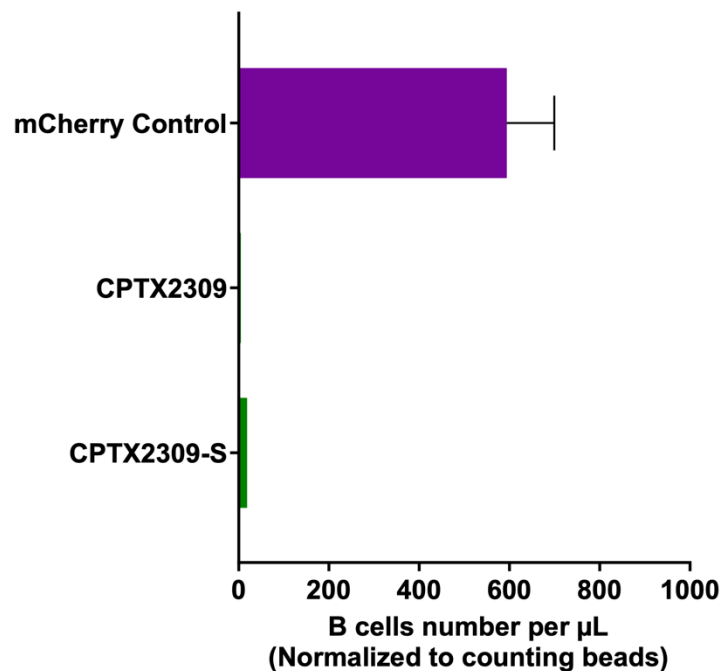
B CELL DEPLETION CORRELATES WITH CAR EXPRESSION IN CD8 T CELLS



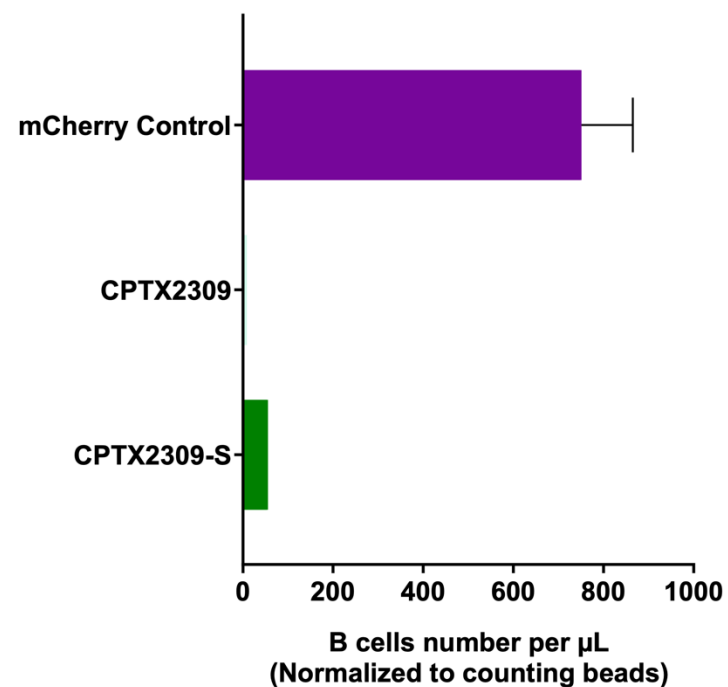
CAR T cells detectable at 1 hour with peak expression at 6 hours post tLNP administration

A Non-Human Primate Surrogate (CPTX2309-S; an anti-CD20 NHP-Cross Reactive CAR) is **Comparable to CPTX2309** (anti-CD19 CAR)

