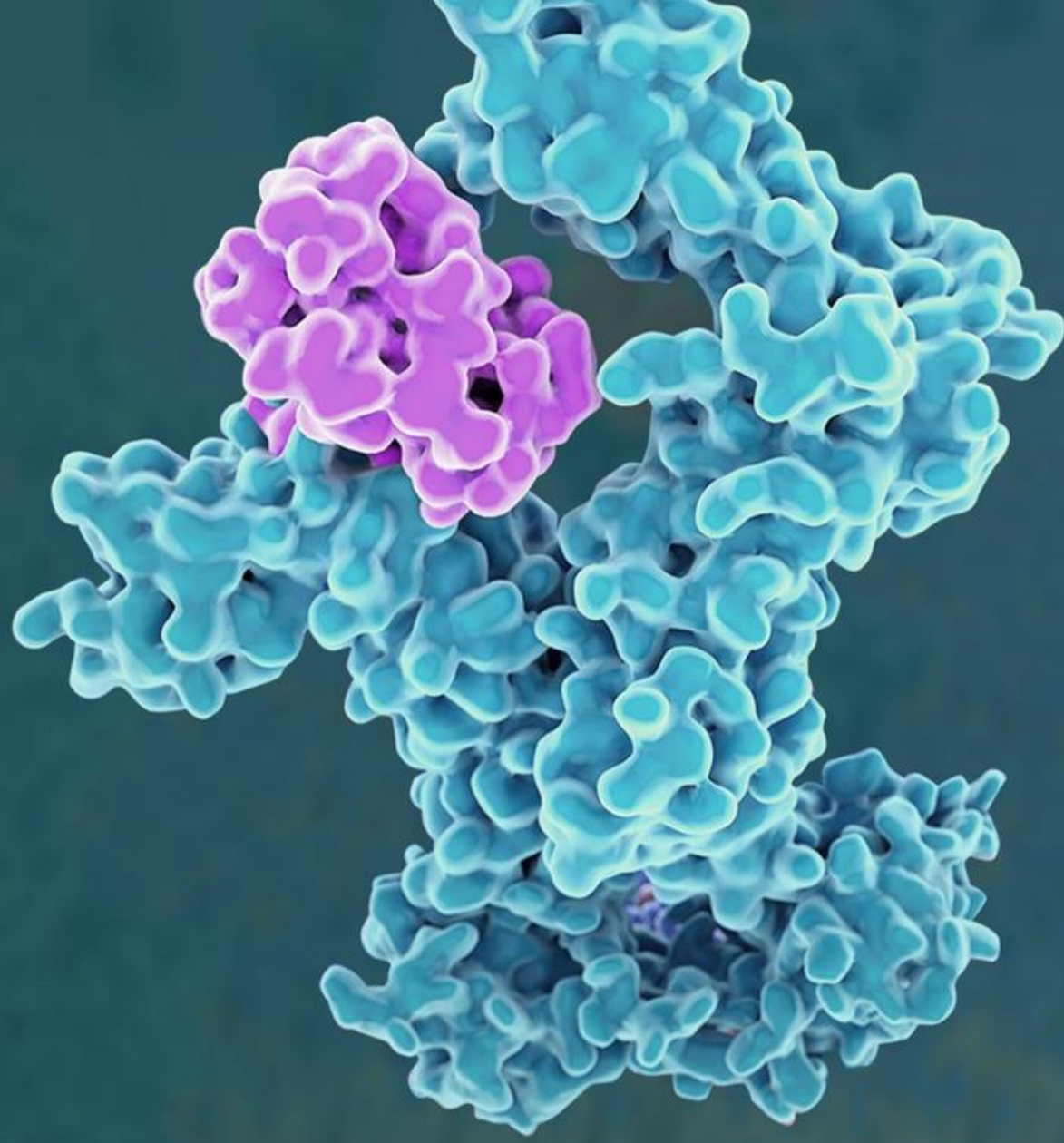
Sep 06, 2024

MDNA209, a High Affinity IL-2β Biased IL-2/IL-15 Super-antagonist, for the Treatment of Autoimmune Diseases



MEDICENNA

The Promise of IL-2 Therapy (IL-2 2024, Paris, France)

Directed Evolution Platform Generated Unique Tunable Superkines

IL-2 Superkines

Super-Agonists

- MDNA11 (Albumin-fused IL-2^{SK}) *Ph1/2 ABILITY Study*
- MDNA113 (Bifunctional IL-2^{SK})

Super-Antagonists

- **MDNA209**

MDNA209 Provides Differentiated MoA

MDNA209 *directly* blocks signaling to pathologic effector immune cells

IL-2R Agonism

Agonists of T regulatory cells

- Boosts immune suppressive activity
- Relies on regulatory T-cells (*not always involved in acute autoimmunity)*
- Does not *directly* reduce the destructive activity of self-reactive T or NK cells

IL-2R/15R Antagonism : MDNA209

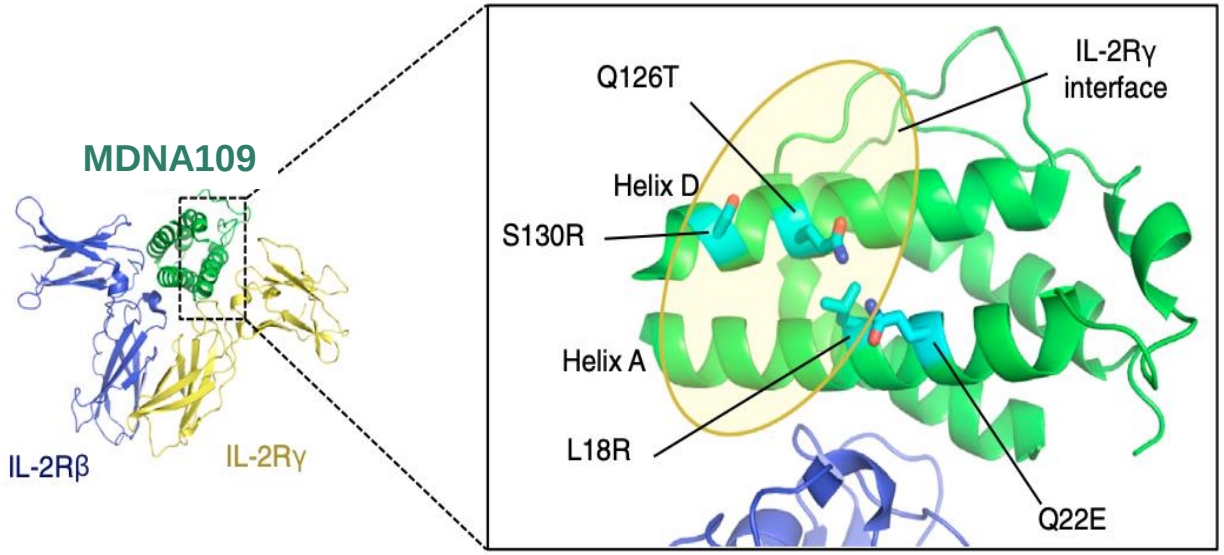
Antagonist of Effector Cells

- Blocks immune effector activity
- *Directly* targets IL-2R/IL-15R signaling in $CD4^+$ T, $CD8^+$ T, and NK cells
- Opportunity for broad use in autoimmune indications

