A Novel Bispecific CD8 Treg Modulator Targeting Cytolytic CD8 Regulatory T cells Reduces Pathogenic CD4 T cells and Inflammation in Translational Models of Intestinal Autoimmune and Inflammatory Disease

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MOZART THERAPEUTICS

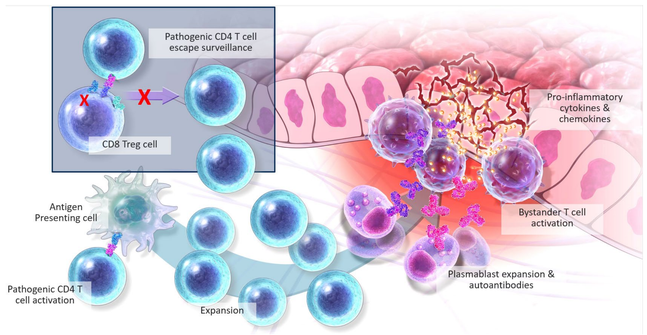
Orchestrating The Immune System

INTRODUCTION:

We have characterized a regulatory CD8 Treg network in autoimmune patients, in which cytolytic CD8 Treg eliminate pathogenic CD4 T cells, reducing inflammation and ameliorating disease in response to pathogenic CD4 T cell activation.

We postulate that the CD8 Treg network is dysfunctional in patients with Celiac and Crohn's disease. This dysregulation allows pathogenic CD4 T cell escape from elimination, resulting in pathogenic CD4 T cell expansion, the initiation of a cascade of inflammatory pathological consequences, and the perpetuation of tissue destructive inflammation. Here we demonstrate the use of bispecific CD8 Treg modulators that may have the therapeutic potential to restore the elimination of pathogenic CD4 T cells in disease.

In autoimmunity, regulatory CD8 T cells fail to control expansion of pathogenic CD4 T cells resulting in downstream inflammation

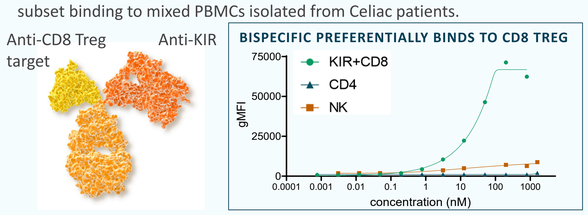


METHODS

- Bispecific CD8 Treg Modulators were designed and evaluated for targeted binding by Octet and flow cytometry.
- The functional impact of CD8 Treg modulators was evaluated using flow cytometry, Incucyte, and supernatant analysis in PBMCs isolated from Celiac and Crohn's disease patients.
- CD8 Treg Modulators were also tested in celiac patient duodenal tissue biopsies and xenograft animal models of acute GVHD.

RESULTS

Figure 1. Bispecific CD8 Treg modulator construct and cell binding. A bispecific CD8 Treg modulator was designed and evaluated for immune cell subset binding to mixed PBMCs isolated from Celiac patients.



Mozart's CD8 Treg Modulator induces an anti-inflammatory cytolytic regulatory CD8 T cell network

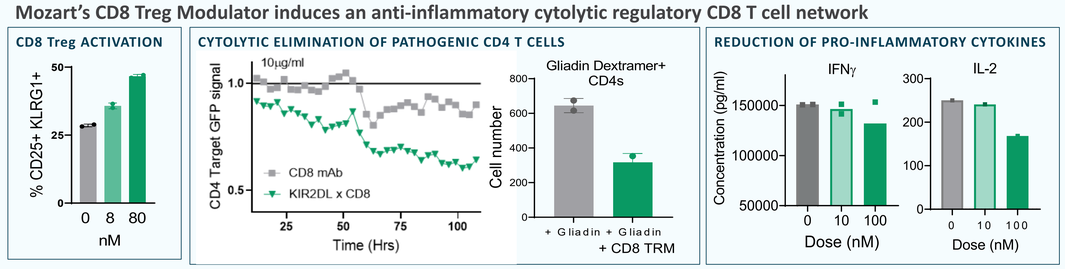


Figure 2 A bispecific CD8 Treg modulator directed to select inhibitory KIRs activates CD8 Treg, results in pathogenic CD4 T cell elimination, and reduces inflammatory cytokines in the presence of gliadin peptide stimulation. CD8 T cells were isolated from Celiac donor PBMCs and expanded in the presence of gliadin-pulsed APCs and cytokines. Cells were stimulated with gliadin peptides for 6 days in the presence of cytokines with a restimulation on Day 6. On day 12, re-stimulated PBMCs were treated with anti-CD3/CD28 with the CD8 Treg modulator and death was evaluated 5 days later. CD8 T cells were then enriched for KIR+CD8 Tregs and incubated with the CD8 Treg modulator for 48-72 hrs with gliadin-specific CD4 target cells. The CD8 Treg modulator resulted in activation of CD8 Tregs, increased and specific elimination of a gliadin responsive CD4 cell line. Gliadin specific CD4 cells were also eliminated in a celiac PBMC culture.