DONOR A



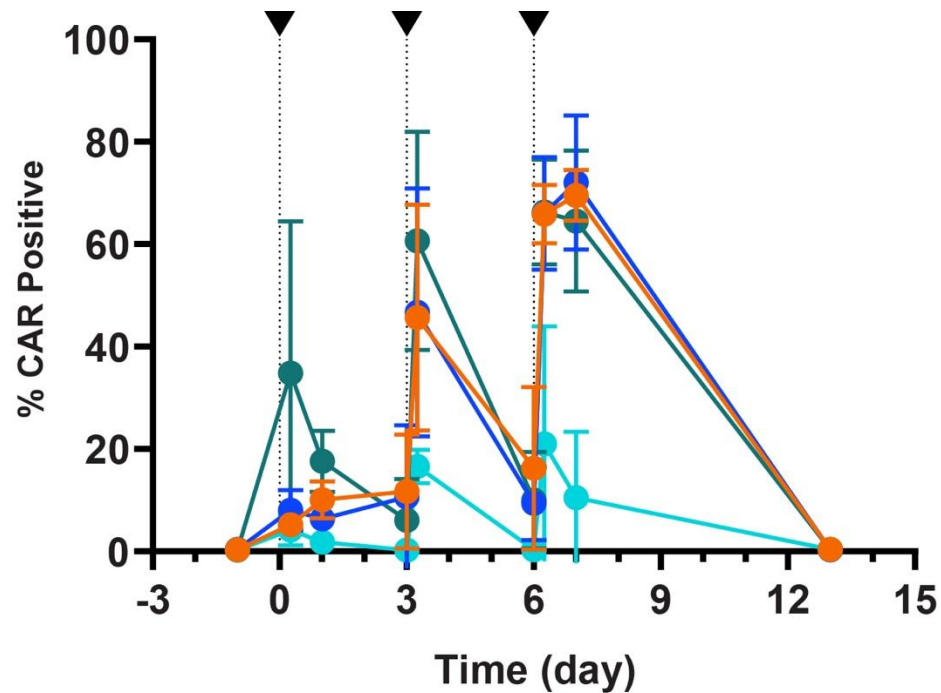
DONOR B



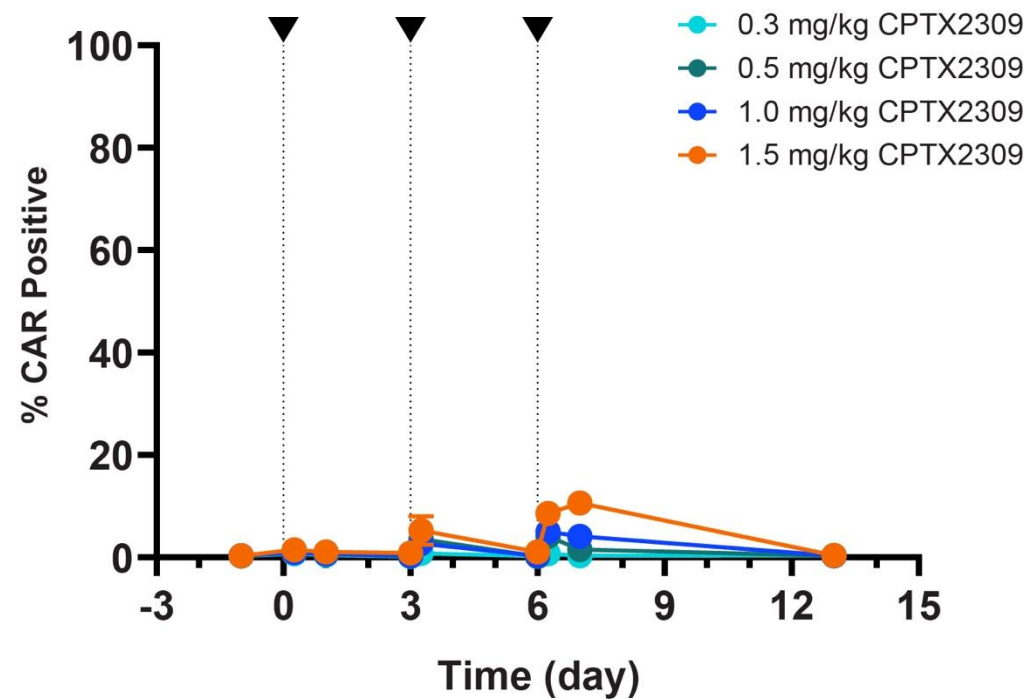
PBMC from healthy donors comprising both T and B cells, were transfected at 3 dose levels in vitro – 0.2, 0.6, and 2 µg – for 1 hour followed by measurement of B cells at 72 hours; data shown from 0.6 µg.

CPTX2309-S Treatment in NHP Results in Robust and Preferential Engineering of CD8+ T cells

CAR EXPRESSION IN CD8+ T CELLS (DOSE REGIMEN Q72H X 3)



CAR EXPRESSION IN CD4+ T CELLS (DOSE REGIMEN Q72H X 3)



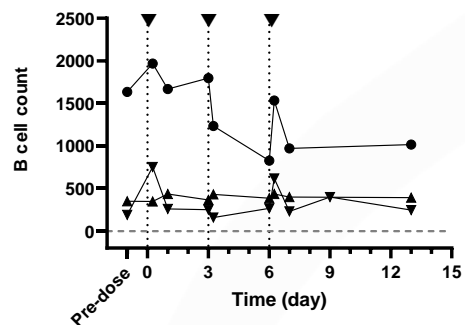
Minimal engineering of CD4+ T cells observed – note that NHP have a measurable % of double + (CD4+ CD8+) cells

