Nonclinical Assessment of Toxicity and Pharmacology of MTX-101, a Novel KIRxCD8 Targeting Bispecific CD8 Treg Modulator, in Humanized Mouse

MOZART THERAPEUTICS
Orchestrating The Immune System

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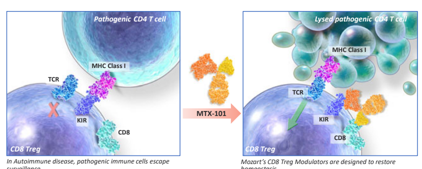
Background

Using humanized mice as a relevant toxicology species has the potential to reduce the number of toxicology studies for IND-enabling assessments that will inform safety and Pharmacokinetic (PK)/ Pharmacodynamic (PD) assessments to support dosing in humans in clinical trials. The humanized mouse model can also be valuable for testing targets with insufficient cross-reactivity to other species and non-human primates (NHP).

MTX-101 is an antibody-based bispecific CD8 regulatory T cell (CD8 Treg) modulator in development for the treatment of autoimmune disease with limited cross-reactivity to targets in animal models. It selectively targets an autoimmune checkpoint, inhibitory KIR2DL1/2/3 (KIR), and CD8 that are co-expressed on the surface of CD8 Treg cells. MTX-101 enhances the CD8 Treg mediated killing of pathogenic CD4 T cells to prevent inflammation in disease, without broad immunosuppression or increase of unwanted immune cell activation or pro-inflammatory cytokines.

Here we demonstrate the use of humanized CD34+ cord blood-engrafted NOD.Cg-Prkdc IL2rg Tg(IL15) /SzJ (CD34+ NSG-Tg(Hu-IL15)) mice as a model and viable toxicology species for the nonclinical safety assessment of MTX-101. The CD34+ NSG-Tg(Hu-IL15) mouse model has physiological levels of human IL-15 and supports long-term engraftment of human CD45+ immune cells, including NK cells and KIR expressing CD8 Treg. Compared to other humanized models, acute macrophage activation is not observed in CD34+ NSG-Tg(Hu-IL-15) mice, making this model ideal for long-term toxicology studies.

MTX-101 is Targeting a Novel Network to Restore Immune Balance in Autoimmune Disease



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

Methods

- MTX-101 was tested in healthy female CD34+ NSG-Tg(Hu-IL15) mice, with animal care and use conducted in accordance with applicable regulations and guidelines (Aryee, et al. and Abeynaike, et al).
- PK profiles were assessed following single doses (up to 10 mg/kg MTX-101) in female BALB/cJ and CD34+ NSG-Tg(Hu-IL15) mice.
- For assessment of toxicity, immunotoxicity, and PK/PD parameters, repeat doses (weekly, n= 5 doses) of MTX-101 (5 or 50 mg/kg) or vehicle were administered intravenously (IV) via the tail vein to CD34+ NSG-Tg(Hu-IL15) mice over 4 weeks at 5 mL/kg.
- Immunotoxicity analyses included flow cytometry and multiplex V-plex Mesoscale Discovery (MSD) based cytokine assays.
- For PK analyses, quantitation of MTX-101 in BALB/cJ and CD34+ NSG-Tg(Hu-IL15) mice was performed using a sandwich ELISA.
- Quantitation of MTX-101 in BALB/cJ was performed with serum samples, while CD34+ NSG-Tg(Hu-IL15) mice underwent a microsampling procedure, yielding about 45% of the value expected from serum collections from mice and allowing serial sampling of small volumes from all individual mice following dosing.

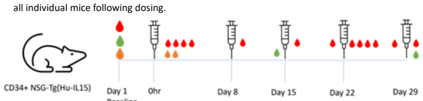


Figure 1 In the repeat dose study, human CD34+ cord blood cells from two independent donors were engrafted into female NSG-Tg(Hu-IL15) mice and screened for inclusion on study at 12 weeks. Mice with >25% hCD45, >3% hCD3 and >2% hCD56 were accepted for the study. After shipment and acclimation, animals received weekly IV dose at 5 or 50 mg/kg of MTX-101 or vehicle (~17-18 weeks post engraftment). Blood samples were collected from subsets of animals for PK (n=3/dose/donor), serum cytokine analysis (n=3/dose/donor) and immunophenotyping (n=5/dose of one donor) at specific time points following dose, and terminal blood and spleen were collected on Day 29. Toxicity assessment was evaluated at terminal timepoints (n=6/dose/donor).

