Pre-Clinical Characterization of MTX-101, a Novel Bispecific CD8 Treg Modulator with the Potential to Restore CD8 Treg Functions in Patients with Rheumatological Autoimmune Diseases

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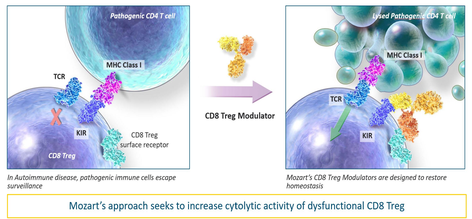
Background/Purpose: In healthy individuals, activation of a regulatory population of CD8 T cells (herein referred to as CD8 Treg) leads to selective elimination of aberrantly activated self-reactive CD4 T cells to maintain immune balance. The CD8 Treg network appears dysfunctional in autoimmune diseases and insufficient to kill self-reactive CD4 T cells, in part due to expression of inhibitory KIR2DL1/2/3 that serves as an autoimmune checkpoint.

We have developed MTX-101, a bispecific CD8 Treg modulator targeting CD8 and KIR2DL1/2/3, which are co-expressed on the surface of CD8 Treg. MTX-101 selectively binds CD8 Treg and enhances their killing of self-reactive CD4 T cells by blocking KIR2DL1/2/3 binding to its ligand.

Enhanced CD8 Treg function prevents self-reactive CD4 T cell expansion and inflammation, without increasing unwanted immune cell activation or pro-inflammatory cytokines.



In Autoimmune disease, pathogenic immune cells escape surveillance

Mozart's CD8 Treg Modulators are designed to restore homeostasis

Mozart's approach seeks to increase cytolytic activity of dysfunctional CD8 Treg

Methods: We used PBMCs derived from patients with Psoriasis (Ps), systemic lupus erythematosus (SLE), Sjögren's (SS), ankylosing spondylitis (ASp), and psoriatic arthritis (PsA) or Healthy donors (H) to examine the prevalence and cytolytic capacity of KIR2DL1/2/3 expressing CD8 T cells. Purified CD8 Treg were used to assess MTX-101 effects on CD8 Treg killing function in vitro. Humanized mice engrafted with CD34+ HSC were used to examine target binding and activation in vivo; PK parameters were assessed in Cynomolgus macaques.

CD8 Treg are present in autoimmune diseases and show defects in their cytotoxic function that can be reversed by their activation

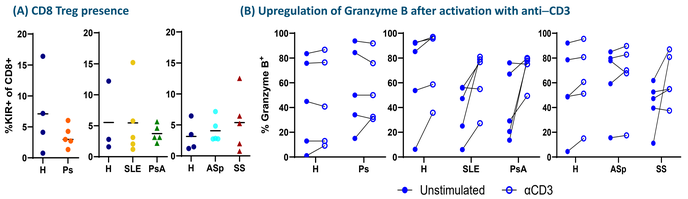


Figure 1: (A) CD8 Treg are present in the peripheral blood of healthy (H) and autoimmune donors (Psoriasis, SLE, Psoriatic Arthritis, Ankylosing Spondylitis and Sjogren's Syndrome). Shown is the proportion of CD8 Treg out of total CD8 T cell population. **(B)** Peripheral blood cells were rested overnight or activated with anti-CD3 (OKT3). Level of intracellular Granzyme B was then assessed.

No MTX-101-related observations in pilot toxicology study following repeated doses up to 50 mg/kg in CD34+ HSC engrafted NSG-Tg(IL-15) mice

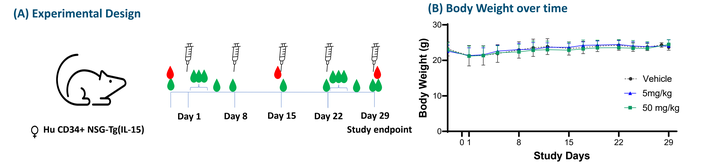


Figure 3: (A) Experimental design for multidose pilot toxicology study in NSG-Tg(IL-15) mice engrafted with CD34+ hematopoietic stem cells (HSCs) from two independent cord blood donors. At 12 weeks, mice with >25% hCD45, >3% hCD3 and >2% hCD56 were accepted for the study. Animals received IV doses on Days 1, 8, 15, 22, 29 of 0, 5 or 50 mg/kg of MTX-101.