CPTX2309-S Treatment in NHP Results in **Deep Depletion of B cells** in Blood and Tissue

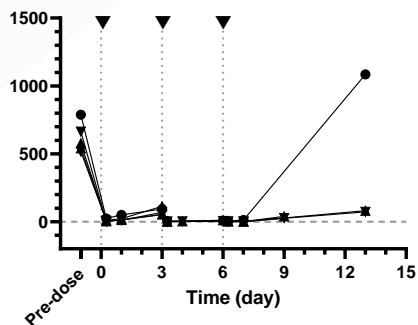
B CELL COUNT

B CELL DEPLETION IN PERIPHERAL BLOOD AT DIFFERENT DOSES (3xQ72hr). EACH LINE REPRESENTS AN INDIVIDUAL ANIMAL

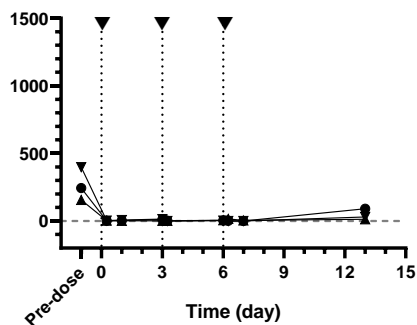
PBS Treated Control



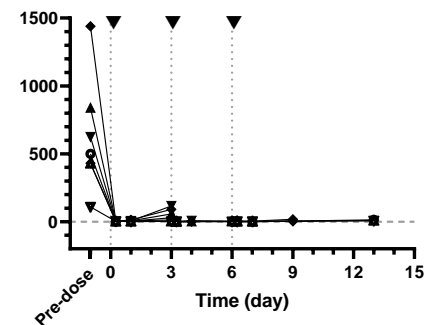
0.3 mg/kg



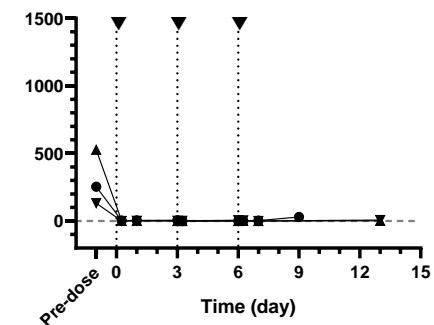
0.5 mg/kg



1.0 mg/kg

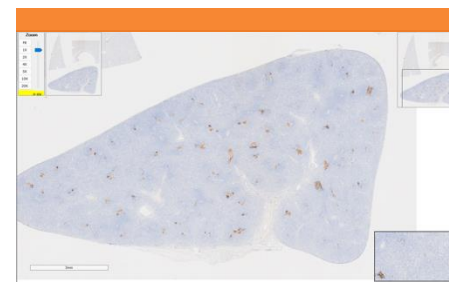
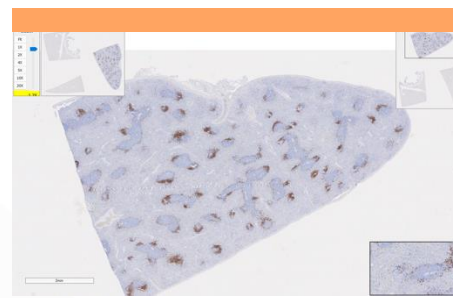
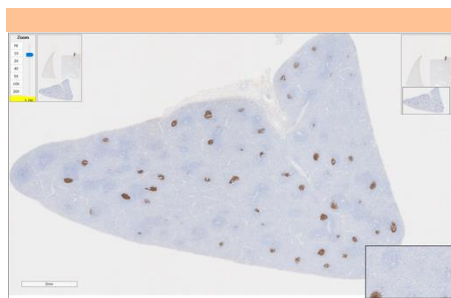
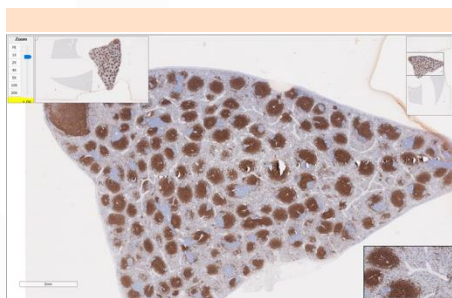


