Profound B Cell Depletion and Repopulation with Predominantly Naïve B Cells in Non-Human Primates Achieved Through a Novel In Vivo CD8-Targeted Lipid Nanoparticle mRNA CAR

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BACKGROUND

Ex vivo chimeric antigen receptor (CAR) T cell therapies have revolutionized cancer treatment and have demonstrated significant clinical activity in various autoimmune disease indications. Despite the success of current CAR T therapies, challenges in cell manufacturing, scalability, utilization of integrating viral vectors, and the need for lymphodepleting chemotherapy highlight the necessity for an off-the-shelf *in vivo* CAR technology applicable for broader use. To that aim, we utilized our CellSeeker™ tLNP platform to develop an *in vivo* anti-CD19 CAR mRNA product delivered by a CD8-targeted lipid nanoparticle (tLNP) (CPTX2309) and evaluated the activity of a cross reactive anti-CD20 CAR surrogate (CPTX2309-S) in non-human primates (NHPs), including the ability to achieve potential immune reset (deep B cell peripheral and tissue depletion).

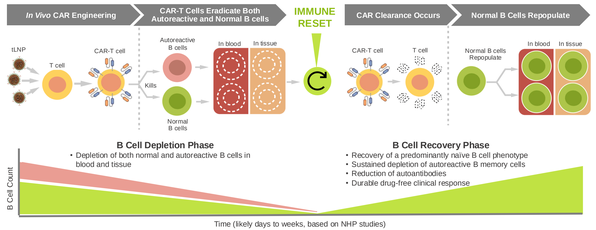
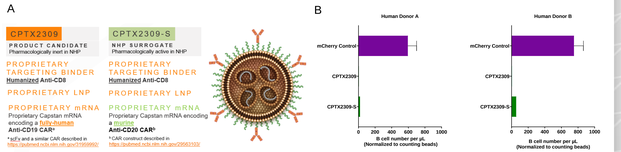


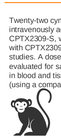
FIGURE 1 | CPTX2309-S TRANSFECTED T CELLS RAPIDLY ELIMINATE B CELLS IN VITRO

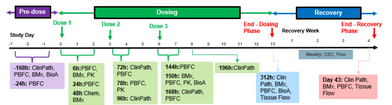


A) Comparison of CPTX2309 product candidate and CPTX2309-S NHP surrogate. B) Primary human PBMCs comprising both T and B cells, were transfected with 0.6 µg of tLNP for 1 hour followed by measurement of B cells at 72 hours.

FIGURE 2 | SCHEMATIC OF NHP STUDIES OF CPTX2309-S

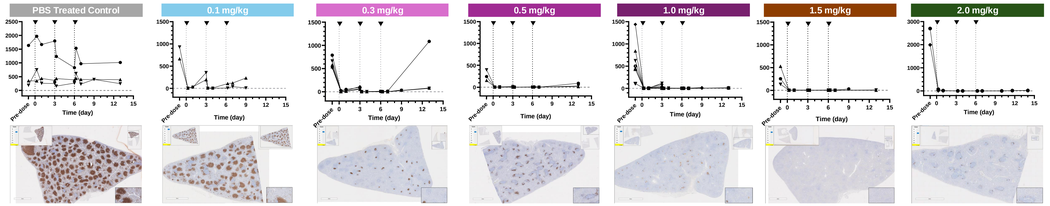
Twenty-two cynomolgus macaques were intravenously administered escalating doses of CPTX2309-S, which showed comparable activity with CPTX2309 *in vitro*, across three separate studies. A dose range of 0.1 – 2.0 mg/kg was evaluated for safety and tolerability. B cell depletion in blood and tissues, as well as B cell repopulation (using a compact dose regimen of 3x q72hr)





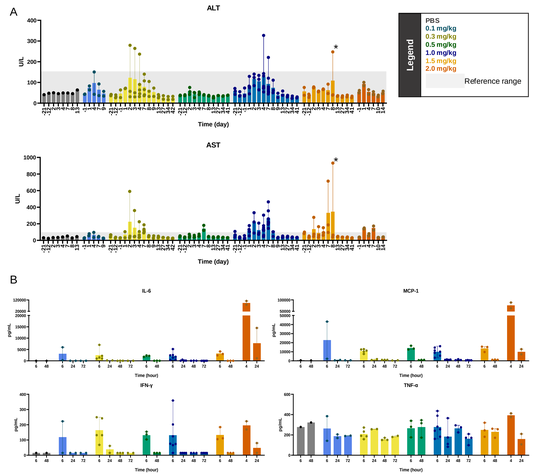
Representative study details for three combined studies included in this presentation. Abbreviations: BioA = ADA and PK assays; BMx = biomarkers; ClinPath = Clinical Pathology consisting of complete blood count, serum chemistry and coagulation testing; h = hour; PBFC = peripheral blood flow cytometry

