MOZART THERAPEUTICS

Orchestrating The Immune System

Evaluation of CD8 Treg Prevalence and MTX-101-Mediated Activation of Peripheral Blood Mononuclear Cells from Newly-Diagnosed Pediatric and Adult T1D Patients

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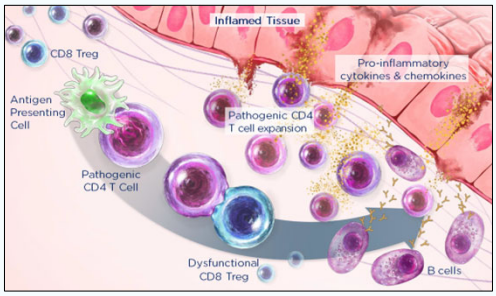
BACKGROUND/AIMS:

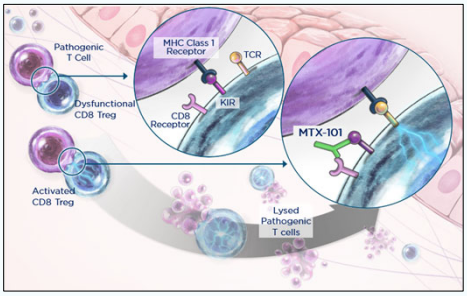
- Regulatory CD8 T cells (CD8 Treg) selectively kill pathogenic T cells to maintain peripheral immune tolerance without impairing anti-viral or anti-bacterial immunity
- CD8 Treg are dysfunctional in patients with autoimmunity, in part due to expression of inhibitory KIR receptors (KIR2DL1/2/3), which serve as autoimmune checkpoints
- We have shown that peripheral blood mononuclear cells (PBMC) isolated from adults with T1D have increased expression of KIR on CD8 Treg relative to healthy donors
- T1D patient CD8 Treg have downstream dysfunctions of their cytolytic capacity and signaling pathways that promote CD8 T cell expansion
- In T1D patients that respond to immunotherapies, KIR+ CD8 Treg prevalence is increased after treatment and correlates with improved outcomes
- This study sought to characterize CD8 Treg isolated from patients with newly-diagnosed Stage 3 T1D, and responses to a bispecific CD8 Treg modulator, MTX-101

Figure 1. Model of a dysfunctional CD8 Treg network (left) and the postulated mechanism of action for MTX-101 (right). When CD8 Treg fail to eliminate pathogenic T cells, pathogenic T cells can expand, secrete proinflammatory cytokines, recruit and activate other inflammatory cells, and induce autoantibody production. This cascade results in the destruction of healthy tissue. Addition of MTX-101, a bispecific antibody targeting inhibitory KIR and CD8, supports TCR-restricted CD8 Treg activation and cytolytic capacity, restoring immune tolerance and preventing downstream inflammation.

Model of Dysfunctional CD8 Treg Network



Model of MTX-101 Mechanism of Action



METHODS:

- Using flow cytometry, we characterized CD8 Treg isolated from the peripheral blood of newly-diagnosed Stage 3 T1D patients (n=20 aged 12–18; n=9 aged 18–40)
- CD8 Treg activation and secreted cytokines were evaluated in the presence or absence of MTX-101

CD8 Treg Prevalence in Newly-Diagnosed T1D Patients

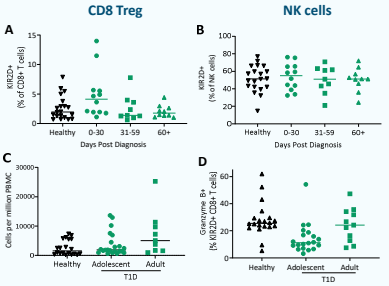


Figure 1. CD8 Treg prevalence and Granzyme B content are reduced in adolescent T1D PBMC compared to adult T1D PBMC. Immunophenotyping was performed on PBMC from T1D patients and healthy age-matched controls (HC). (A, B) CD8 Treg and KIR2D+ NK cell prevalence. (C) CD8 Treg number in total PBMC in adult vs. adolescent T1D patients and healthy controls. (D) Granzyme B content of CD8 Treg.

MTX-101 Selectively Activates CD8 Treg in T1D Patient PBMC

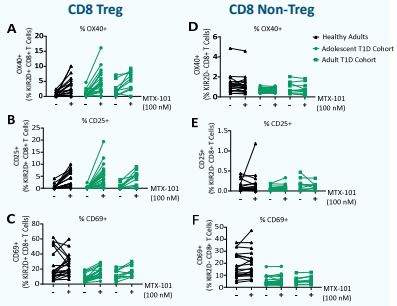


Figure 2. CD8 Treg are selectively activated following exposure to MTX-101. Total PBMC from adolescent T1D patients, adult T1D patients, and HC were cultured +/- 100 nM MTX-101 for 24hr prior to staining for activation markers CD25, CD69, and OX40. (A-C) Percentage of CD8 Treg expressing activation markers. (D-F) Activation markers on CD8+ non Treg cells.

