Pre-clinical Pharmacologic and Tolerability Characterization of MTX-101, a Novel KIRxCD8 Targeting Bispecific CD8 Treg Modulator

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MOZART THERAPEUTICS

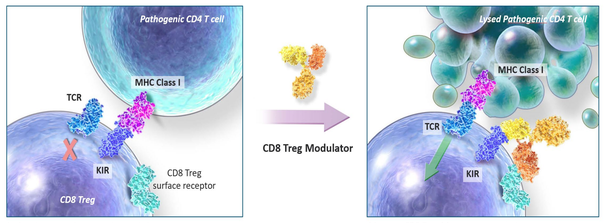
Orchestrating The Immune System

Introduction

In healthy individuals, CD8 Treg activation leads to selective elimination of self-reactive CD4 T cells. 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 KIR2DL(1/2/3). We have developed MTX-101, a bispecific CD8 Treg modulator targeting CD8 and KIR2DL(1/2/3) molecules. MTX-101 targets CD8 Treg, activating the cells, thereby reducing inflammation without increasing unwanted immune cell activation or pro-inflammatory cytokines. Our early data suggest that MTX-101-mediated enhancement of CD8 Treg function is a broadly applicable and a promising CD8 Treg specific therapeutic to restore immune balance for the treatment of autoimmune diseases, including gastrointestinal indications (eg., celiac disease, Crohn's and ulcerative colitis).

Previously, we evaluated the binding and specificity profiles of MTX-101 in vitro and in vivo. No activation of immune cells or increased proinflammatory cytokines were observed in vitro and improved outcomes were demonstrated in an acute inflammatory GVHD model. Here, we present data in humanized cord blood CD34+-engrafted NSG(human IL-15Tg) mice, where the pharmacokinetic profile of MTX-101 was consistent with antibody-like molecules. We evaluated binding, impact to immune cell phenotypes, pharmacology and early tolerability of MTX-101 in humanized mice and observed detectable binding on immune cells in peripheral blood and terminal tissues, with no resulting activation. Importantly, no induction of proinflammatory cytokines in the serum was observed following a single dose of MTX-101. Evaluation of PK/PD and biomarker readouts in this model will inform clinical dosing and development of MTX-101 to improve treatment outcomes in autoimmune disease patients.

MTX-101 is targeting a novel regulatory CD8 T cell network to restore immune balance in autoimmune disease



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

- MTX-101, a novel CD8 Treg Modulator, was tested in healthy humanized CD34+ NSG(IL15Tg) mice at approximately 17-18 weeks post engraftment with two independent human donor cells.
- The in vivo pharmacological impact of MTX-101 was evaluated using flow cytometry and human U-PLEX Meso Scale Discovery (MSD) assays.
- Quantitation of MTX-101 in humanized CD34+ NSG(IL15Tg) mice was performed using a sandwich ELISA using samples collected with a microsampling procedure. Concentrations achieved with microsamples yielded about 45% of value expected from serum collections from mice and allowed serial sampling of small volumes from all individual mice following dosing.

Single dose PK/tolerability study design

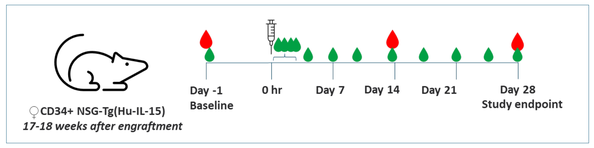


Figure 1 Female, human CD34+ cord blood cells from two independent donors were engrafted into NSG(human IL-15Tg) mice and screened for inclusion on study at 12 weeks. Mice with >25% hCD45, >3% hCD3 and >2% hCD56 were accepted for the study. After shipment and acclimation, animals received a single IV dose at 1 or 10 mg/kg of MTX-101 (~17-18 weeks post engraftment). Blood samples were collected from subsets of animals 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, 8, 24, 96, 168, 252, 336, 420, 504, 558 and 696 hrs post dose
- Flow cytometry time points (🩸): predose on day 0 and on Day 14 and 28