- PK time points (red drop): to evaluate exposure, microsamples were collected pre-dose and following dosing on Day 1 and 22 at 0.5, 2, 24, 96, 168 hours; time points were also collected post-dose on Day 8, 15 and 29
- Flow cytometry time points (green drop): pre-dose on Day 1 and Day 15 and post-dose on Day 29
- Serum cytokine time points (orange drop): pre-dose on Day 1 and at 8 and 24 hr post-dose

MTX-101 binding to CD8 Treg and NK cells without releasing cytokines

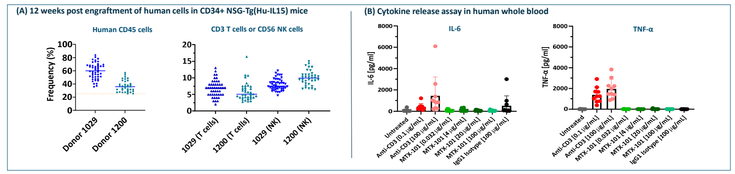


Figure 2A depicts the percentages of hCD45, CD3 T, and CD56 NK cells at 12 weeks post engraftment in the mice enrolled for the study. CD34+ cells from donors 1029 and 1200 were engrafted into NSG-Tg(Hu-IL-15) mice aged 4 weeks, followed by a 12-week post engraftment assessment for hCD45+, CD19+, CD3+, CD33+, and CD56+ at the Jackson Laboratory. Mice exhibiting >25% hCD45, >3% hCD3, and >2% hCD56 were subsequently shipped to the testing facility. See Figure 1 for study design.

Figure 2B The objective of the study was to evaluate the potential of MTX-101 to trigger cytokine release in primary human peripheral blood mononuclear cells (PBMCs) derived from ten healthy human donors in the soluble and wet-coated presentation formats. A range of MTX-101 concentrations (0.032 µg/mL, 0.16 µg/mL, 0.8 µg/mL, 4 µg/mL, 20 µg/mL, and 100 µg/mL) was evaluated in the assays. Anti-CD3 antibody (OKT3 clone; positive control) treatment, human IgG1 antibody (negative control) treatment, and a no treatment control were also included in the assay. All treatments were evaluated in triplicate in both the soluble and wet-coated plate formats. Tissue culture supernatants from treated PBMC samples were collected after 24 hours of treatment. The levels of IL-2, IL-6, IL-8, IL-10, TNF-α, and IFN-γ were measured using MSD platform. All donors were responsive to positive control anti-CD3, demonstrating that these donors have the capacity to release cytokines in response to an immunomodulatory stimulus. In both treatment formats, there was no dose-responsive cytokine release (IL-2, IL-6, IL-8, IL-10, TNF-α, and IFN-γ) stimulated by MTX-101 above the level of isotype or untreated controls, for all donors.

After multiple doses of MTX-101, the presence of human immune cells in peripheral blood of CD34+ NSG-Tg(Hu-IL-15) mice remained consistent with vehicle group

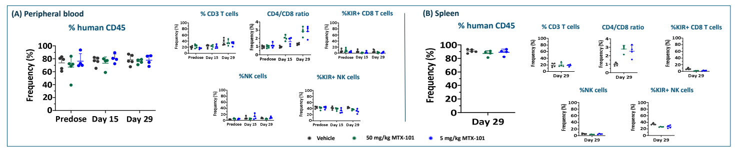


Figure 3 Frequency of human immune cell subsets were assessed at baseline, Day 15, and Day 29 in peripheral blood (**A**) and spleen (**B**) following weekly IV administration of vehicle or MTX-101 at 5 or 50 mg/kg (see Figure 1 for study design). Over the course of the study the percentage of total hCD45, CD3, CD4, CD8, NK (CD56+CD8-), KIR+ CD8+ and KIR+ NK cells in blood (**A**) and spleen (**B**) remained similar between 0, 5 and 50 mg/kg MTX-101. Data are presented for individuals with mean bars ± SD.

