PRECLINICAL TESTING OF A BISPECIFIC ANTIBODY TARGETING INHIBITORY KIR AND ICOS DESIGNED FOR THE TREATMENT OF INFLAMMATORY BOWEL DISEASE PATIENTS

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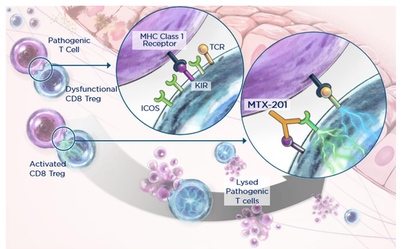
MOZART THERAPEUTICS

Orchestrating The Immune System

INTRODUCTION

Regulatory CD8 T cells (CD8 Treg) are responsible for the selective killing of self-reactive and pathogenic T cells to maintain peripheral immune tolerance. In autoimmune disease, CD8 Treg fail to control the expansion of pathogenic T cells that subsequently cause tissue destruction. This CD8 Treg dysfunction is due in part to the expression of inhibitory KIR receptors (KIR2DL1/2/3), which serve as autoimmune checkpoints and insufficient ICOS (inducible T cell co-stimulator) signaling, which is a primary costimulatory receptor for CD8 Treg. In inflammatory bowel disease (IBD) patient peripheral blood mononuclear cells (PBMC), the expression of ICOS on CD8 Treg is increased relative to that in healthy controls, with downstream dysfunctions of their cytolytic capacity and signaling pathways that promote CD8 T cell longevity and memory. Here we present pre-clinical characterization of the CD8 Treg modulator MTX-201, a bispecific antibody that binds to inhibitory KIR and ICOS that are co-expressed by CD8 Treg. MTX-201 binding antagonizes inhibitory KIR signaling and promotes ICOS co-stimulation, resulting in restored CD8 Treg activation and function in inflamed tissues.

MTX-201 Targets CD8 Treg to Restore Immune Balance in Autoimmune Disease



MTX-201 ***inhibits KIR and agonizes ICOS*** to selectively potentiate CD8 Treg functionality and ameliorate inflammation in highly inflamed autoimmune disease

METHODS

- The functional impact of MTX-201 was examined in highly inflammatory acute GvHD mouse model.
- The phenotype of CD8 Treg from IBD donors was examined by flow cytometry.
- Binding and impact of MTX-201 to IBD donor PBMC was evaluated by RNA Seq, flow cytometry, and supernatant cytokine analysis.
- MTX-201 was tested in IBD patient intestinal tissue biopsy organoids.
- MTX-201 impact on response to common viruses and bacterial antigens was examined in vitro.
- Tolerability and pharmacokinetic studies for MTX-201 were performed with NSG-Tg(Hu-IL15) mice engrafted with human CD34⁺ cord blood cells.

MTX-201 selectively binds and increases CD8 Treg in mouse model of acute GvHD leading to increased survival and decreased clinical disease score