1.5 mg/kg



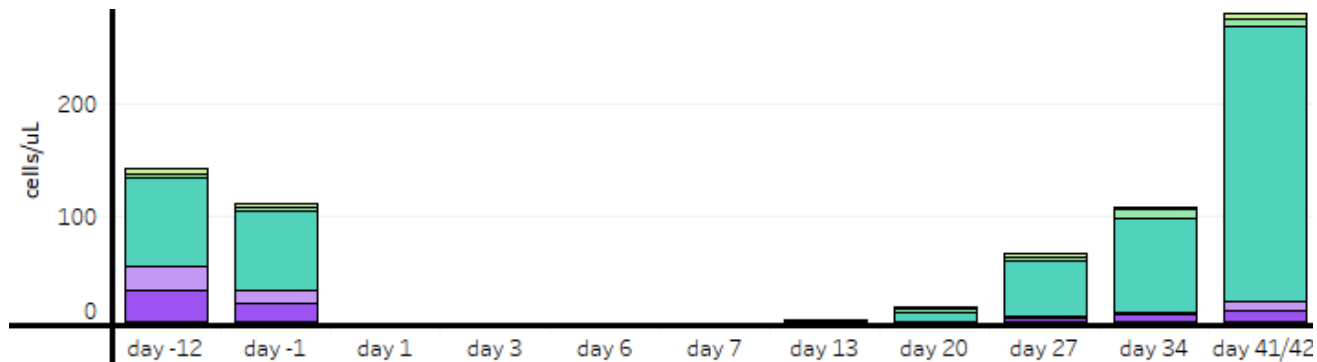
SPLEEN

IMMUNOHISTOCHEMISTRY STAINING OF SPLEEN USING ANTI-CD20 OF REPRESENTATIVE ANIMAL FROM EACH GROUP

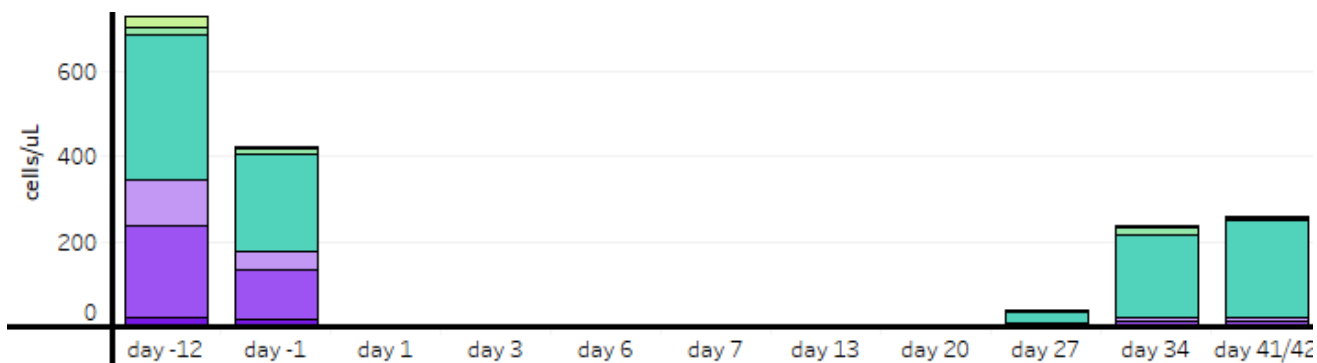


Repopulation of B cells after Depletion in NHP Blood Consists of Predominantly Naïve B cells

Animal 1 (1 mg/kg)



Animal 2 (1 mg/kg)



Naive

Mature

LEGEND

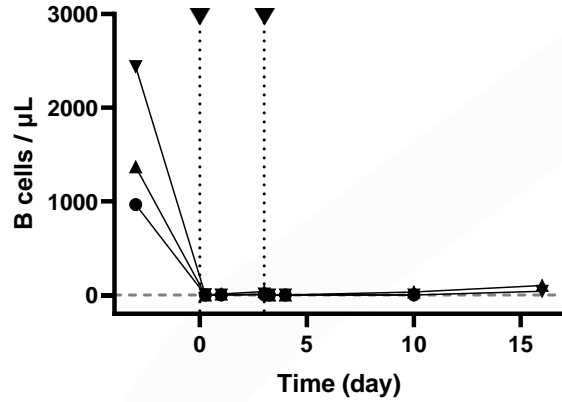
- Transitional**
(CD10+)
- Immature and CD21+ Double Negative**
(IgD- CD27- IgG-)
- Naïve**
(IgD+ CD27-)
- Unswitched Memory**
(IgD+ CD27+)
- Class-Switched Memory**
(IgD- CD27+)
- IgG+ Double Negative**
(IgD- CD27- IgG+)