Blood samples were collected for PK (n=4/dose/donor) and immunophenotyping (n=5/dose/donor) at specific time points following dose, and terminal blood and spleen were collected on Day 28.

- PK time points (🩸): predose and at 0.5, 2, 24, 96, 168 hr following dosing on Days 1 and 22; additional PK time point were collected on Day 15, 29 (0.5 hr post)
- Flow cytometry time points (🩸): predose on Day 0 and 15 and post dose on Day 29

(B) No difference in body weight over time was observed after dosing with MTX-101 vs. vehicle control

Evidence of MTX-101-related pharmacology in CD8 Treg observed following dosing in CD34+ HSC engrafted NSG-IL-15 mice

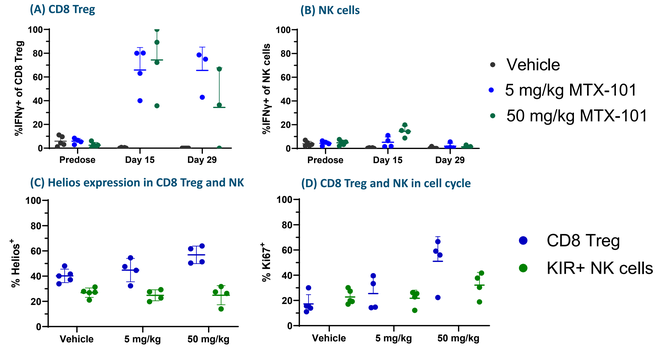


Figure 5: MTX-101 induced IFNγ production by **(A)** CD8 Treg and **(B)** NK cells as measured by flow cytometry for intracellular cytokine production. **(C)** MTX-101 also increased the proportion of Helios+ cells in the CD8 Treg population but not in NK cells. **(D)** MTX-101 induced proliferation of CD8 Treg but not NK cells. Experimental design is shown in Figure 3A.

MTX-101 modulates the function of CD8 Treg

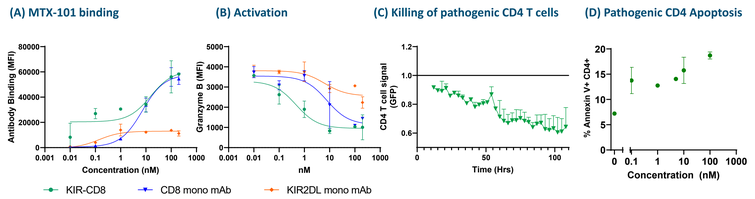


Figure 2: (A) PBMCs from a Celiac donor were thawed, rested and incubated with MTX-101 or each of the monospecific constituent molecules. MTX-101 binding to CD8 Treg was detected using an anti-human IgG1 Fc secondary antibody. **(B)** Granzyme B degranulation following intracellular staining in the same experiment. **(C)** CD8 Treg elimination of celiac-responsive CD4 T cells as detected by a decrease in GFP signal following addition of MTX-101, relative to control. **(D)** Elimination of gliadin-responsive Celiac donor CD4 T cells as detected by increase in Annexin V+ CD4 T cells at 48hrs following addition of MTX-101