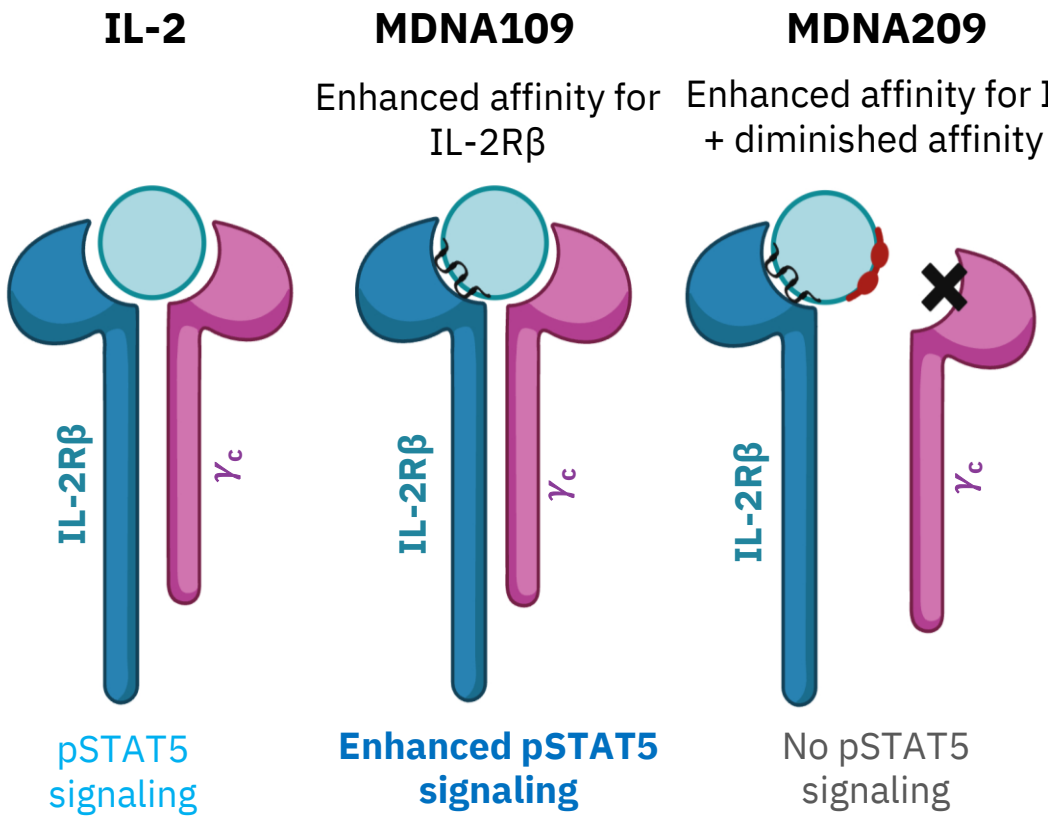
MDNA209 is a Long Acting IL-2 Antagonist With Diminished γ_c Receptor Binding

MDNA209 contains mutations with the following effects:

- Enhanced binding to IL-2Rβ outcompetes IL-2
- Diminished engagement with γ_c blocks downstream signaling
- **'Dominant-negative' IL-2 antagonist**



Mitra et al., Immunity (2015)



MDNA209/IL-2Rβ Complex Does Not Bind ©$_c$

- Long-acting MDNA209 retains high affinity for IL-2Rβ
- MDNA209 blocks formation of functionally active IL-2Rβγ_c complex