Dose regimen = 3 doses, 72 hours apart

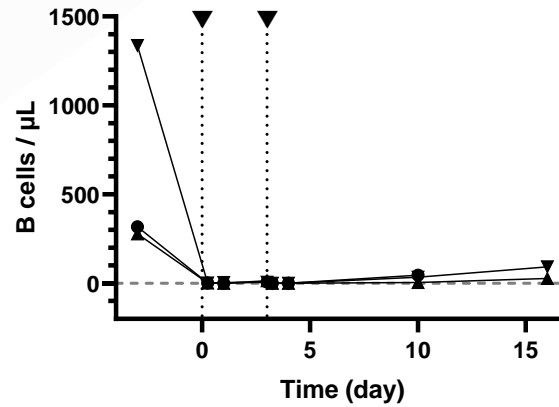
Each graph represents a single animal. Axes top and bottom rows are scaled differently. B cells were categorized based on IgD and CD27 expression.

Deep B Cell Depletion is Achievable with Two Doses

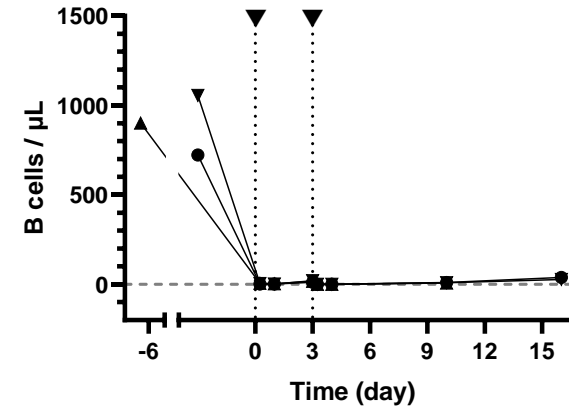
1.0 mg/kg Dosed 2x



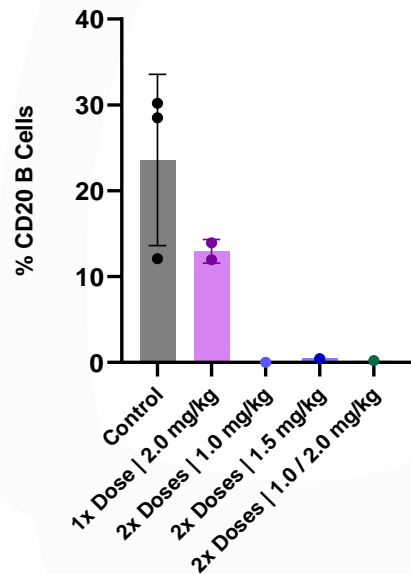
1.5 mg/kg Dosed 2x



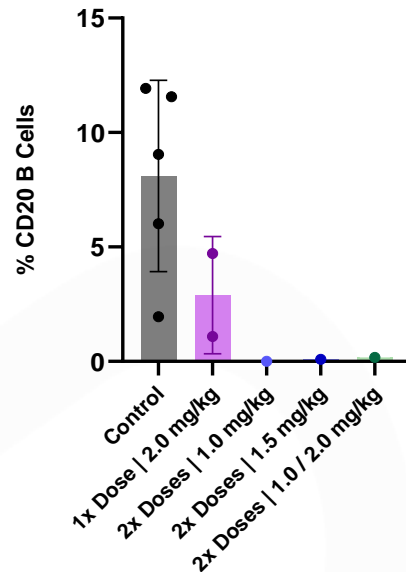
1.0/2.0 mg/kg



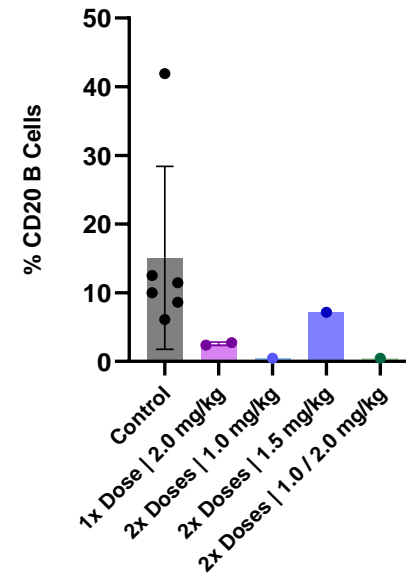
Spleen



Bone Marrow



Lymph Node



Legend

- Control | Spleen (n = 3)
- Control | Bone Marrow (n = 5)
- Control | Lymph Node (n = 6)
- 1x dose | 2.0 mg/kg (n=2)
- 2x Doses | 1.0 mg/kg (n=1)
- 2x Doses | 1.5 mg/kg (n=1)
- 2x Doses | 1.0/2.0 mg/kg (n=1)