Binding of MTX-101 to peripheral blood cells co-expressing the targets

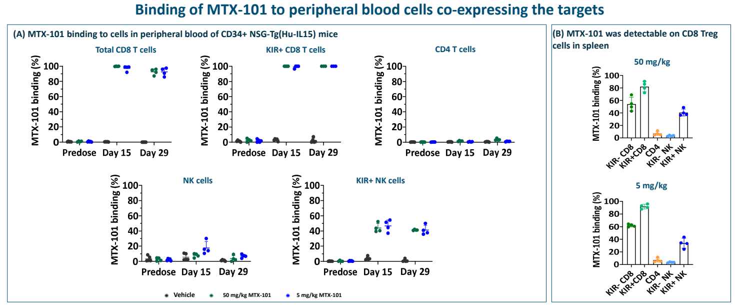


Figure 4 Binding of MTX-101 to KIR+ and KIR- of CD8 and NK cells via Fc detection using an anti-human IgG1 Fc secondary antibody were found on cells in blood (**A**) and spleen (**B**). Sustained binding was observed to cells expressing both targets in peripheral blood (**A**) . Summary of frequency of binding of MTX-101 for to KIR+ and KIR- of CD8 and NK cells in spleen at terminal timepoint (**B**). As expected, no binding was observed to CD4 T cells either in blood or spleen (**B**). Data are presented for individuals with mean bars ± SD.

Pharmacologic impact following single or multiple doses of MTX-101 in CD34+ NSG-Tg(Hu-IL-15) mice demonstrates selective & functional CD8 Treg engagement

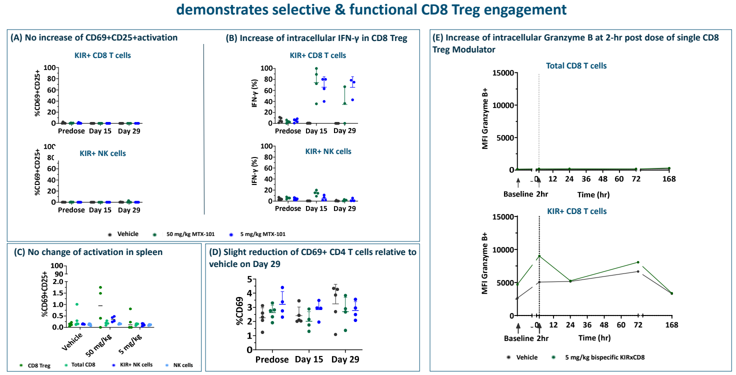


Figure 5 Detection of CD69+CD25+ activation was assessed in peripheral blood (**A**) and spleen (**C**). No increase of CD69+CD25+ in total CD8, KIR+ CD8+, CD4+, NK, and KIR+ NK cells was detectable after multiple doses of MTX-101 (**A and C**).
An increase of intracellular IFN-γ in CD8 Treg cells in the blood was measurable on Day 15 and Day 29 in animals treated with MTX-101. Additionally, a slight uptake of IFN-γ was noted in KIR+ NK cells on Day 15 for both treatment groups but level returned to baseline on Day 29 (**B**). At Day 29, a reduction of CD69+ in total CD4 T cells was observed (**D**).

Figure 5E The top panel represents Total CD8 T cells, while the bottom panel represents KIR+ CD8 Treg cells. Granzyme B levels were assessed in an independent study (n=3/dose/donor) using a preliminary research stage lot of KIRxCD8 bispecific candidate protein. Mice were administered a single dose of bispecific KIRxCD8 antibody or vehicle. A transient increase in median fluorescent intensity (MFI) of Granzyme B in peripheral blood was observed at the 2-hour time point post-dose in CD8 Treg cells following bispecific KIRxCD8 antibody treatment.

No increase of pro-inflammatory cytokines with MTX-101 in CD34+ NSG-Tg(Hu-IL-15) mice at doses tested

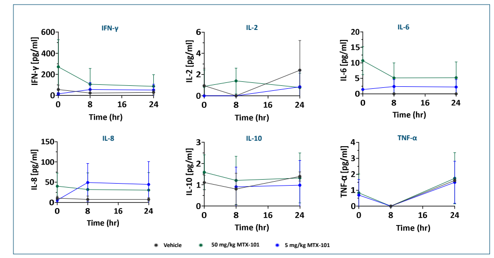
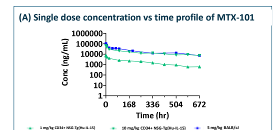


Figure 6 MTX-101 did not increase the expression of pro-inflammatory serum cytokines after single dose of 5 or 50 mg/kg MTX-101 at 8 and 24 hr. Data are presented as mean bars ± SD.