MTX-101 selectively binds CD8 Treg in CD34+ HSC engrafted NSG-IL-15 mice

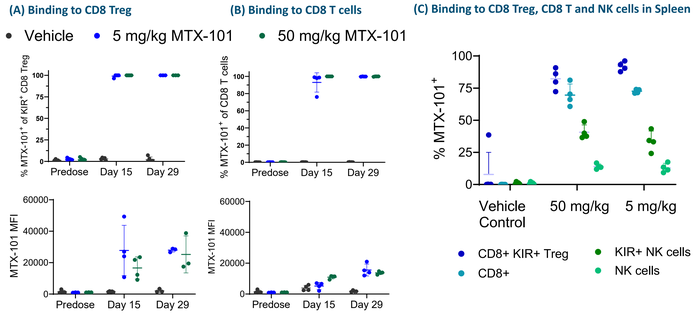
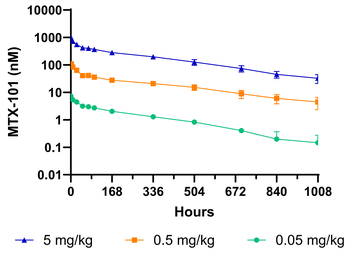


Figure 4: Binding of MTX-101 to **(A)** CD8 Treg and **(B)** CD8 T cells in the peripheral blood of mice CD34+ HSC engrafted NSG-IL15 mice predose on Day 15 (7 days after Day 8 dosing) and 30 min post dose on Day 29. **(C)** Binding of MTX101 to CD8 Treg, CD8 and NK cell populations from the spleen of CD34+ engrafted NSG-IL15 mice at terminal time point (Day 29, 30min post dose). Experimental design shown in **Figure 3A**.

MTX-101 shows linear relationship between dose and C_{max}/AUC in a single-dose Cyno PK study



	0.05mg/kg	0.5mg/kg	5mg/kg
C_{max} (µg/mL)	0.905	13.7	128
AUC_{0-t} (hr*µg/mL)	144	2318.4	21264
AUC_{0-inf} (hr*µg/mL)	153.84	2544	22704
$t_{1/2}$ (hr)	231.6	276	249.6
C_L (mL/hr/kg)	0.325	0.200	0.223
Vz (mL/kg)	108	77.6	80

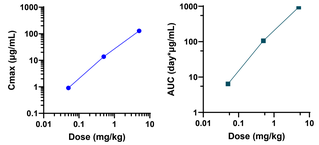


Figure 6: Cynomolgus monkeys were injected with a single dose of 5, 0.5 or 0.05mg/kg MTX-101. Blood, processed to serum, was taken at time points after dose, and construct concentration assessed by ECL assay (MSD-based method). PK parameters were calculated from concentration vs time data using Phoenix WinNonLin, analyzed using an IV bolus administration model.

Results:

- CD8 Treg are present in all patients and indications, regardless of disease status.
- CD8 Treg appear impaired in autoimmune disease, showing reduced responsiveness to stimulation and expression of CD8 Treg functional proteins relative to healthy donor CD8 Treg.
- MTX-101 selectively binds to and activates CD8 Treg in human PBMC and tissues.
- Consistent with previous data (Gardell et al, AAI 2023), MTX-101 increases cytolytic capacity, activation, and prevalence of healthy donor and autoimmune patient-derived CD8 Treg.
- MTX-101 shows selective CD8 Treg binding, without unwanted immune cell activation or body weight loss in tolerability studies up to the highest dose evaluated (50mg/kg) in a lymphoreplete, physiologically relevant humanized mouse model.
- Treatment with MTX-101 selectively increases the Helios content and proliferation of the CD8 Treg population, but not of NK cells, which may serve as clinical biomarkers.
- MTX-101 demonstrates a half-life of ~11 days following a single dose in NHP; consistent with observations in WT (Balb/c) and humanized mice (Pham et al, AAI, 2023).

Conclusions:

- **CD8 Treg are present and appear to be dysfunctional in the rheumatological indications tested**
- **MTX-101 disrupts inhibitory KIR, an autoimmune checkpoint, and can restore CD8 Treg function**
- **In non-clinical safety studies, MTX-101 was well-tolerated at all doses tested (up to 50 mg/kg)**
- **Our data support the therapeutic potential of MTX-101 for the treatment of autoimmune diseases including rheumatological indications with the objective to achieve durable re-balancing of the immune system**

Disclosures: All authors are employees of Mozart Therapeutics. Follow up questions can be directed to Kristine Swiderek, Chief Scientific Officer, Mozart Therapeutics (kswiderek@mozart-tx.com)