Receptor binding by SPR

Construct	Binding K_D (nM)
	IL-2Rβ
IL-2	434
MDNA109	4.7
MDNA209	3.6

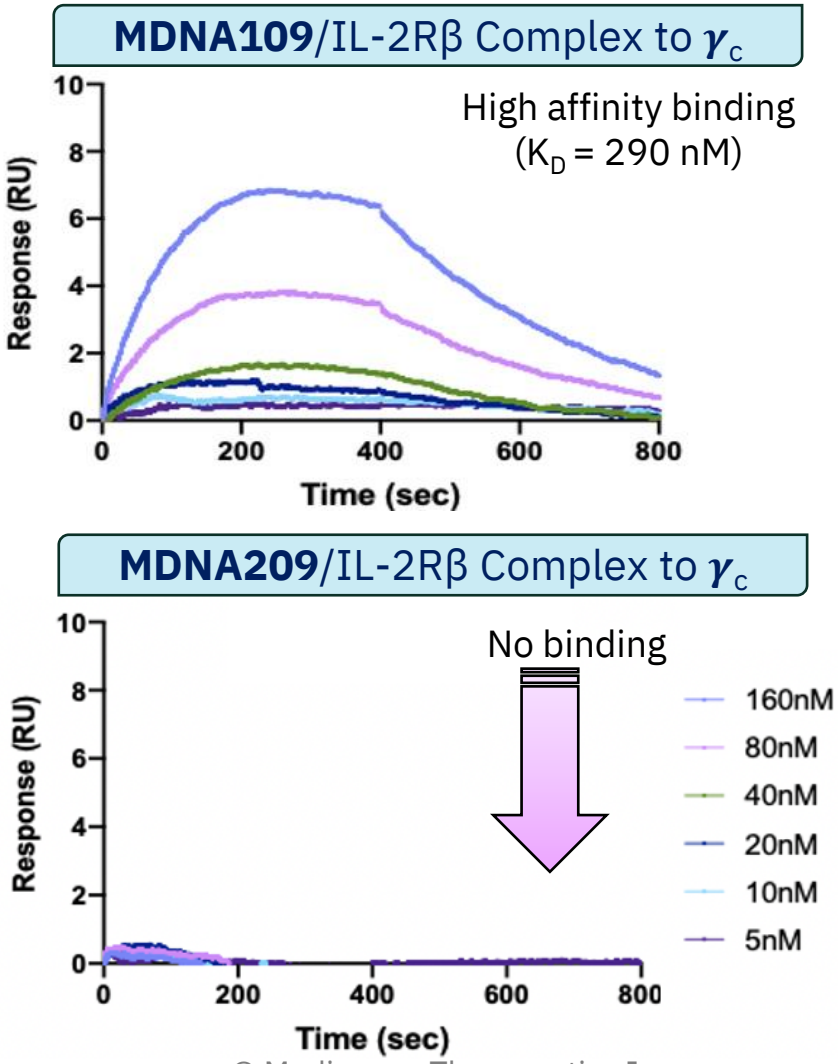
100x

High affinity binding to IL-2Rβ

MDNA109
IL-2 Super-agonist

MDNA209
IL-2 Antagonist

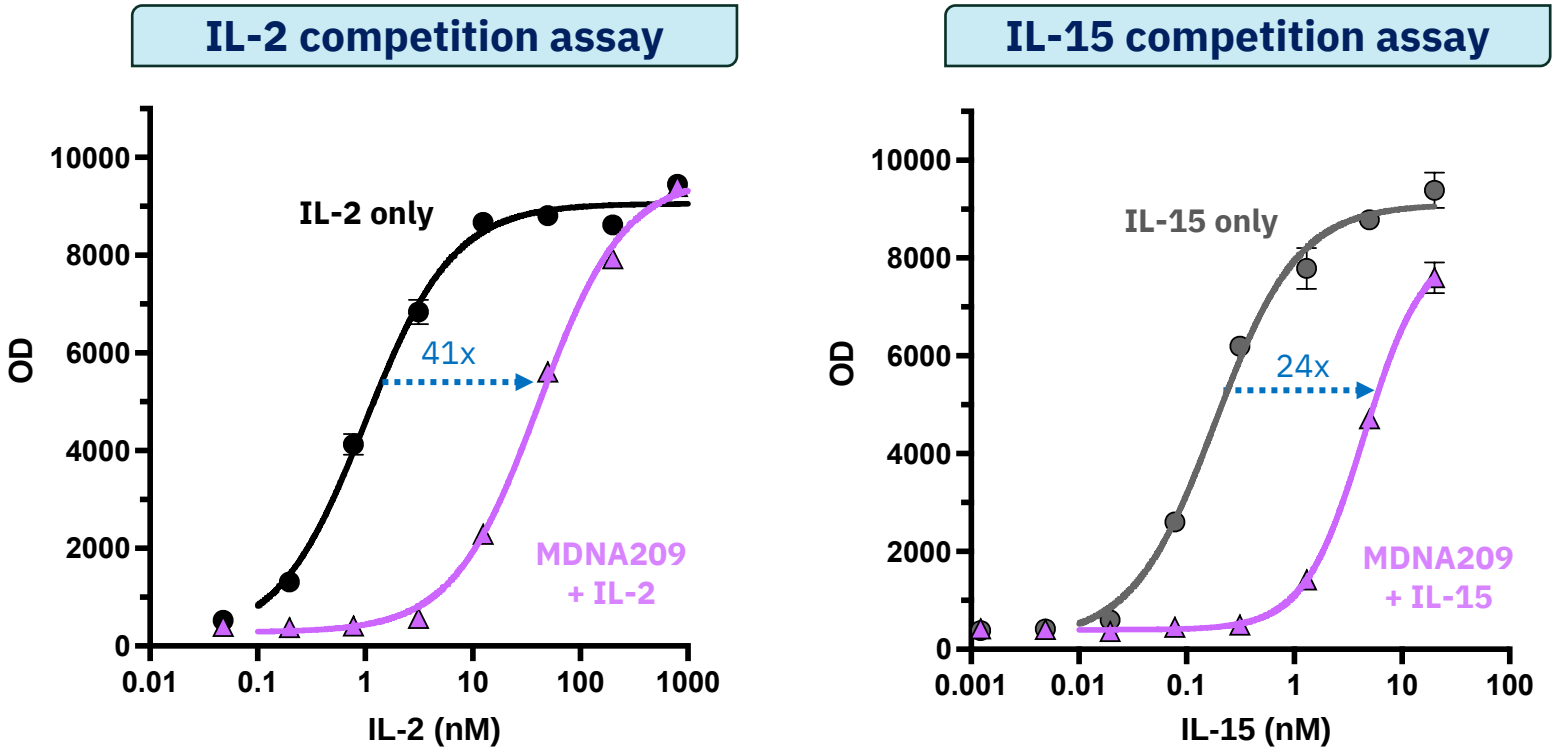
Co-operative binding by SPR



- Receptor affinity measured using SPR (Surface Plasma Resonance)

MDNA209 Potently Inhibits IL-2 and IL-15 Induced IL-2Rβγ Signaling

IL-2Rβγ Jurkat cell assay



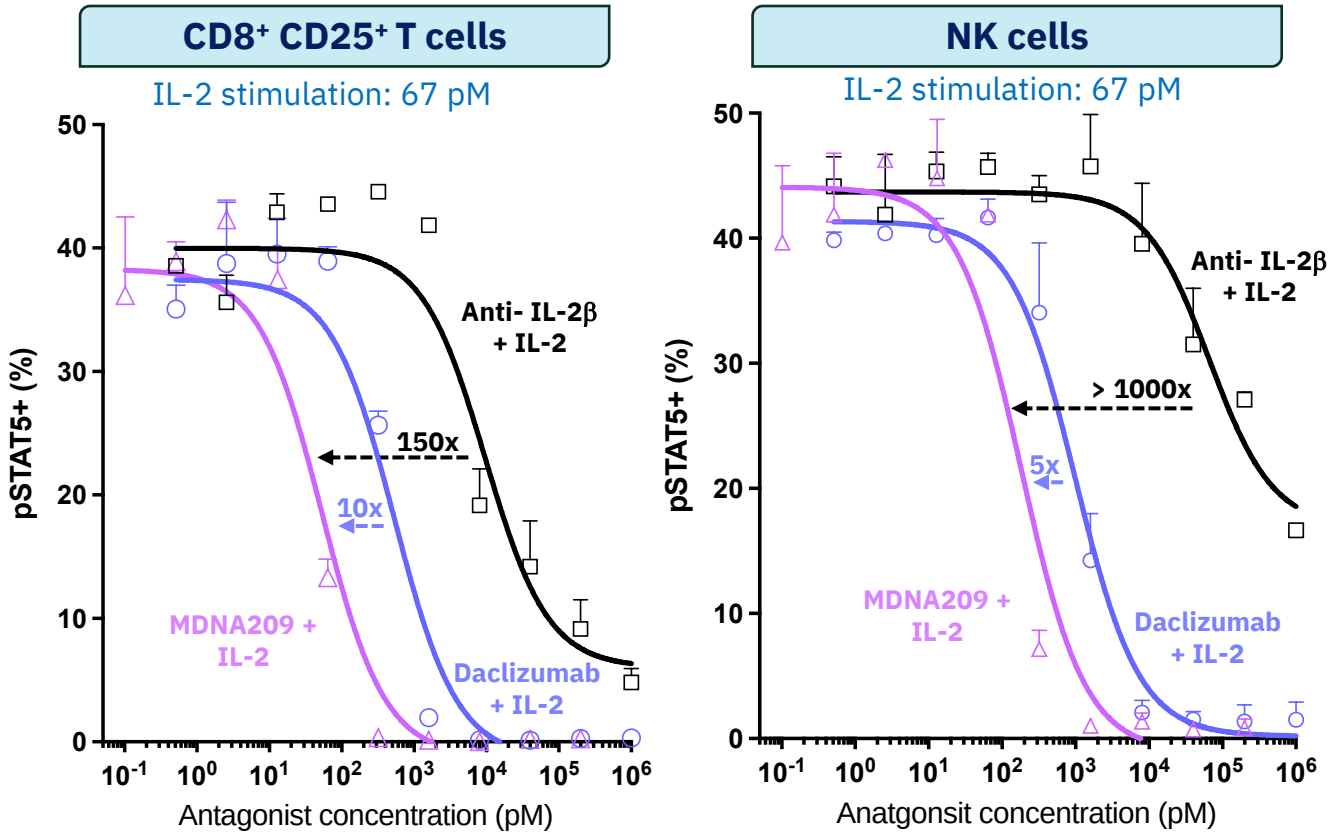
	EC_{50} (nM)	
Sample	IL-2 assay	IL-15 assay
IL-2	1.01	-
IL-15	-	0.19
MDNA209	41.0	4.43