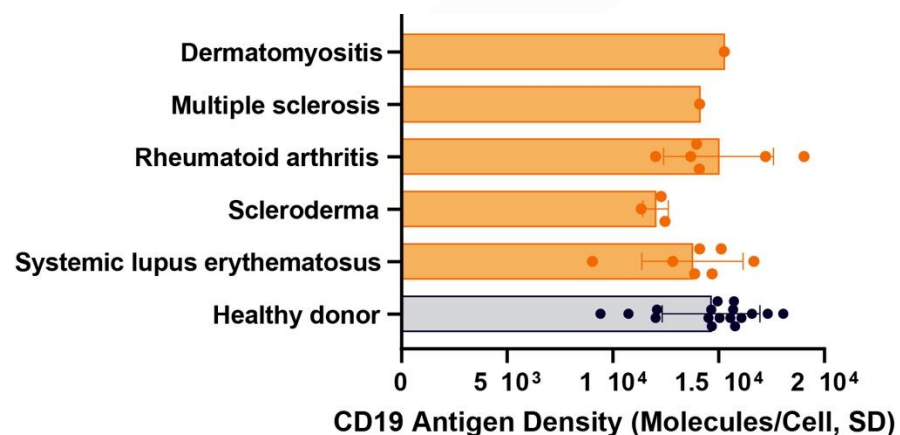
Evaluating the Efficacy of CPTX2309 in Autoimmune Patient Immune Cells

- PBMC (n=33) were collected from donors, including patients on immunosuppressive therapy as shown in the table below
- 22 age/gender/ethnicity matched healthy donors were included as control

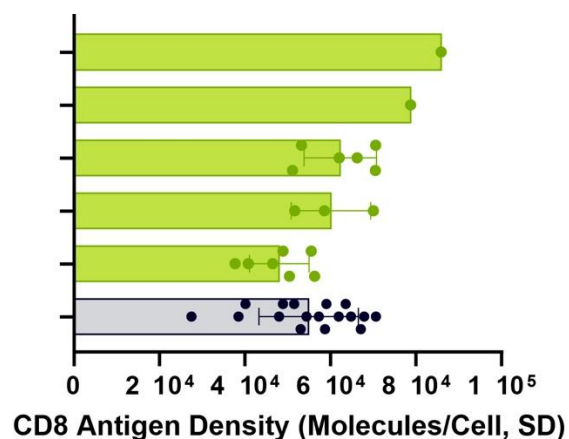
AUTOIMMUNE DISEASE	n
Antisynthetase syndrome	3
Dermatomyositis	3
Immune-mediated necrotizing myopathy	3
Multiple sclerosis	1
Rheumatoid arthritis	6
Scleroderma	8
Sjogren's syndrome	1
Systemic lupus erythematosus	8

Comparable Immune Cell Phenotyping Between Autoimmune Disease and Healthy PBMC

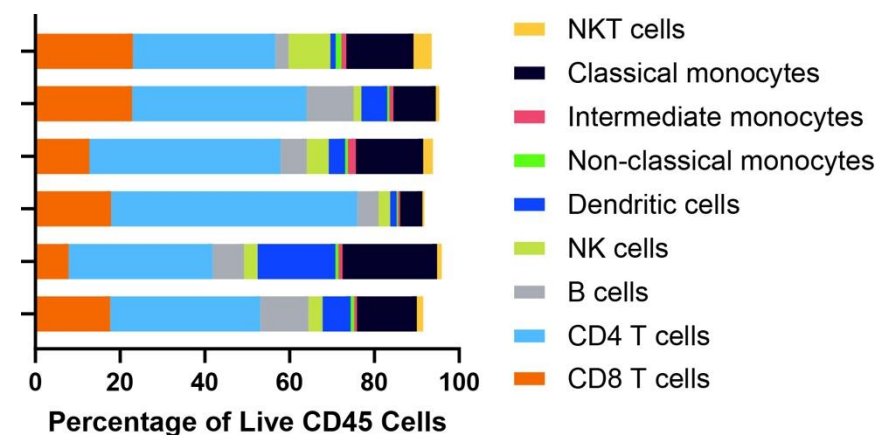
COMPARABLE CD19 ANTIGEN DENSITY ON CD19+ CELLS