Mozart's CD8 Treg Modulator enhances the functions of tissue resident CD8 Treg in Celiac donor organoids

In collaboration with Dr. James Lord, BRI

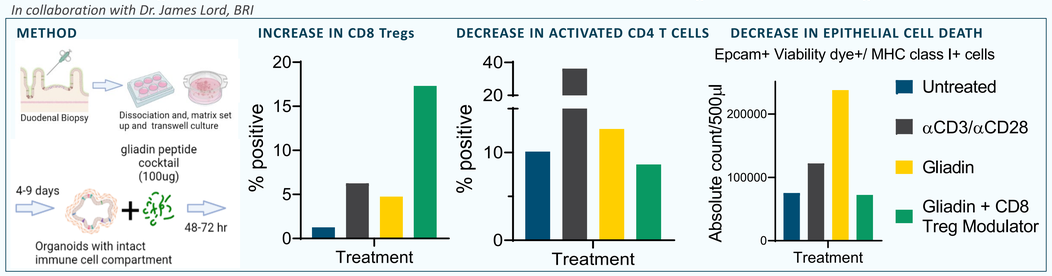


Figure 3. Bispecific CD8 Treg modulator increased CD8 Treg prevalence and activation, decreased proinflammatory cytokine production, and decreased epithelial cell death in duodenal biopsy organoid cultures. Duodenal biopsies from celiac patients were grown in a collagen matrix with essential growth factors to generate organoid cultures. After a week of culture, organoid immune cells were stimulated with gliadin peptides in the presence or absence of 100nM CD8 Treg modulator to induce activation of the celiac responsive CD4 T cells. 48-72 hr later, organoids were digested to a single cell suspension and analyzed using flow cytometry for CD8 Treg prevalence, activated CD4 T cells, and epithelial cell death.

Mozart's CD8 Treg Modulator enhances CD8 Treg network anti-inflammatory functions in patients with Crohn's disease

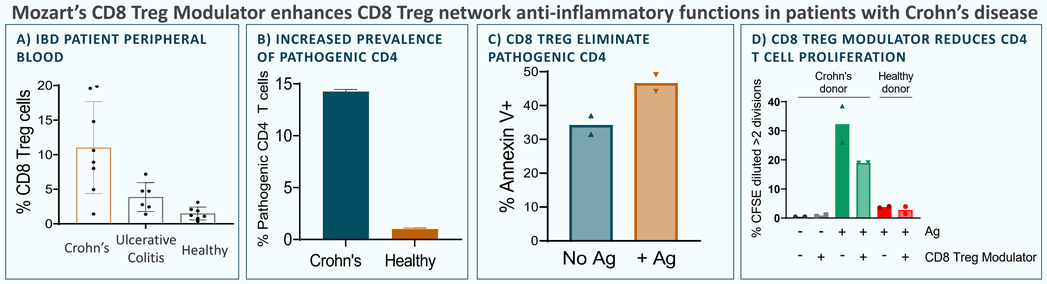
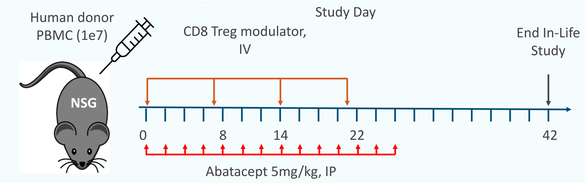
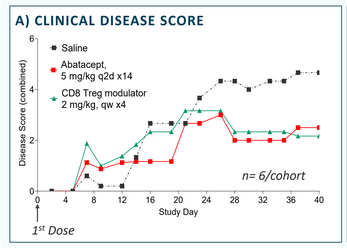
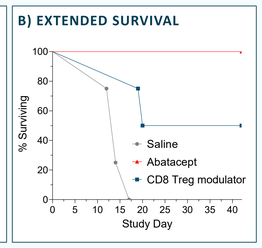


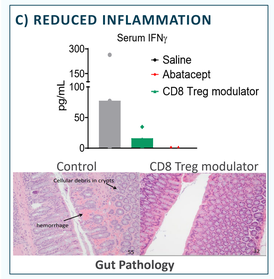
Figure 4. CD8 Treg Modulator reduces inflammation and enhances CD8 Treg functions in vitro using Crohn's disease patient PBMCs A) Peripheral blood from patients with Crohn's disease was evaluated for the prevalence of CD8 Treg using flow cytometry B) Peripheral blood from patients with Crohn's disease was evaluated for the prevalence of pathogenic CD4 T cells using flow cytometry C) Pathogenic CD4 and CD8 Treg (1:1) were stimulated with Flagellin antigen and pathogenic CD4 T cells analyzed using flow cytometry for Annexin V as a marker of apoptosis 48 hours later D) PBMCs from a Crohn's patient (green) or a healthy donor (red) were stained antigen stimulated in the presence or absence of CD8 Treg modulator and pathogenic CD4 T cell proliferation was evaluated six days later.

Bispecific CD8 Treg modulator ameliorates disease in Human PBMC-NSG™ GvHD Model









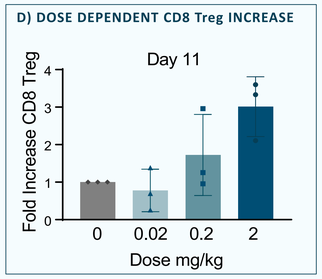


Figure 4. CD8 Treg Modulator ameliorates inflammation in vivo A) CD8 Treg modulator reduced clinical disease scores. B) CD8 Treg Modulator treated mice had delayed disease onset and increased survival. C) Mice treated with CD8 Treg modulator had reduced serum pro-inflammatory cytokines, including IFNγ and IL-2, and reduced disease pathology in disease affected tissues D) Peripheral blood analyzed four days after the second CD8 Treg Modulator injection showed a dose dependent increase in CD8 Treg prevalence.

CONCLUSIONS

- Mozart's novel bispecific CD8 Treg modulator enhanced CD8 Treg functions in vitro, in vivo, and in ex vivo patient tissues
- CD8 Treg Modulator treatment led to decreased inflammation via selective elimination of pathogenic CD4 T cells that initiate Celiac and IBD inflammatory cascades
- Our data suggest that this network can be targeted by immune-modulating biologics to suppress pathogenic T cells, reducing disease severity and frequency of flares in patients with Celiac disease and IBD.

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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