Pre-clinical pharmacologic and tolerability characterization of a novel KIRXCD8 targeting bispecific CD8 Treg Modulator

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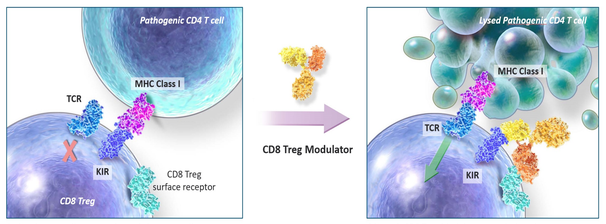
MOZART THERAPEUTICS

Orchestrating The Immune System

Introduction

We have characterized a subset of CD8 T cells (CD8 Treg) with immunosuppressive characteristics in inflammatory disease settings. Here we describe the binding and specificity profiles of a novel bispecific CD8 Treg Modulator in vivo, and initial assessment of tolerability and pharmacology in a humanized mouse model. We evaluated the binding, pharmacokinetics (PK), and early tolerability of CD8 Treg Modulator targeting CD8 and KIR2DL1/2/3. Our early data suggest that KIR-mediated enhancement of CD8 Treg function using a bispecific CD8 Treg Modulator is a broadly applicable and is a promising CD8 Treg specific therapeutic modality for the treatment of autoimmune disease.

CD8 Treg Modulator is Targeting a Novel 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

- A novel CD8 Treg Modulator was tested in healthy humanized CD34+ NSG-Tg(Hu-IL15) mice.
- The in vivo pharmacological impact of the CD8 Treg Modulator was evaluated using flow cytometry and human U-plex Meso Scale Discovery (MSD) assays.
- Quantitation of the CD8 Treg Modulator in humanized CD34+ NSG-Tg(Hu-IL15) and BALB/cJ mice was performed using a sandwich ELISA.

Exploratory single dose tolerability study design

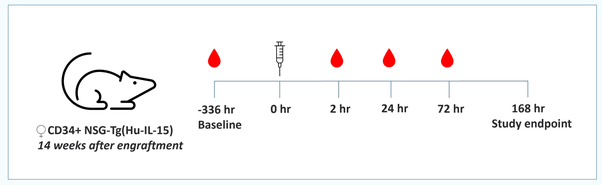


Figure 1 Animals received of 5 mg/kg CD8 Treg Modulator, KIR single arm, CD8 single arm, KIR bivalent, vehicle, or 0.5 mg/kg OKT3 (N=6 per group). At study endpoint, terminal blood and tissues including spleen, bone marrow, and lung were collected.

Frequency of human immune cells in peripheral blood and terminal tissues remained stable after single dose of CD8 Treg Modulator

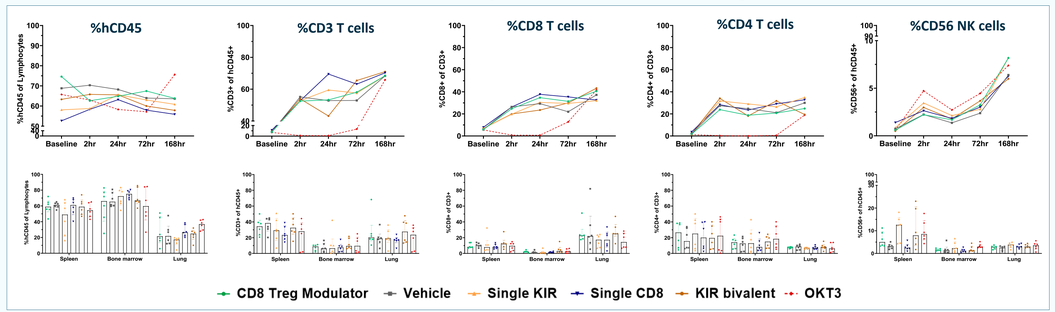


Figure 2 Frequency of human immune cells (hCD45+mCD45-), including T and NK cells, were assessed by flow cytometry at baseline, 2, 24, 72, and 168-hr post-dose in peripheral blood and terminal tissues remained stable after administration of CD8 Treg Modulator. Data are presented as median.