COMPARABLE CD8 ANTIGEN DENSITY ON CD8+ CELLS



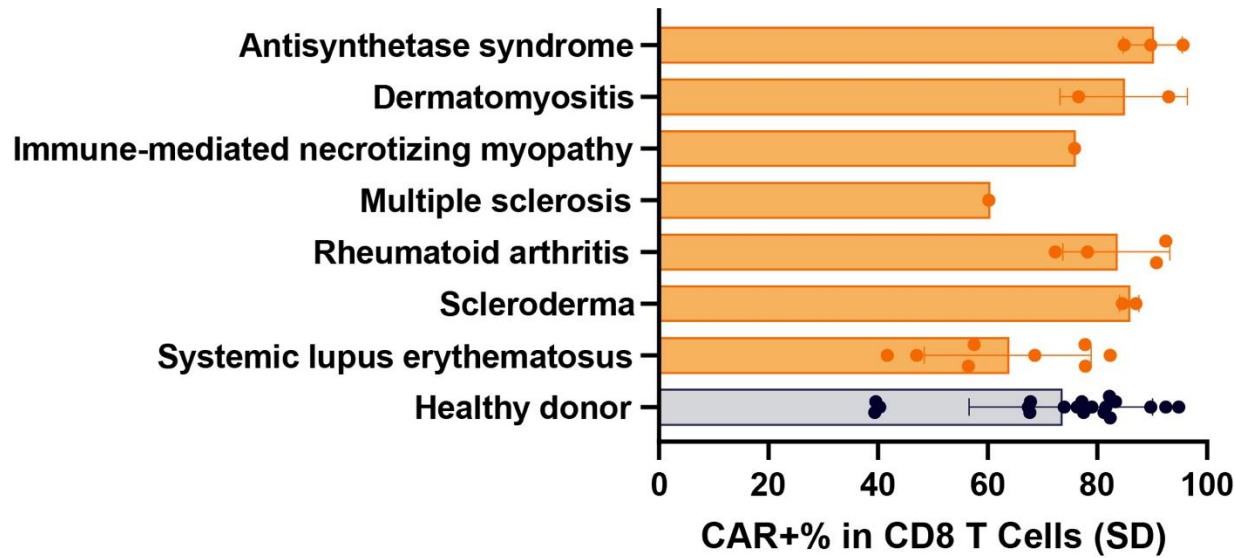
COMPARABLE IMMUNE CELL COMPOSITION



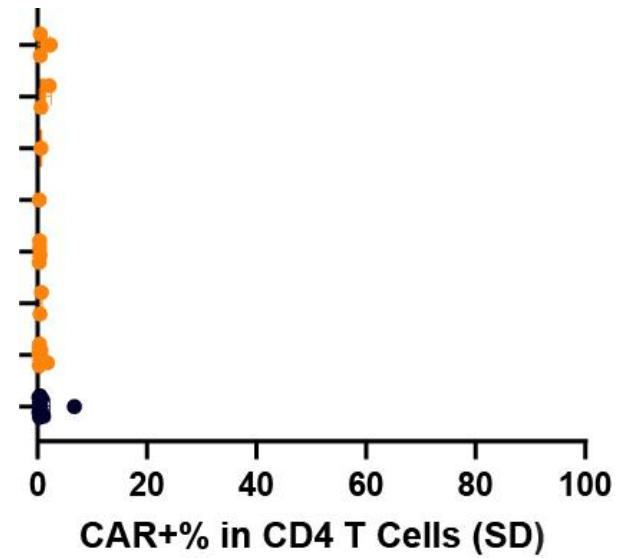
PBMCs were stained for immunophenotyping antibodies immediately after thaw. CD19 or CD8 antigen density was measured by staining PBMC at a saturated concentration of PE-conjugated anti-CD19 or anti-CD8 antibody. The number of molecules/cell was calculated from a standard curve generated with BD Quantibrite™ PE Quantitation Kit. Samples from patients on B cell depleting therapies not included in this analysis due to lack of sufficient cell number. Each circle represents a distinct donor with blood samples collected at a distinct time.

Effective and Preferential Engineering of CD8+ T cells Across Autoimmune Disease Patient Samples with CPTX2309