Pharmacokinetic profile and body weight of CD34+ NSG-Tg(Hu-IL-15) mice treated with MTX-101

(A) Single dose concentration vs time profile of MTX-101



Parameter	Units	CD34+ NSG-Tg(Hu-IL-15)		BALB/cJ
Dose	mg/kg	10	1	5
Sample type	n/a	Microsample		Serum
Tmax	hr	0.5	0.8	0.5
Cmax	µg/mL	75	7.68	106
AUCinf	hr*µg/mL	7824	1431.8	15500
t1/2	day	11.1	10.5	11.1
CL	mL/hr/kg	1.275	0.763	0.323
Vz	mL/kg	490	264	124
Number evaluated per Study Group/ dose level		1	5	9

(B) Body weight of MTX-101-treated mice

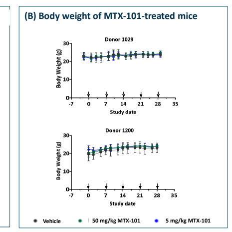


Figure 7A Following a single dose, MTX-101 was detectable through 672-hr of CD34+ NSG-Tg(Hu-IL15) at 10 or 1 mg/kg (serial microsample) and in BALB/cJ mice at 5 mg/kg (serum) and was consistent between donors and strains. Blood collection for serum in BALB/cJ mice was staggered as n=3 per timepoint. Data are presented as mean of individuals at individual time points shown in graph, while only mice with AUC%Extrap <20% were used to calculate PK parameters shown in table (CD34+ NSG-Tg(Hu-IL-15Tg): n=4, 1mg/kg; n=1, 10 mg/kg; BALB/cJ: n=9).
Figure 7B CD34+ NSG-Tg(Hu-IL-15) mice treated weekly with 0, 5, and 50 mg/kg MTX-101 were monitored for their body weight changes. No differences in the body weight were observed among the treatment groups or donors 1029 and 1200.

Conclusions

MTX-101 is a promising therapeutic candidate for the treatment of autoimmune disease. Data support the use of the humanized mouse model for safety assessment and understanding of a PK/PD relationship, and to inform clinical development.

- MTX-101 was well-tolerated following single or multiple doses in CD34+ NSG-Tg(Hu-IL-15) mice with no impact to body weight, in-life observations or terminal toxicity assessments.
- MTX-101 binding to CD8 Treg, Total CD8 T cells and KIR+NK cells was measurable in peripheral blood and spleen of CD34+ NSG-Tg(Hu-IL15) mice.
- MTX-101 did not increase activation of NK cells, CD4 T cells or CD8 T cells or cause an increase in detectable pro-inflammatory serum cytokines.
- MTX-101 may selectively increase expression of Granzyme B in CD8 Treg cells at early time points, suggesting an impact to their cytolytic capacity.
- A decrease of activated CD4 T cells was observed at late timepoints, supporting the postulated mechanism of action of MTX-101.
- Concentration of MTX-101 indicated high exposure and PK parameters are consistent with antibodies with a $T_{1/2}$ of about 11.5 days following a single dose.
- Data derived from the CD34+ NSG-Tg(Hu-IL-15) mouse model align with in vitro and in vivo findings for MTX-101, highlighting the utility of this model for non-clinical safety assessment.
- Our data underline the use of the CD34+ NSG-Tg(Hu-IL-15) mouse to assess non-clinical safety of development candidates targeting human immune system receptors with limited or restricted cross-reactivity in conventional toxicology species.

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References:

Li, et al. KIR $^+$ CD8 $^+$ T cells suppress pathogenic T cells and are active in autoimmune diseases and COVID-19. *Science.* 2022 DOI: 10.1126/science.abi9591
Aryee, et al. Enhanced development of functional human NK cells in NOD-scid-IL2rg^{null} mice expressing human IL15. FASEB, 2022. https://doi.org/10.1096/fj.202200045R
Abeynaike, et al. Human Hematopoietic Stem Cell Engrafted IL-15 Transgenic NSG Mice Support Robust NK Cell Responses and Sustained HIV-1 Infection. Viruses 2023. https://doi.org/10.3390/v15020365