MTX-101 modulates function of CD8 Treg cells without activation of NK cells

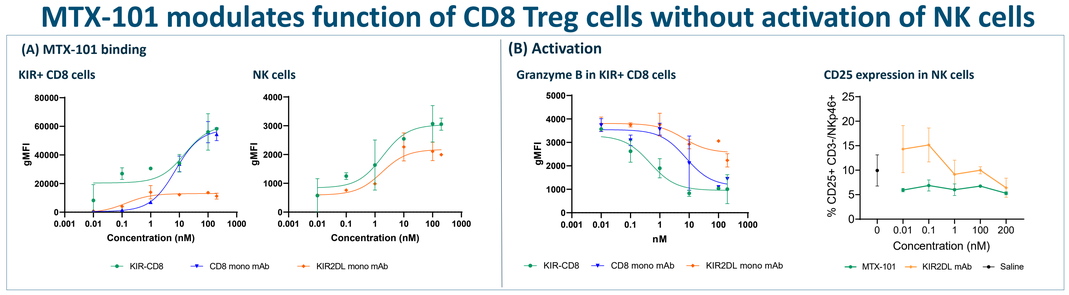


Figure 2: MTX-101 binds to KIR+CD8 T cells (CD8 Tregs) causing a reduction in intracellular Granzyme B compared to control monospecific mAb. MTX-101 binds to NK cells without causing an increase of activation as measured by upregulation of CD25 (bivalent anti-KIR antibody as control for activation). PBMCs from a Celiac donor were thawed, rested and incubated with MTX-101 or each of the monospecific control molecules. A nonblocking anti-KIR antibody was used to selectively gate on the KIR+ CD8s within the total PBMCs, while anti-NKp46 antibody was used to define NK cells. MTX-101 binding to different cell populations was detected using an anti-human IgG1 Fc secondary antibody **(A)**, anti-Granzyme B following intracellular staining **(B, left)** or surface staining with anti-CD25 antibody **(B, right)**. Healthy Donor PBMCs were used for evaluation of NK cell activation with bivalent anti-KIR2DL mAb as control, and data were generated with a research stage lot of candidate protein **(B, right)**.

Frequency of human immune cells in peripheral blood remains steady after single dose of MTX-101 in huCD34+ NSG(IL15Tg) mice

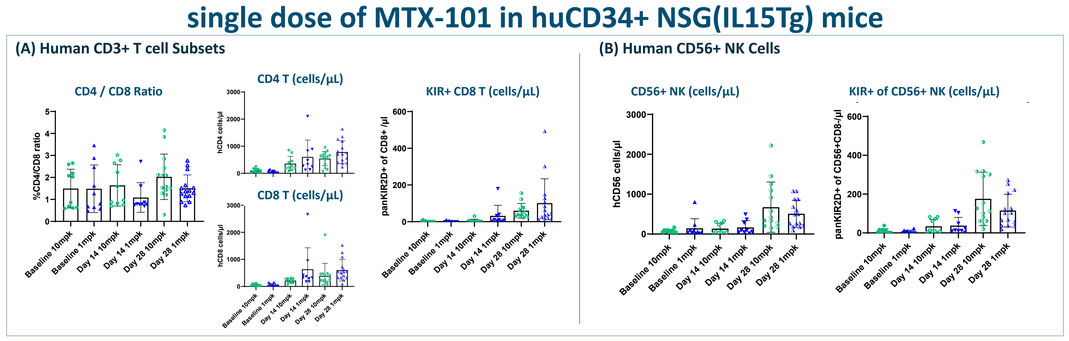


Figure 3 Frequency of Immune cell subsets, including CD4+ or CD8+ T-cell subsets **(A)** and NK cells **(B)**, were assessed by flow cytometry at baseline, Day 14 and Day 28 in peripheral blood following IV administration of MTX-101. Enrolled study mice at baseline had a mean of 62% hCD45, 5% CD3, and 7% CD56. An increase in the number of CD8 Treg cells in peripheral blood was observed after a single dose of MTX-101 without impact to the CD4/CD8 ratio. An increase KIR2D+ NK cells was also observed at late time points relative to baseline levels. Notable changes also occurred in total CD4 and CD8 T cells, which may be indicative of a still evolving immune system in these mice following engraftment (Abeynaike, et al). Additional studies comparing MTX-101 to untreated controls are ongoing.