MTX-101 Activates Adolescent CD8 Treg

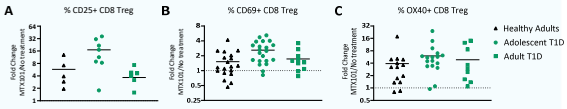


Figure 3. CD8 Treg activation markers are higher in newly-diagnosed adolescent T1D patients than in newly-diagnosed adult T1D patients following MTX-101 exposure. Total PBMC from adolescent T1D patients, adult T1D patients, and HC were cultured with 100 nM MTX-101 for 24hr prior to staining for activation markers CD25, CD69, and OX40. (A-C) Shown is fold change in activation marker expression on CD8 Treg following MTX-101 exposure relative to no treatment.

MTX-101 Selectively Regulates CD8 Treg Cytolytic Capacity

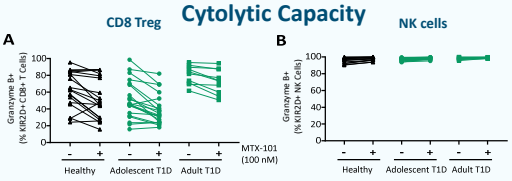


Figure 4. Granzyme B is altered by MTX-101 in CD8 Treg but not KIR2D+ NK cells. Total PBMC from adolescent and adult T1D patients and HC were cultured +/- 100 nM MTX-101 for 24hr prior to intracellular Granzyme B analysis. (A) Granzyme B levels in CD8 Treg. (B) Granzyme B levels in KIR2D+ NK cells.

MTX-101 Induces Cytokine Responses in PBMC from T1D Patient PBMC

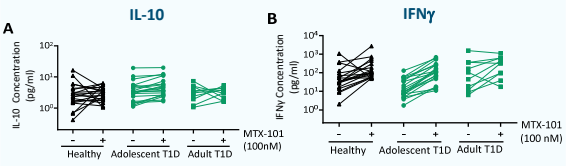


Figure 5. Newly-diagnosed adolescent and adult patients respond to MTX-101 by producing proinflammatory cytokines. Total PBMC from T1D patients or HC were cultured +/- 100 nM MTX-101 for 24hr and supernatants were analyzed using MSD. Following MTX-101 exposure (A) IL-10 secretion and (B) IFNγ secretion.

MTX-101 Selectively Reduces Pathogenic T cell Responses

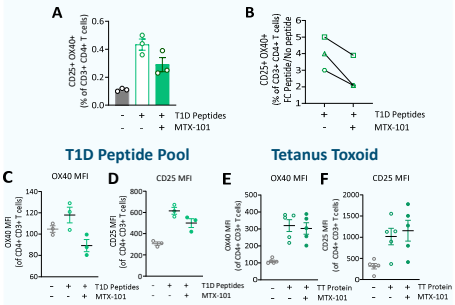


Figure 6. MTX-101 reduces activation induced marker (AIM) expression in T1D patient PBMC in response to autoantigenic T1D peptides but not tetanus toxoid protein. PBMC from adult T1D patients (>5-year post diagnosis) were stimulated with pooled T1D peptides, tetanus toxoid protein or control media in the presence/absence of MTX-101. Activation markers were measured at 44hr post stimulation. (A) Percentage and (B) Fold Change of CD25+ OX40+ CD4 T cells following incubation with T1D peptides. (C-F) CD25 and OX40 levels (MFI) following stimulation with T1D peptides (C, D) and tetanus toxoid (E, F).

RESULTS AND CONCLUSIONS

- Newly-diagnosed adult and adolescent T1D patient PBMC had equivalent CD8 Treg prevalence compared to healthy donors, but impaired cytolytic effector proteins at baseline.
- Addition of MTX-101 enhanced CD8 Treg activation in both populations, with a more pronounced activation of adolescent CD8 Treg.
- Addition of MTX-101 reduced pathogenic T cell responses to autoantigenic peptide stimulation.
- Collectively, our data support the development of MTX-101 as a therapeutic to restore CD8 Treg functions and preserve C-peptide in newly-diagnosed T1D patients.

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References: Li, et al. DOI: 10.1126/science.abi9591; Gardell et al, 2024 DOI: 10.3389/fimmu.2024.1452537