Prevalence of populations following exposure to CD8 Treg Modulator

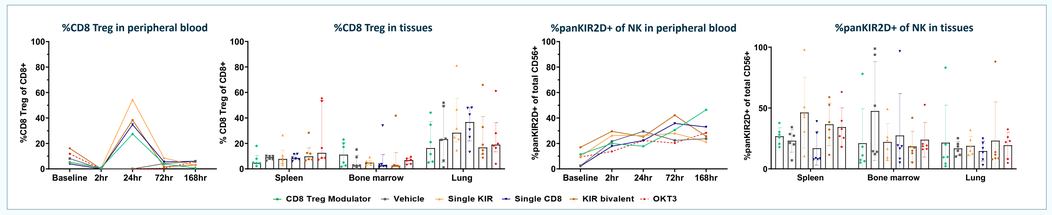


Figure 3 Similar transient fluctuation of CD8 Treg cells in peripheral blood was observed after a single dose of CD8 Treg Modulator and all control molecules tested. Frequency of panKIR2D in NK cells was similar between all treatment groups in peripheral blood and terminal tissues.

Sustained binding to peripheral blood cells expressing both targets of the CD8 Treg Modulator

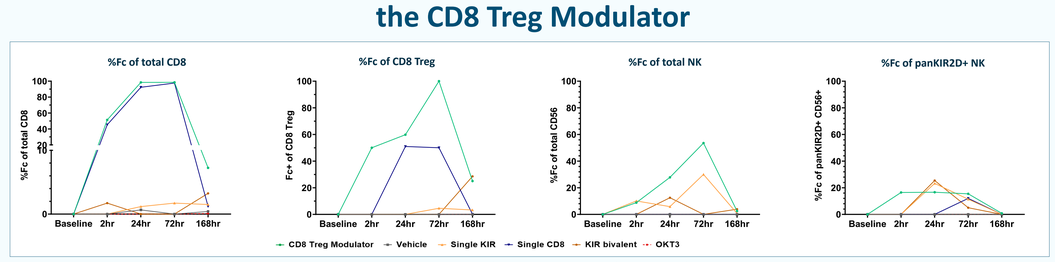


Figure 4 Detection of CD8 Treg Modulator on total CD8, CD8 Treg, NK, and panKIR2D+ NK cells increased at 2-hr post-dose, remained high at 72-hr timepoint in peripheral blood, and decreased at 168-hr timepoint.

Detection of drug (anti-hFc) binding in terminal tissues

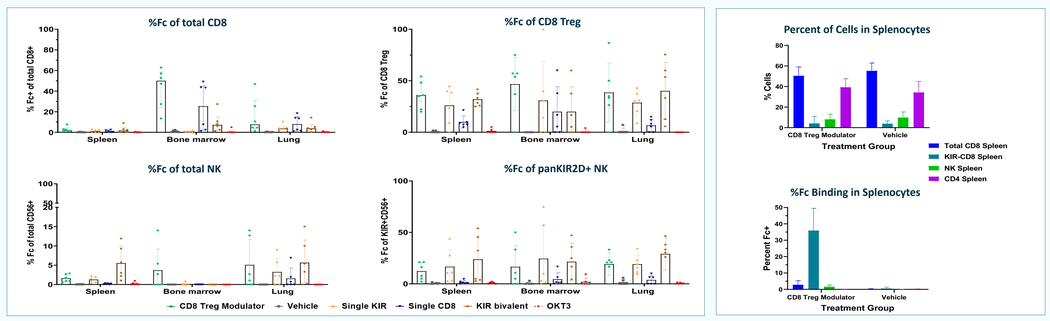


Figure 5 Binding of CD8 Treg Modulator to total CD8, CD8 Treg, NK, and panKIR2D+NK cells were found on cells in terminal tissues. Sustained binding was observed to cells expressing both targets. Data are presented as median (left panel). Summary of prevalence and binding of CD8 Treg Modulator vs. vehicle control for total CD8, KIR+CD8, CD4 T cells, and NK cells in spleen at terminal timepoint (mean ± SD) (right panel).