Sustained binding to peripheral blood cells expressing both targets of MTX-101

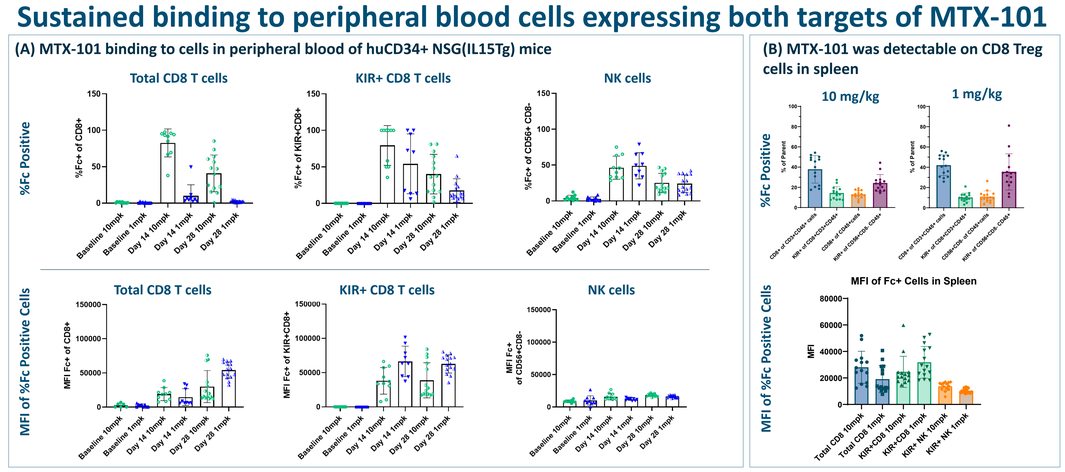


Figure 4 Binding of MTX-101 to total CD8, CD8 Treg, NK, and panKIR2D+NK cells via Fc detection were found on cells in blood and spleen. **(A)** Sustained binding was observed to cells expressing both targets in peripheral blood. **(B)** Summary of prevalence and MFI of binding of MTX-101 for total CD8 T cells, KIR+CD8, and NK cells in spleen at terminal timepoint. Data are presented for individuals with mean bars ± SD. As expected, no binding was observed to CD4 T cells either in blood or spleen.

Pharmacologic impact following a single dose of MTX-101 in huCD34+ NSG(IL-15Tg) mice

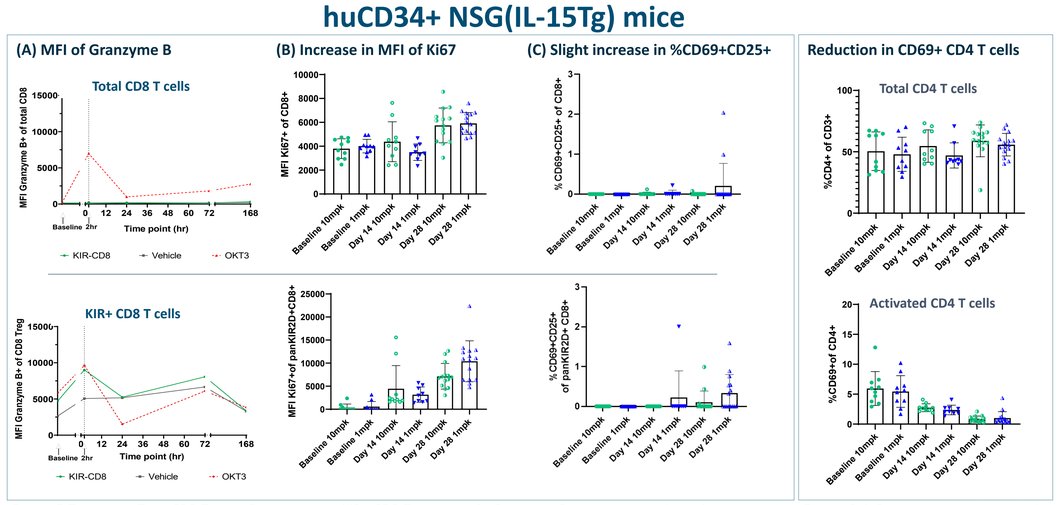


Figure 5 Top panel, Total CD8 T cells; Bottom panel, KIR+ CD8 Treg cells. **(A)** Granzyme B was evaluated in an independent study (n=3/dose/donor) with a research stage lot of candidate protein. A transient increase of median fluorescent intensity (MFI) Granzyme B was detected the 2-hr timepoint post-dose in CD8 Treg cells following CD8 Treg modulator treatment. **(B-C)** In MTX-101 treated mice, a slight increase in Ki67 MFI and CD69+CD25+ Treg cells were observed over the course of the study (relative to total CD8 population).