Fixed antagonist (30nM) and titration of IL-2 or IL-15

IL-2R$\beta\gamma$ Jurkat cells express intermediate affinity heterodimeric IL-2 receptors
Cells were cultured in presence of increasing concentrations of IL-2 and a fixed concentration (30nM) of MDNA209

MDNA209 Inhibits IL-2R Signaling in Human PBMCs

- MDNA209 inhibits IL-2 induced signaling to a greater extent than IL-2Rα or IL-2Rβ blocking antibodies



pSTAT5 Signaling in Human PBMCs

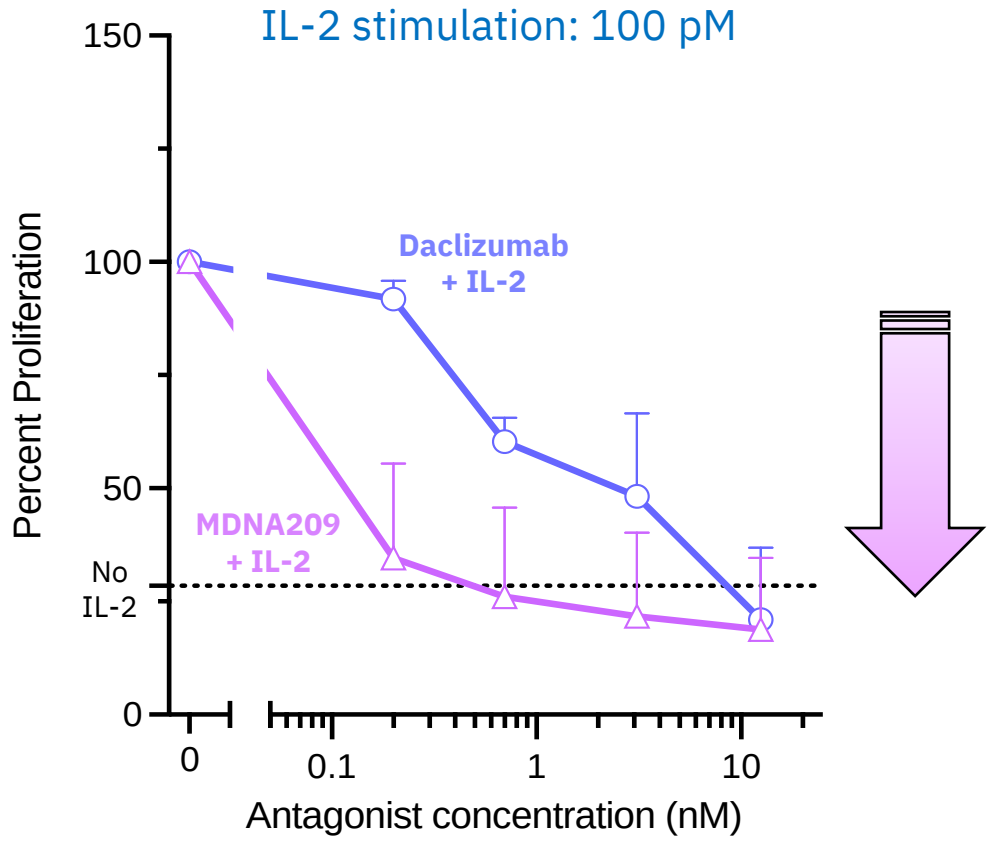
	Average IC_{50} (pM)			
Antagonist	CD8+ CD25+	Treg	CD4+	NK
MDNA209	53.37	22.70	52.94	182.5
Daclizumab	535.1	3266	628.9	993.7
Anti-IL-2β	8219	ND	7167	ND

ND= not determined

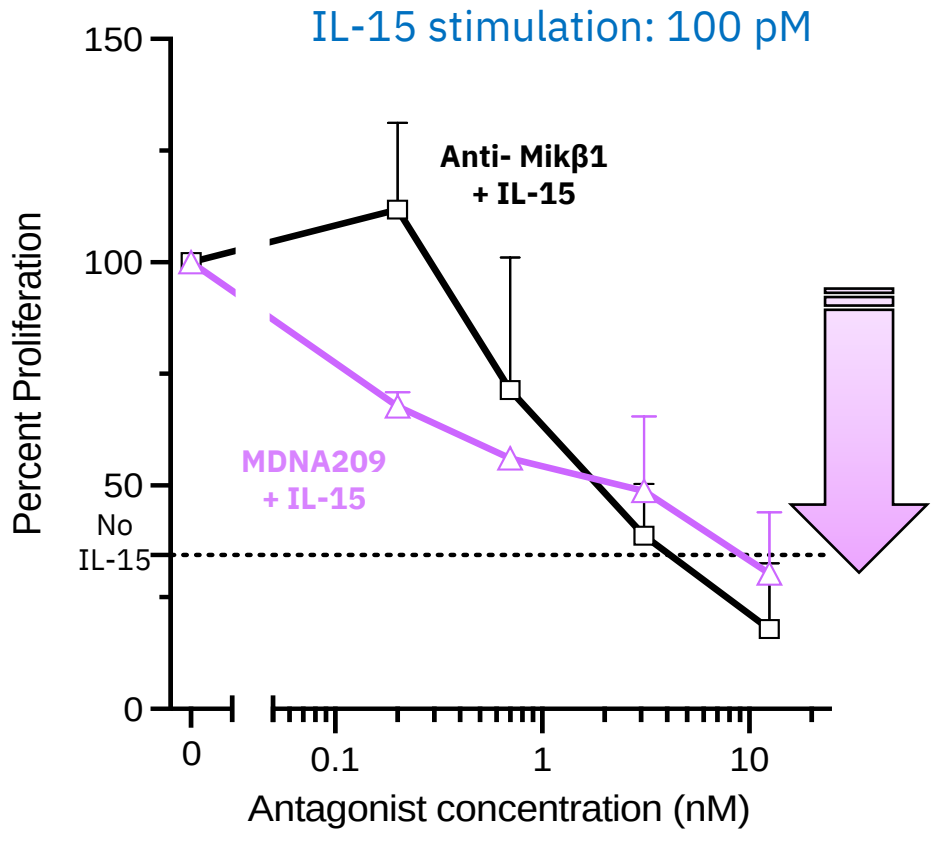
PBMCs (2 donors) were stimulated with rhIL-2 (67pM) and increasing concentrations of antagonists for 15 minutes. p-STAT5 expression was measured by flow cytometry
Daclizumab: biosimilar blocks IL-2 binding to CD25/IL-2Rα alpha receptor; Anti-IL-2β: blocks IL-2 binding to CD122/IL-2β receptor

