#Tu1727: MRT-6160, a VAV1-Directed Molecular Glue Degrader, Inhibits Disease Progression in a T-cell Transfer Mediated Colitis Model Concomitant with Reduced Calprotectin Expression

Adam N. R. Cartwright[2], Shailee Vora[1], Lucas Gyger[2], Foram Desai[1], Xudong Wang[1], Katie May[1], Sophia Nguyen[1], Daniel Lam[1], Peter Trenh[1], Xavi Lucas[2], Mary Zlotosch[1], Elisa Liardo[2], Daric Wible[1], Ilaria Lamberto[1], Bradley Demarco[1], Chris King[1], Debora Bonenfant[2], Sharon Townson[1], Steven De Beukelaer[2], Eswar Krishnan[1], John Castle[2], Markus Warmuth[1], Owen Wallace[1], Filip Janku[1], Laura McAllister[2], Alison Paterson[1], Marisa Peluso[1]

[1]Monte Rosa Therapeutics Inc., 321 Harrison Ave, Boston, MA 02118, United States
[2]Monte Rosa Therapeutics AG, WKL-136.3, Klybeckstrasse 191, 4057 Basel, Switzerland

VAV1 is a critical guanine nucleotide exchange factor in T-cell receptor signaling and activity

Engaged TCR
LAT
IL-2
IL-17A
CD69
VAV1
P
NFAT
Cytokines

- VAV1 expression is highly restricted to immune cells
- VAV1 is a pivotal scaffolding protein and signaling molecule downstream of the TCR
- CRISPR-mediated KO[1] or germline genetic loss[2] of VAV1 is associated with decreased functions of T cells
- VAV1 degradation is predicted to impact T cell and B cell function and treat a broad set of autoimmune and inflammatory diseases

MRT-6160 is a rationally designed molecular glue degrader that selectively degrades VAV1

Cereblon
MGD, e.g. MRT-6160
Cereblon + MGD
Neosubstrate e.g. VAV1
Ternary complex
Ubiquitination
Ternary complex
Ubiquitin chain
Proteasome-mediated degradation of neosubstrate

Molecular glue degraders (MGD) function to induce structural changes in ubiquitin ligases, such as cereblon, to drive the formation of ternary complexes with a non-natural target protein, termed neosubstrate.

Following binding of cereblon to the target protein, this target is then ubiquitin tagged and subsequently degraded via the proteasome-mediated degradation machinery of the cell.

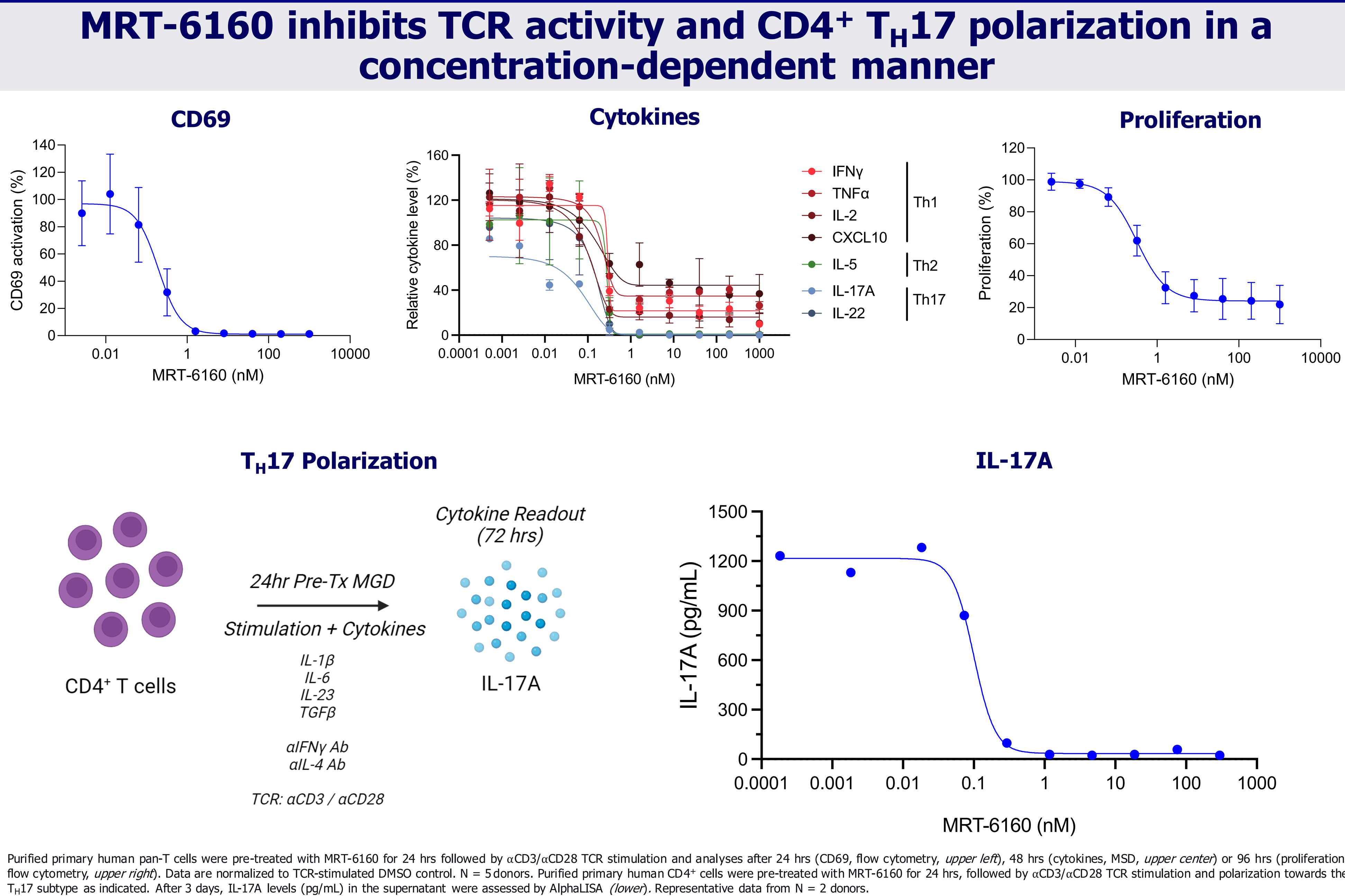
MGDs can induce degradation of otherwise 'undruggable' proteins as the mechanism does not require a classical binding pocket contrary to conventional protein inhibitors, significantly increasing the target space and utility across a range of diseases.