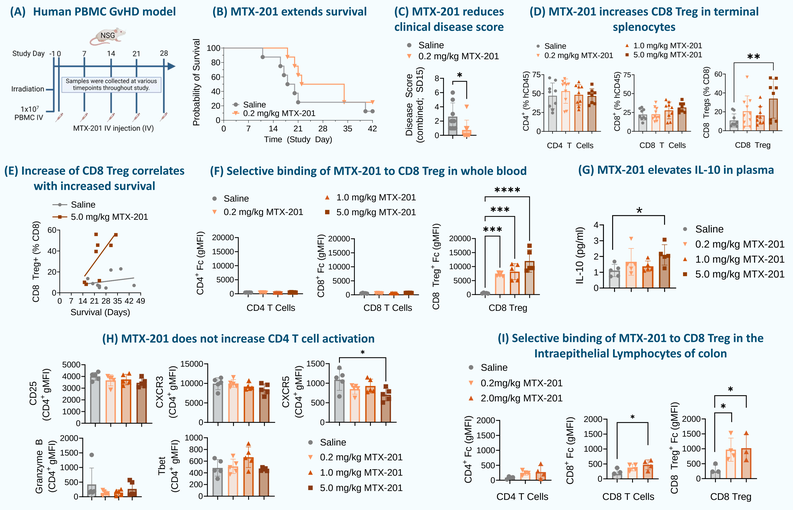
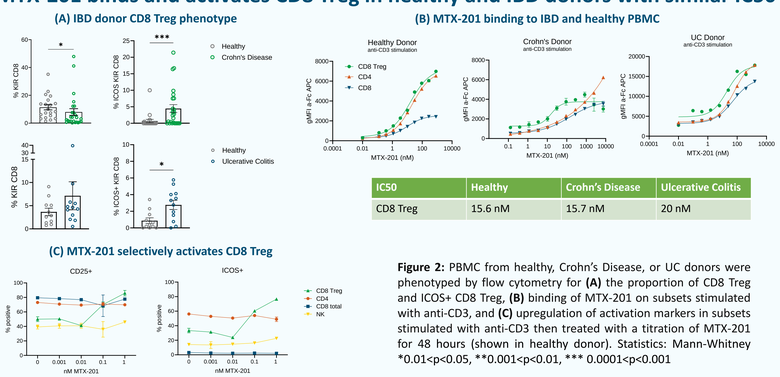


Figure 1: (A) Model overview of study design. **(B)** Survival Curve (Statistics out to SD33 p= 0.0895: Gehan-Breslow-Wilcoxon). **(C)** SD15 combined disease score based on weight, paleness, skin, fur, activity, and posture (Statistics: Unpaired T-test). **(D, F, H-I)** Assessed using flow cytometry. **(D)** Terminal Splenocytes (variable study day) T cell population frequencies. **(E)** The frequency of CD8 Treg vs survival. r^2=0.47 for MTX-201 treated and 0.059 for saline. **(F)** Bound MTX-201 at SD14 (7 days post dose) in peripheral blood shown as the geometric mean (gMFI) of bound human anti-IgG-Fc on T cells populations. **(G)** MSD analysis of SD14 (7 days post dose) plasma for IL-10. **(H)** The gMFI of CD25, CXCR3, CXCR5, Granzyme B, T-bet on CD4 T cells at SD14. **(I)** Bound MTX-201 at SD16 (2 days post dose) in the Intraepithelial Lymphocytes (IEL) of the colon represented by the gMFI of bound human anti-IgG-Fc on T cell populations on SD14. Statistics: One-Way ANOVA with multiple comparison analysis * 0.01<p<0.05 , ** 0.001<p<0.01, *** 0.0001<p<0.001, **** p<0.0001

MTX-201 binds and activates CD8 Treg in healthy and IBD donors with similar IC50



IC50	Healthy	Crohn's Disease	Ulcerative Colitis
CD8 Treg	15.6 nM	15.7 nM	20 nM

Figure 2: PBMC from healthy, Crohn's Disease, or UC donors were phenotyped by flow cytometry for **(A)** the proportion of CD8 Treg and ICOS+ CD8 Treg, **(B)** binding of MTX-201 on subsets stimulated with anti-CD3, and **(C)** upregulation of activation markers in subsets stimulated with anti-CD3 then treated with a titration of MTX-201 for 48 hours (shown in healthy donor). Statistics: Mann-Whitney *0.01<p<0.05, **0.001<p<0.01, *** 0.0001<p<0.001

MTX-201 results in a reduction in IBD antigen-activated CD4 T cells in vitro

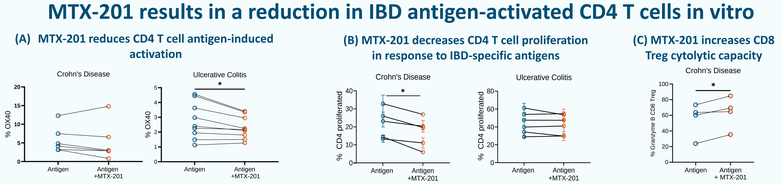


Figure 3: (A) MTX-201 effects on antigen specific CD4 activation in IBD donor PBMC stimulated for 48 hours with $OmpC_{321-340}$ and flagellin. **(B)** MTX-201 decreased proliferation of antigen-specific CD4 cells in a 7-day assay of IBD PBMC stimulated with antigen, IL7, and IL15. **(C)** IBD PBMC were expanded for 12 days with antigen, IL2, and IL15 before restimulation with autologous APC pulsed with antigen. Statistics: Paired T-test *0.01<p<0.05, **0.001<p<0.01, *** 0.0001<p<0.001