MDNA209 Blocks IL-2 and IL-15 Induced PBMC Proliferation

IL-2 Induced PBMC Proliferation Assay

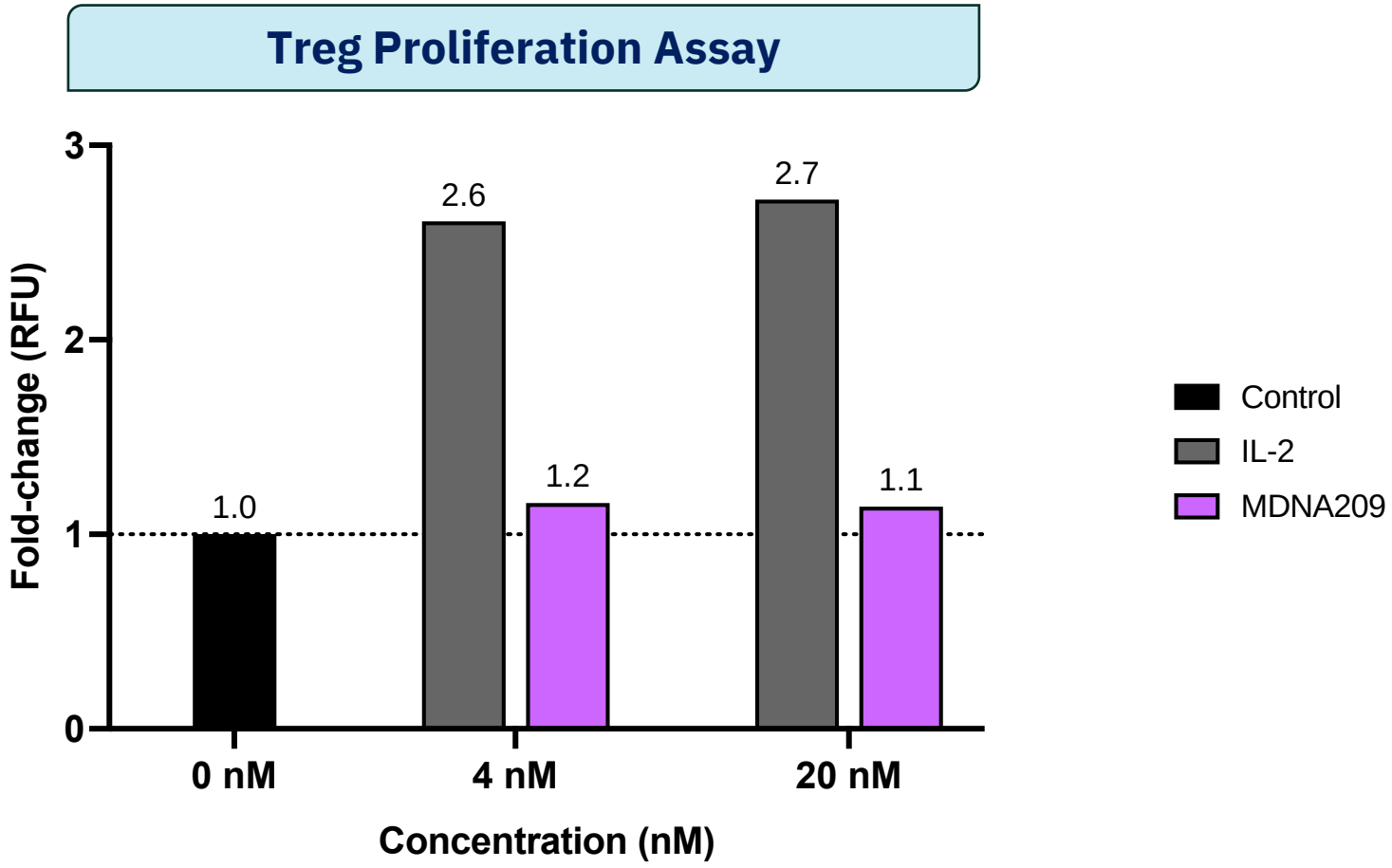


IL-15 Induced PBMC Proliferation Assay



PBMCs (N = 2 donors) pre-activated with anti-CD3/CD28 were re-stimulated with IL-2 or IL-15 (0.1nM) in presence of different concentration of MDNA209, Daclizumab or Mikβ1 antibodies for 72h. Thymidine incorporation assay