CAR EXPRESSION IN DISEASE CD8 T CELLS



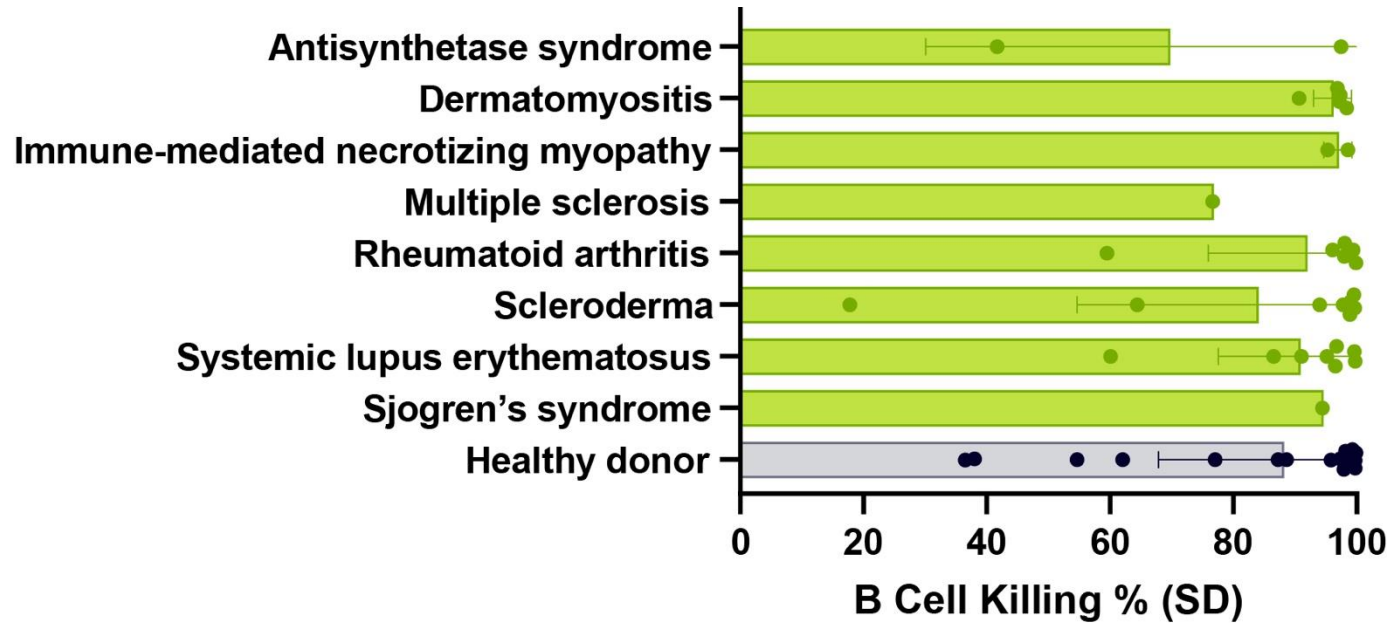
CAR EXPRESSION IN DISEASE CD4 T CELLS



2e5 T cells isolated from patient PBMC or matched healthy donor were transfected with 0.6 µg CPTX2309 in vitro for 1 hour followed by measurement of CAR expression at 24 hours. Each circle represents a distinct donor with blood samples collected at a distinct time.

T Cells From Autoimmune Disease Subjects can be Successfully Engineered with CPTX2309 to Generate Functional CD8 CAR+ T Cells that Kill Primary B Cells

B CELL KILLING PERCENTAGE



PBMCs (4×10^5) comprising both T and B cells, were transfected with $0.6 \mu\text{g}$ CPTX2309 *in vitro* for 1 hour followed by measurement of B cell killing at 72 hours. B cell killing % is normalized to the non transfected control. Samples from patients on B cell depleting therapies not included in this analysis due to <100 baseline B cells in the non transfected control. Each circle represents a distinct donor with blood samples collected at a distinct time.

Summary

- CPTX2309 is a novel in vivo anti-CD19 CAR product candidate, in mRNA format, delivered as anti-CD8 antibody-targeted lipid nanoparticle
- Treatment with CPTX2309 or CPTX2309-S results in robust, preferential, and transient engineering of functional CD8+ CAR T cells in vitro and in small and large animal models, with a pronounced pharmacological response
- Repopulation by predominantly naïve B cells after deep and transient depletion of B cells in non-human primate blood and tissue after CPTX2309-S treatment is suggestive of an immune reset, similar to what has been observed with ex vivo CD19 CAR T therapy
- Immunophenotype is similar between various B cell mediated autoimmune disease patient samples compared to healthy donor controls. Antigen density of CD8 on T cells for tLNP targeting and CD19 on B cells for CAR targeting are comparable
- CPTX2309 can effectively engineer functional CD8+ CAR T cells from autoimmune disease patient cells with myositis (ASyS, IMNM, DM), systemic lupus erythematosus, rheumatoid arthritis, scleroderma, Sjogren's syndrome, and multiple sclerosis comparably with healthy donor cells, and irrespective of prior or concomitant treatments
- B cells from autoimmune disease patients and healthy donors are killed with autologous anti-CD19 CAR T cells produced by CPTX2309 treatment
- These data support continued development of CPTX2309 as a novel in vivo CAR treatment for B cell involved diseases

Thank You

Yan Zhang
and the Capstan team

NIH
Andrew Mammen,
Maria Casal-Dominguez, and
Iago Pinal-Fernandez

Scan to view Capstan posters
and presentations at ACR