MTX-201 reduces epithelial cell death in Crohn's tissue-derived air-liquid-interface (ALI) cultures

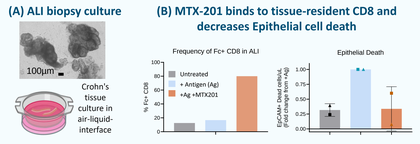


Figure 4: (A) ALI Crohn's intestinal tissue cultures were established from colon biopsies from two separate Crohn's donors and grown for 8-9 days. To model a flare, tissues were given an antigen stimulation (flagellin+$OmpC_{321-340}$) with or without MTX-201 treatment. **(B)** MTX-201 binds to tissue resident CD8 T cells in ALI cultures, detected by anti-Fc. Antigen stimulation increases epithelial cell death and MTX-201 reduces antigen-induced cell death in two separate Crohn's donors (squares or triangles) .

MTX-201 treatment of autologous PBMC-engrafted Crohn's donor organoids improves epithelial condition

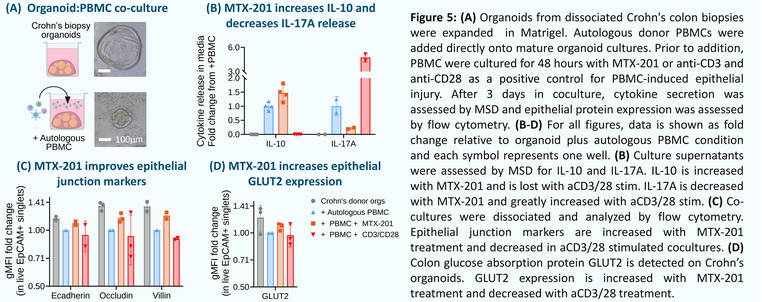


Figure 5: (A) Organoids from dissociated Crohn's colon biopsies were expanded in Matrigel. Autologous donor PBMCs were added directly onto mature organoid cultures. Prior to addition, PBMC were cultured for 48 hours with MTX-201 or anti-CD3 and anti-CD28 as a positive control for PBMC-induced epithelial injury. After 3 days in coculture, cytokine secretion was assessed by MSD and epithelial protein expression was assessed by flow cytometry. **(B-D)** For all figures, data is shown as fold change relative to organoid plus autologous PBMC condition and each symbol represents one well. **(B)** Culture supernatants were assessed by MSD for IL-10 and IL-17A. IL-10 is increased with MTX-201 and is lost with aCD3/28 stim. IL-17A is decreased with MTX-201 and greatly increased with aCD3/28 stim. **(C)** Co-cultures were dissociated and analyzed by flow cytometry. Epithelial junction markers are increased with MTX-201 treatment and decreased in aCD3/28 stimulated cocultures. **(D)** Colon glucose absorption protein GLUT2 is detected on Crohn's organoids. GLUT2 expression is increased with MTX-201 treatment and decreased with aCD3/28 treatment.

MTX-201 does not cause broad immunosuppression

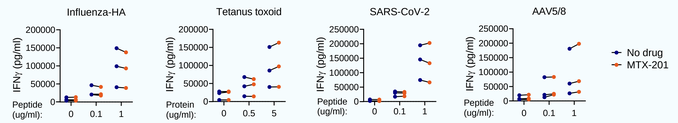
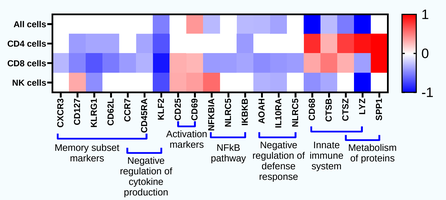


Figure 6: Immune responses to viral and microbial antigens were maintained in the presence of MTX-201. Graphs show IFN-γ levels for three donors and each point represents the mean of two technical replicates. Values with and without MTX-201 were compared and no significance was found by paired t-test.