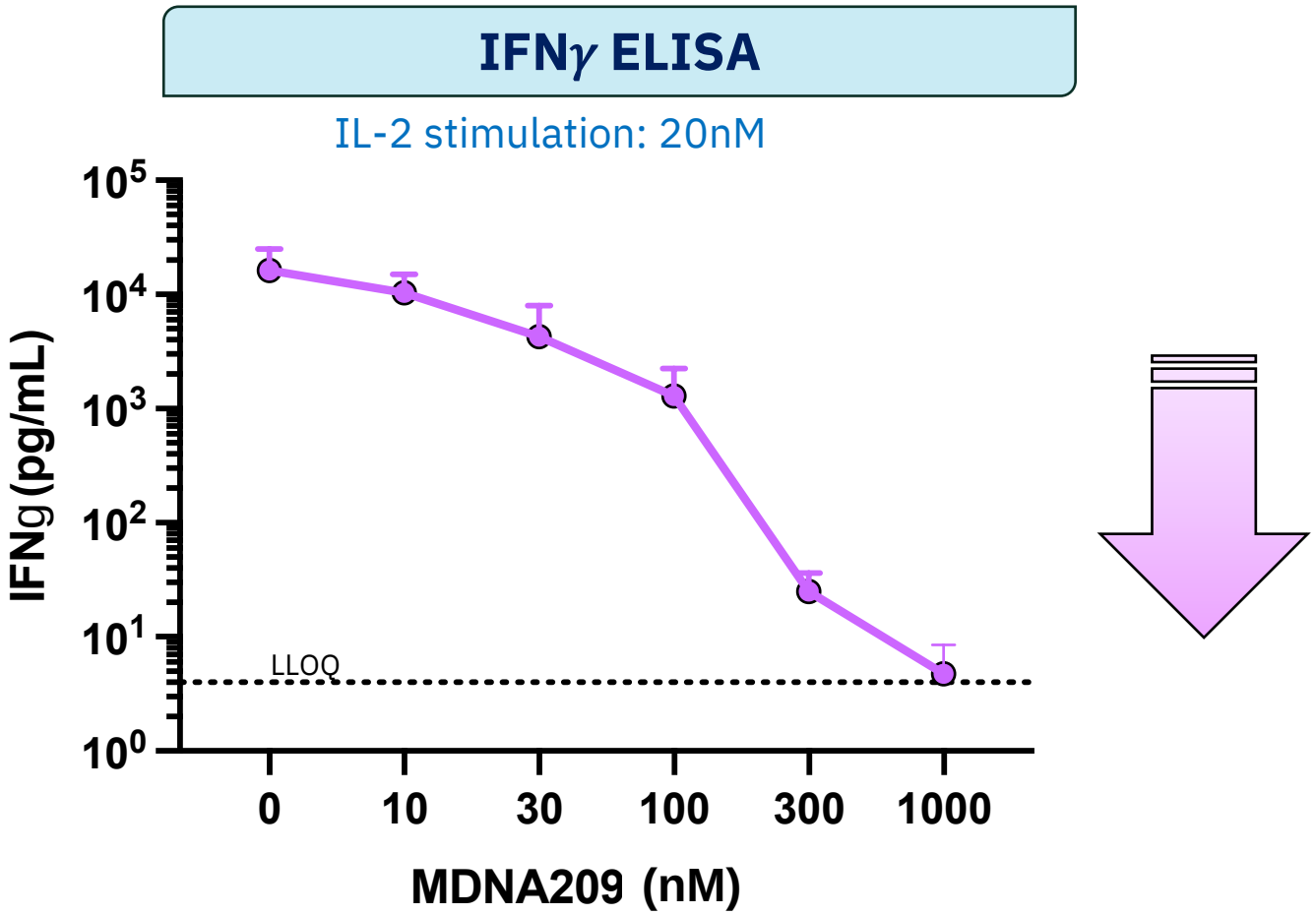
MDNA209 Maintains Treg Cells



Human nTreg cells were activated (anti-CD3/CD28) and cultured for 4 days in presence of IL-2 or MDNA209.

Proliferation was assessed with CyQuant reagent

MDNA209 Inhibits IL-2 Induced IFNγ Secretion in Human PBMCs

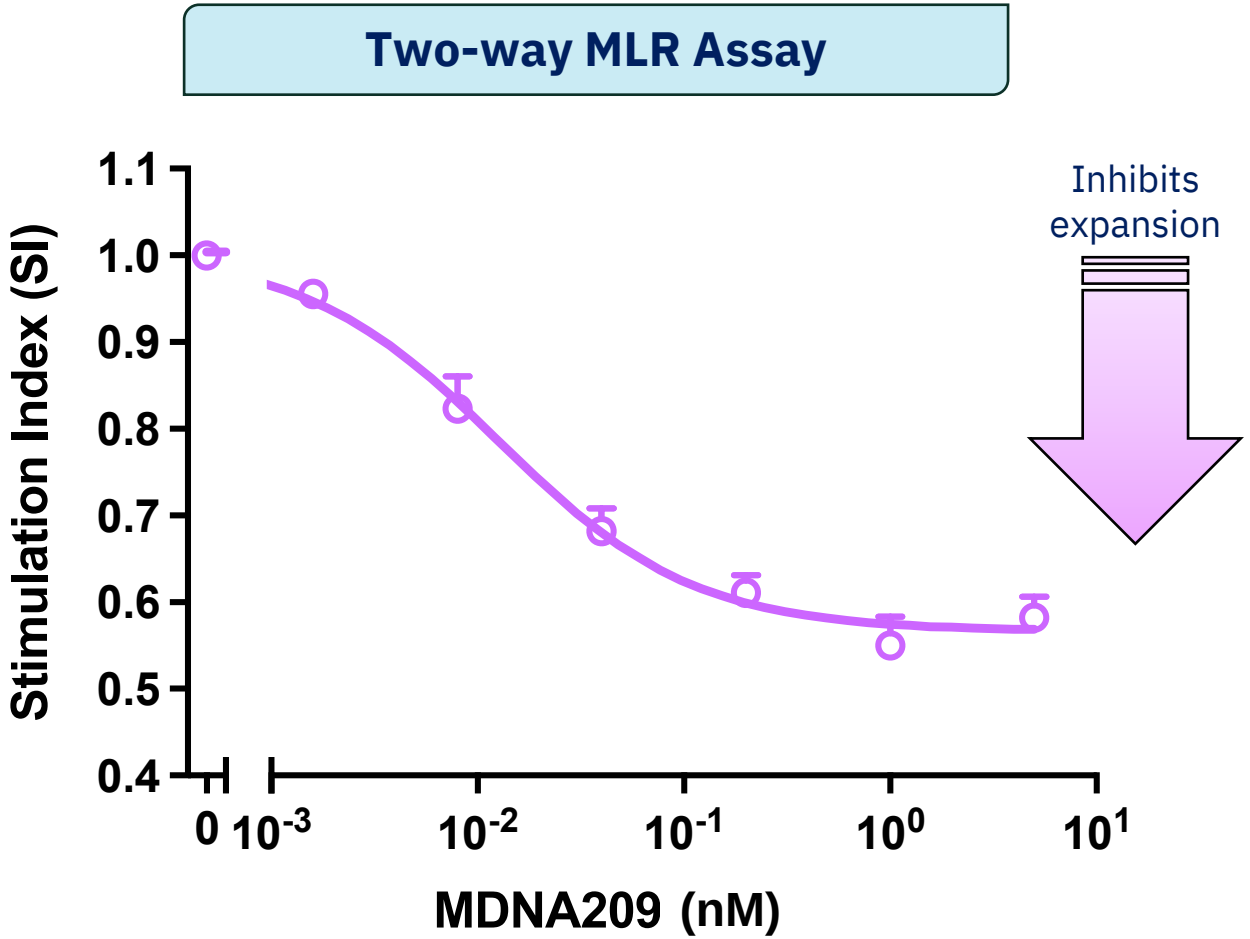


Construct	IC_{50} (nM)
MDNA209	14.9 ± 8.4

N = 4

PBMCs were preincubated for 15 min with MDNA209 followed by stimulation with rhIL-2 (20nM) for 48 hrs. Secreted IFNγ was measured in culture supernatant by ELISA. LLOQ = Lower limit of quantification