FIGURE 3 | RAPID B CELL DEPLETION AFTER DOSING WITH CPTX2309-S



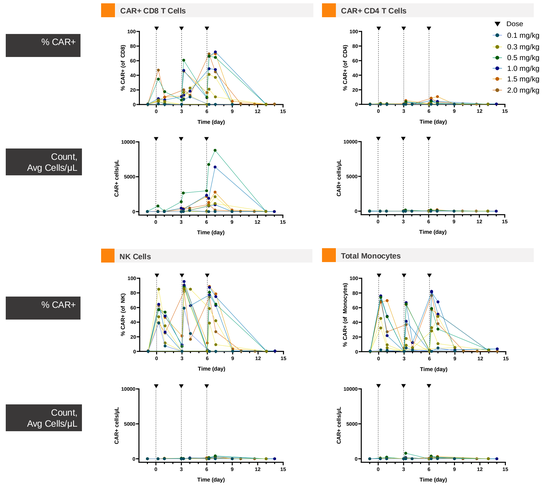
Top) B cells per µL (CD19+ and/or CD20+) in blood after dosing with indicated amounts of CPTX2309-S as measured by flow cytometry and **Bottom**) paired CD20 immunohistochemistry of cynomolgus monkey spleen after indicated dose of CPTX2309-S.

FIGURE 4 | TRANSIENT ELEVATION IN LIVER ENZYMES AND PRO-INFLAMMATORY CYTOKINES AFTER CPTX2309-S TREATMENT



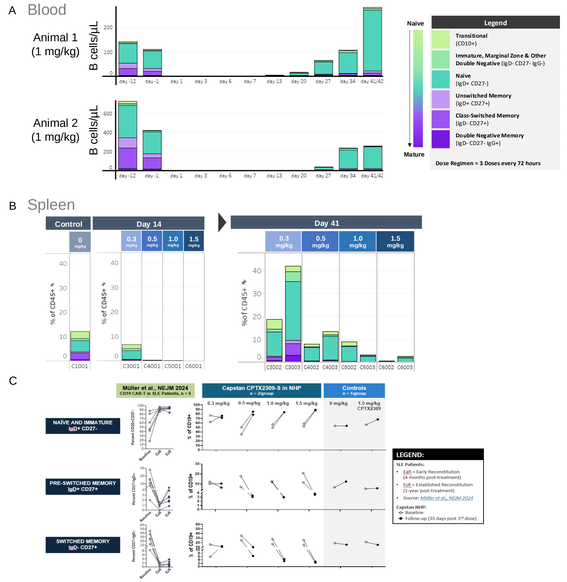
A) Liver enzymes and B) cytokines in animals treated with CPTX2309-S; Data from scheduled necropsy shown; * Elevated levels of ALT and AST were observed after the 3rd dose in one animal terminated early in 1.5 mg/kg cohort, associated with exaggerated pharmacology

FIGURE 5 | DOSE-DEPENDENT CAR EXPRESSION AFTER TREATMENT WITH CPTX2309-S



Averaged CAR expression and CAR+ cells per µL in CD8 T cells (top left), CD4 T cells (top right), NK cells (bottom left) and monocytes (bottom right) after dosing with indicated amounts of CPTX2309-S. Detection reagents varied between studies.

FIGURE 6 | REPOPULATING B CELLS AFTER CPTX2309-S TREATMENT ARE PREDOMINANTLY NAÏVE



(A) B cell subsets in blood, (B) B cell subsets in spleen, and (C) depletion of isotype class-switched B cells and repopulation with naïve B cells, comparable to Müller et al NEJM 2024. Data shown from 1 of 3 studies, (n=12)

CONCLUSIONS

- CPTX2309-S, a test article analogous to our lead product, rapidly delivers an anti-CD20 CAR mRNA payload, resulting in engineering *in vivo* up to 80% of CD8+ cells
- CPTX2309-S had a favorable safety and tolerability profile at doses of 0.1 – 1.0 mg/kg x3
- Peripheral B cell depletion was observed at all doses tested (>0.1 mg/kg), with profound B cell depletion observed in tissues following multiple doses (>0.5 mg/kg x3)
- Recovering B cells in blood and tissues are predominantly of a naïve phenotype following multiple doses of >0.5 mg/kg, suggestive of an immune reset