Figure 6 A Decrease was observed in %CD69+ CD4 cells after a single dose of MTX-101, while no change in total CD4+ T cells was detected (Data presented as median).

No increase of pro-inflammatory serum cytokines with MTX-101 in huCD34+ NSG(IL15Tg) mice

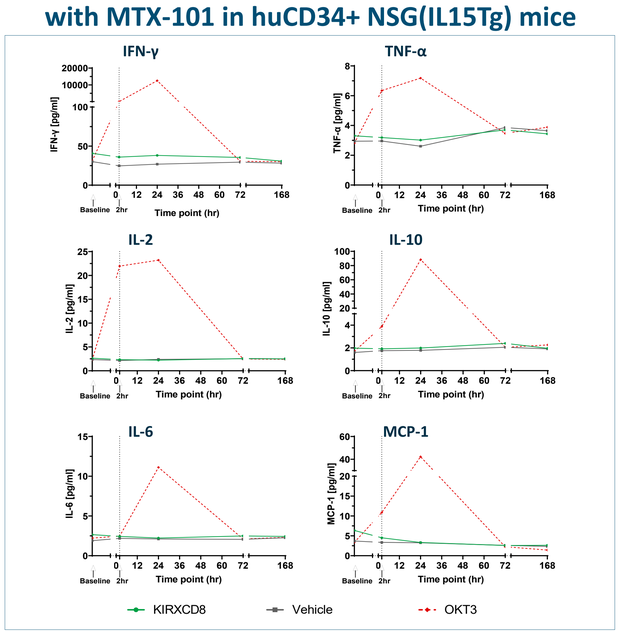
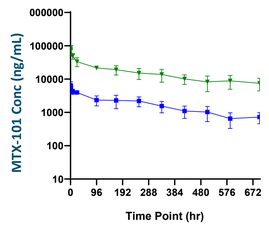


Figure 7 A single dose of CD8 Treg Modulator at 5 mg/kg was evaluated in an independent small study with a research stage lot of candidate protein to evaluate cytokine release and utility of the model (n=3/donor, two donors). OKT3 was used as a positive control at 0.5 mg/kg. MTX-101 did not increase the expression of pro-inflammatory serum cytokines. Data are presented as median.

Pharmacokinetic profile of MTX-101 in huCD34+ NSG(IL15Tg) mice

Single Dose Concentration vs. Time Profile of MTX-101



Parameter	Units	10 mg/kg	1 mg/kg
Half-life $t_{1/2}$	day	11.6	11.5
Clearance (Cl)	mL/hr/kg	1.26	0.738
Volume of distribution (Vz)	mL/kg	508	288

Figure 8 Single dose of 10 or 1 mg/kg MTX101 was detectable in blood (serial microsample collection) of humanized CD34+ NSG(IL15Tg) through 696-hr and was consistent between donors. Data are presented as mean +/- SD of individuals from one donor (n=4 / dose level) shown in graph, while only mice with AUC%Extrap <20% were used to calculate PK parameters shown in table (n=4, 1mg/kg; n=1, 10 mg/kg)

Conclusions

MTX-101 is a promising therapeutic candidate for the treatment of autoimmune disease. Data support safe administration of MTX-101 and use of the humanized mouse model for safety assessment and understanding of a Pharmacokinetics/ Pharmacodynamics (PK/PD) relationship.

- MTX-101 was well-tolerated following a single dose in humanized mice and did not increase activation of NK cells, CD4 T cells or CD8 T cells or detectable pro-inflammatory serum cytokines.
- Following a single dose of MTX-101, an increase in CD8 Treg activation and proliferation at later time points was observed.
- Preliminary data suggest that MTX-101, may selectively increase expression of Granzyme B in CD8 Treg cells at early time points, suggesting an impact on their cytolytic capacity.
- Following a single dose of MTX-101, a decrease of activated CD4 T cells was observed at late timepoints in support of the postulated MoA of MTX-101.
- MTX-101 binding to CD8 Treg cells, Total CD8 T cells and KIR+NK cells was measurable in peripheral blood and spleen of humanized mice with sustained binding to cells expressing both targets.
- Concentration of MTX-101 indicated high exposure in humanized mice with a $T_{1/2}$ of about 11.5 days and antibody like PK parameters.

Contact:
Follow up questions can be directed to Kristine Swiderek, Chief Scientific Officer, Mozart Therapeutics
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References:
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