MDNA209 Inhibits PBMC Proliferation in a Mixed Lymphocyte Reaction



Construct	IC_{50} (pM)
MDNA209	12.7 ± 1

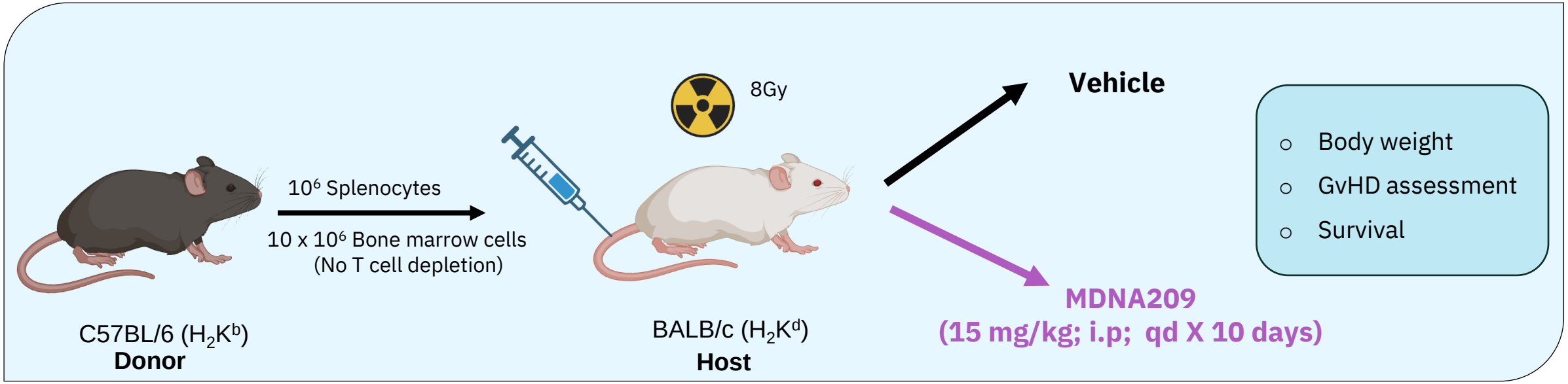
N = 3

PBMCs from different donor pairs (N =3) were co-cultured with increasing concentrations of test constructs for 4 days.
BrdU added on Day 3 and proliferation measured based on BrdU incorporation by ELISA and expressed as stimulation index (y-axis)

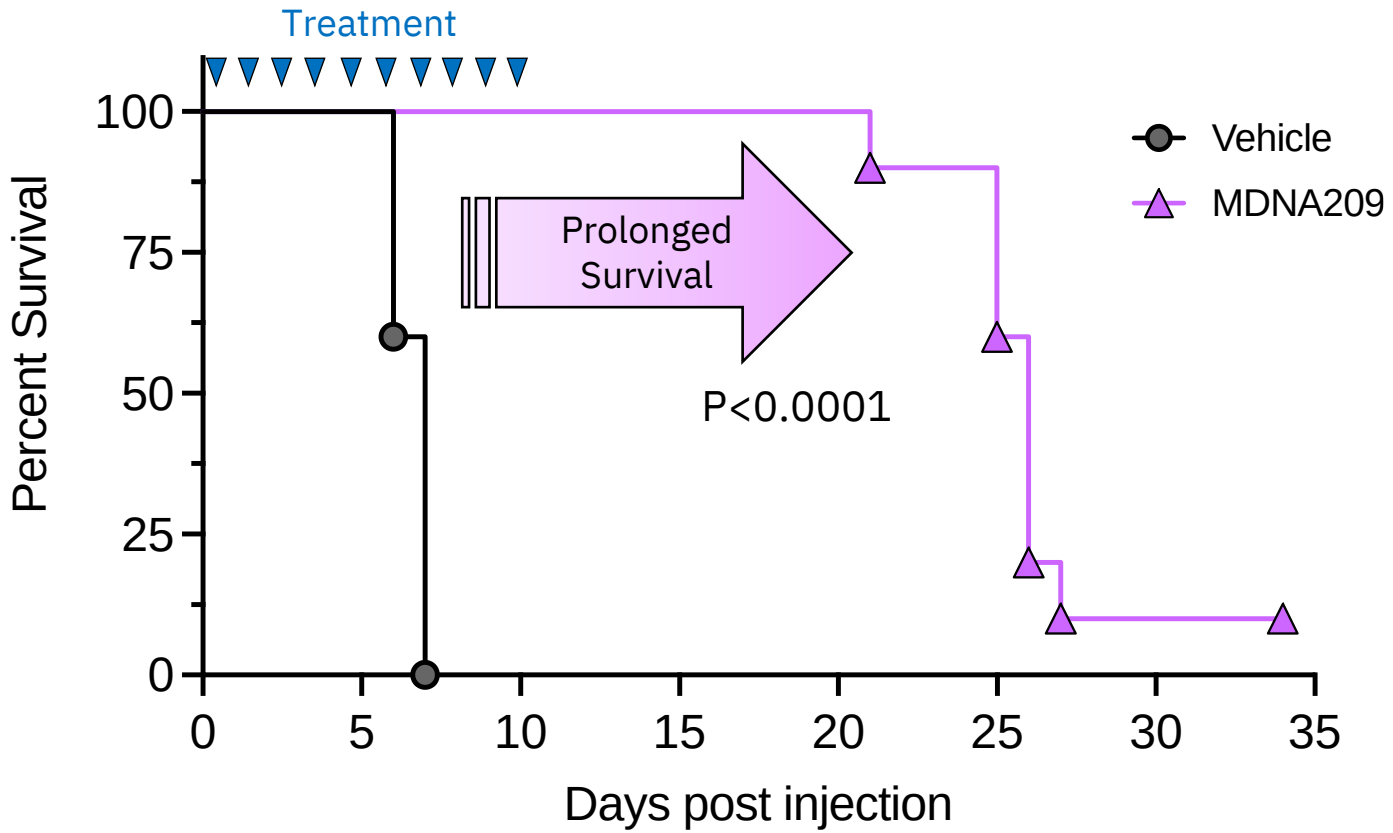
Acute Graft versus Host Disease (GvHD) Allograft Model

- aGvHD involves enhanced T-cell activation, expansion and direct cytotoxic effects of donor T cells on recipient tissues
- inflammatory cascade (cytokine storm) is activated
- systemic syndrome of weight loss, diarrhea, skin changes and high mortality