Expression of Granzyme B, Ki67, and CD69+CD25+ in total CD8 and CD8 Treg

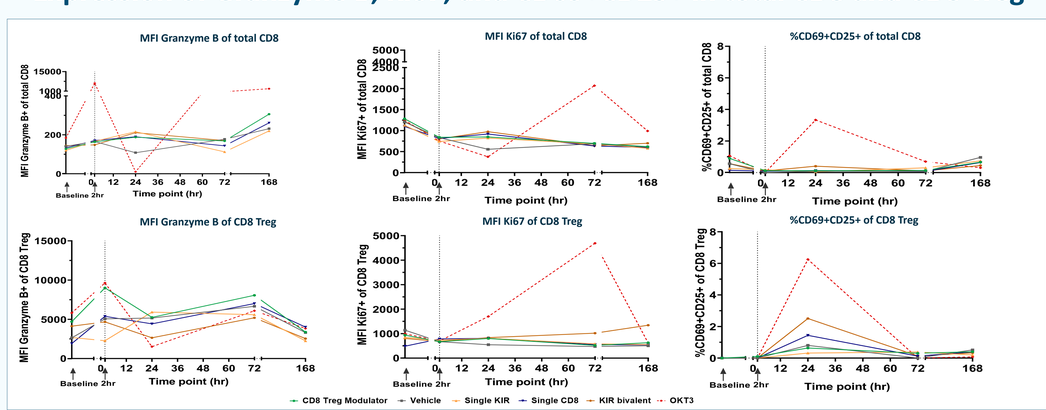


Figure 6 A transient increase of median fluorescent intensity (MFI) Granzyme B was detected the 2-hr timepoint post-dose in CD8 Treg cells following CD8 Treg modulator treatment. No changes in proliferation (Ki67+) of total CD8 and CD8 Treg cells were observed over the course of the study. Similar to vehicle, the frequency of activation markers CD69+CD25+ remained low after single dose administration of CD8 Treg Modulator. No increase of CD69+CD25+ in CD4 T and NK cells was observed after single dose of CD8 Treg Modulator (data not shown). Data are presented as median.

No increase of pro-inflammatory serum cytokines with CD8 Treg Modulator

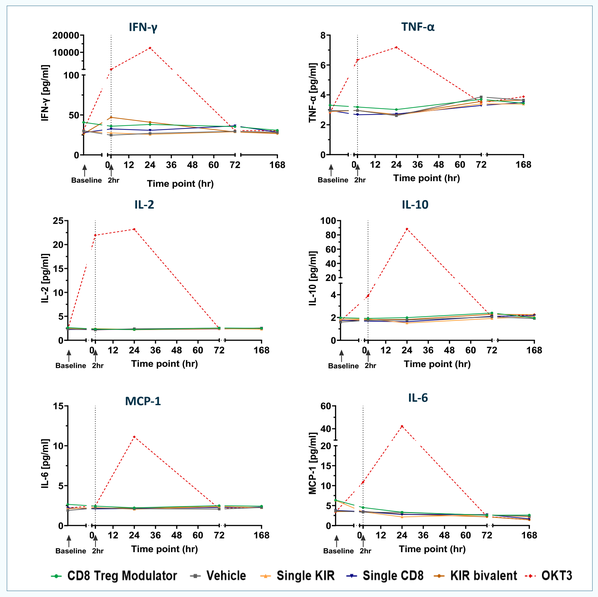
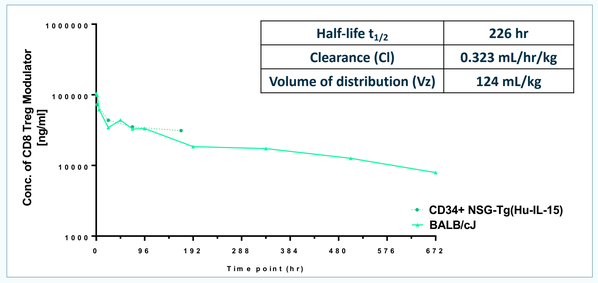


Figure 7 Single dose of CD8 Treg Modulator did not increase the expression of pro-inflammatory serum cytokines. Data are presented as median.

Quantitation of CD8 Treg Modulator in serum of humanized CD34+ NSG-Tg(Hu-IL15) and BALB/cJ mice



Half-life $t_{1/2}$	226 hr
Clearance (Cl)	0.323 mL/hr/kg
Volume of distribution (Vz)	124 mL/kg

Figure 8 Single dose of 5 mg/kg CD8 Treg Modulator was detectable in serum of humanized CD34+ NSG-Tg(Hu-IL15) through 168-hr and BALB/cJ mice, through 696-hr. Data are presented as median.

Exposure of CD8 Treg Modulator was also assessed in human PBMC-NSG GvHD mouse model. Please visit Poster board number 973 "A KIRXCD8 targeting bispecific modulator enhances regulatory T cell functions and reduces inflammation in models of autoimmune disease" for more information.

Conclusions

Exploratory data support that the CD8 Treg Modulator is a promising therapeutic modality for the treatment of autoimmune disease.

- A Single dose of CD8 Treg Modulator was well-tolerated in humanized mice and did not increase serum pro-inflammatory cytokines or activation of NK, CD4, CD8, or CD8 Treg cells.
- CD8 Treg Modulator selectively increased the expression of Granzyme B in CD8 Treg cells indicating potential impact on their cytolytic capacity.
- Drug binding to on-cell targets was measurable in peripheral blood and tissues including spleen, bone marrow, and lung of humanized mice with sustained binding to cells with both targets.
- Serum concentration of CD8 Treg Modulator indicated high exposure in humanized mice and antibody like PK parameters in BALB/cJ mice.

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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