Single-cell RNA sequencing of Crohn's PBMCs treated with MTX-201 shows different effects across cell subsets

Figure 7: PBMC isolated from Crohn's patients were activated with anti-CD3 for 24 hours and then rested for 24 hours in media. Experimental samples were treated with MTX-201 for 48 hours, while control samples were left untreated for that duration. Sample libraries were created with the 10X Genomics Chromium Fixed RNA Profiling protocol. Sequencing was performed on the NovaSeq X Plus. PBMC subsets were defined by celldex MonacoImmuneData markers. Differentially expressed genes of interest between experimental and control groups are displayed and grouped by relevant function.



MTX-201 does not induce toxicity or proinflammatory cytokines with repeat dosing in CD34⁺ NSG-Tg(Hu-IL15) mice

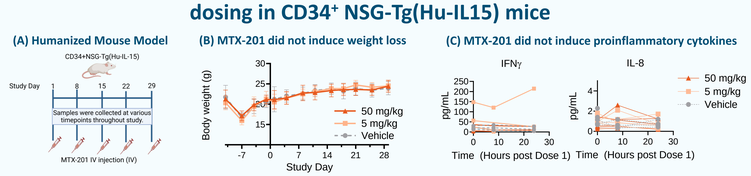


Figure 8: (A) Humanized female NSG-IL-15 transgenic mice were given weekly IV doses of MTX-201. Mice were dosed at 5mg/kg or 50 mg/kg a total of five times over the 29-day study. Blood samples were collected after dose 1 and dose 4 for PK time points (Fig 9). Serum was collected for cytokine analysis pre-dose on Day 1 and at 8hrs and 24hrs post-dose. **(B)** The body weight of animals was monitored over time for the duration of the study. **(C)** The MSD U-plex assay platform was used to detect 10 serum cytokines. IFNγ and IL-8 showed minimal change in cytokine levels. All other cytokines tested were undetectable in the serum.

MTX-201 has antibody-like pharmacokinetic parameters in humanized mice

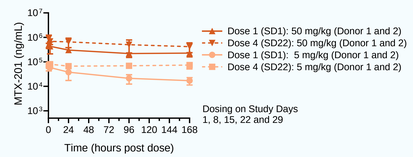


Figure 9: NSG-IL-15 transgenic mice (CD34+ NSG-Tg(Hu-IL15), Fig 8A) microsample data are presented as a mean of individuals (n=3 mice per Human PBMC Donor; two donors) at two dose levels (5 and 50 mg/kg). The graph depicts the MTX-201 concentration levels for the seven days post-dose on Day 1 and Day 22.

RESULTS

- MTX-201 selectively binds CD8 Treg in a mouse model of acute GvHD with an associated extension of survival and decrease in clinical disease score.
- MTX-201 binds and activates CD8 Treg in PBMC without inducing activation of single target expressing cells.
- MTX-201 decreases IBD antigen-activated CD4 T cells in vitro and reduces epithelial death and improves expression of functional epithelial markers in organoid models of IBD.
- Immune responses to common viral and microbial pathogens are retained in the presence of MTX-201.
- MTX-201 has antibody-like PK parameters and did not induce toxicity or proinflammatory cytokines with repeat dosing in an immunocompetent mouse model.

CONCLUSION

MTX-201 targets CD8 Treg to selectively eliminate pathogenic CD4 T cells, decrease inflammation, and reduce subsequent epithelial cell damage in IBD. MTX-201 may represent a differentiated and selective therapeutic approach to restore durable immune balance in patients with inflammatory bowel disease.

Acknowledgements: The authors would like to acknowledge the contributions of Dr. James Lord (Benaroya Research Institute; Seattle, WA) and patients for intestinal biopsies.

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Disclosures: All authors are employees of Mozart Therapeutics.

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