Fully mismatched major histocompatibility complex (MHC) allograft model



MDNA209 Promotes Survival in Graft versus Host Disease Model



Group	Median Survival (days)
Vehicle	7
MDNA209	26

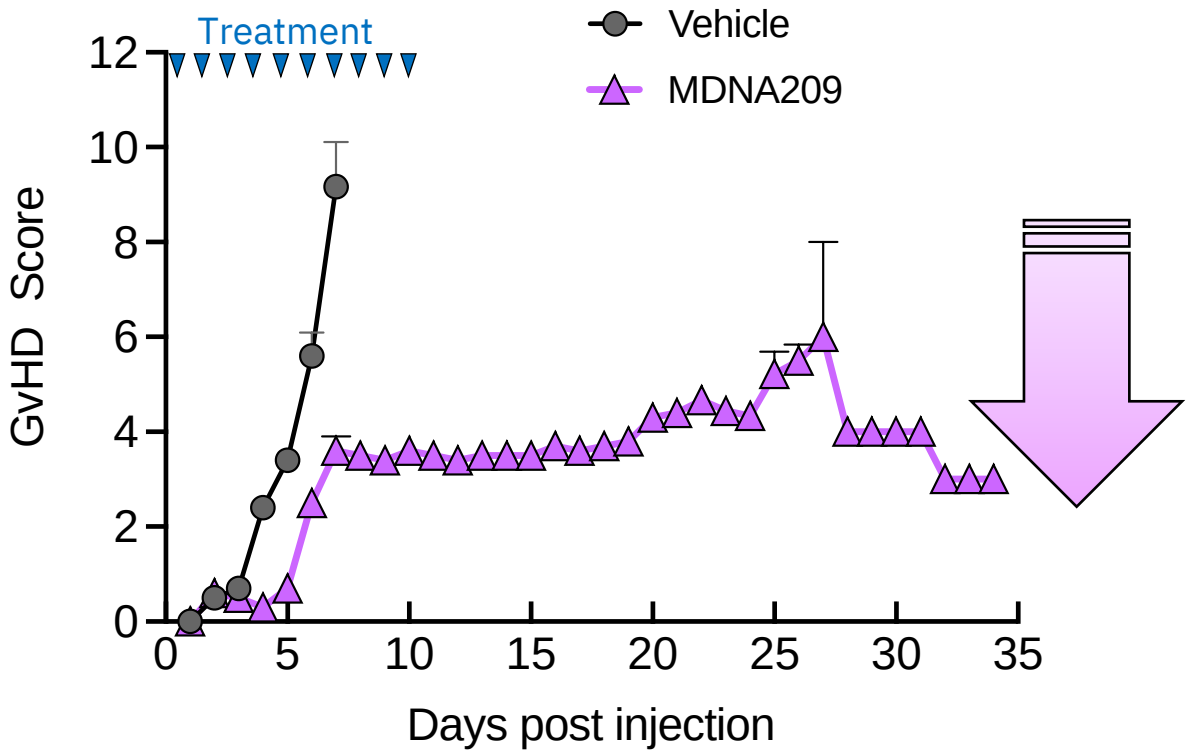
N= 10/group

MDNA209 was administered daily (i.p.) starting day 1 (upto day 10) after GvHD induction

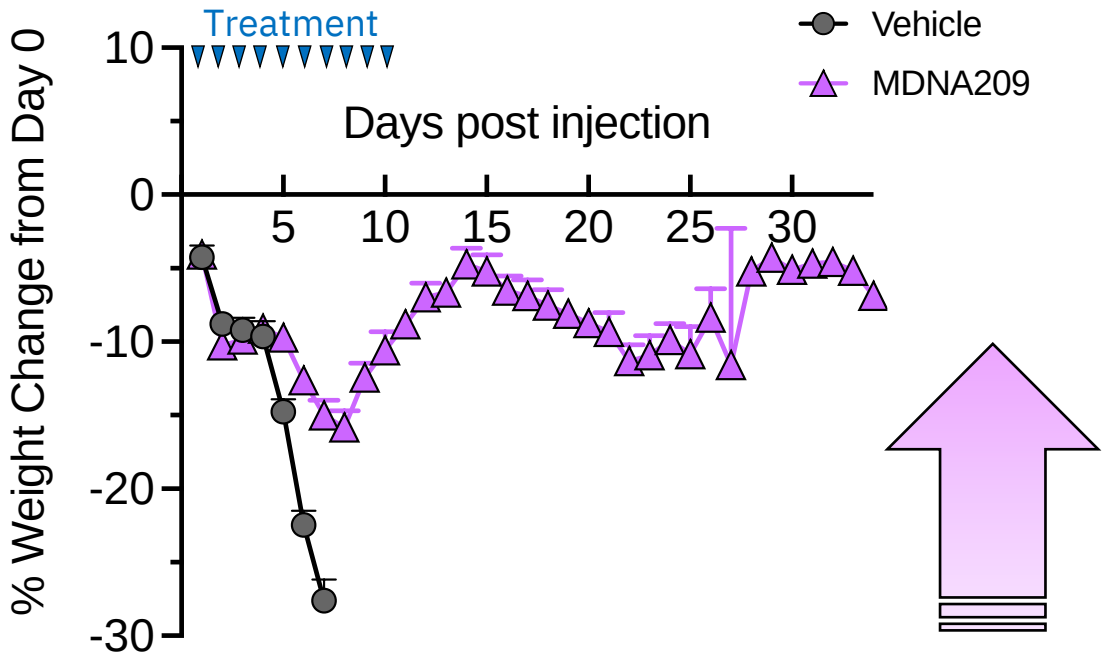
MDNA209 Ameliorates Graft versus Host Disease *in vivo*

- Protective effects of MDNA209 were maintained long after treatment had ended

MDNA209 reduced GvHD clinical score



MDNA209 limited weight loss



GvHD score is based on changes in mobility, body weight, eyes, ear color, skin and fur texture of animals (N=10/group)

Summary

- MDNA209 is an IL-2/IL-15 super-antagonist with enhanced affinity for IL-2Rβ, therefore acting as a receptor clamp to exclude binding of IL-2 and IL-15
- MDNA209 blocks engagement of γ_c, preventing downstream signaling
- MDNA209 inhibits IL-2 and IL-15 induced PBMC proliferation without affecting Treg survival
- MDNA209 impedes human PBMC proliferation in a mixed lymphocyte reaction (MLR) and blocks IL-2 induced release of IFNγ
- MDNA209 significantly increases survival in MHC-mismatched mouse model of acute graft-versus-host disease (aGvHD)
- MDNA209 directly targets disease driving effector immune cells paving an opportunity for broad use in Autoimmune indications

Thank you