Human PBMCs | **Mouse Splenocytes**

p-value (-log10)
Protein fold-change ($\log_2$)
VAV1, GSPT1, VAV3, IKZF1, IKZF3, VAV2, CSNK1A1, ZFP91
Vav1, Zfp91, Gspt1, Ikzf1, Vav3, Ikzf3, Csnk1a1, Vav2

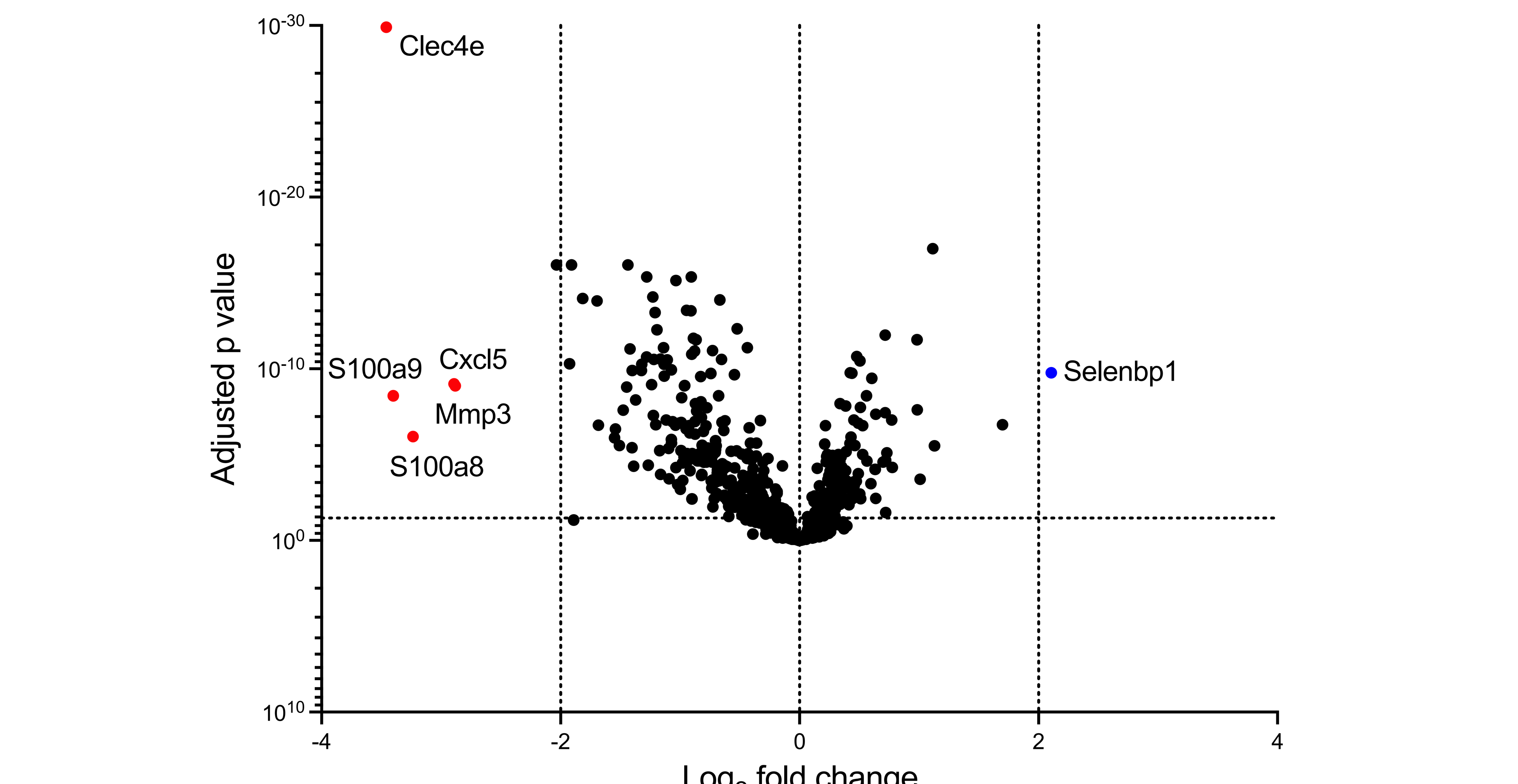
Human PBMCs and mouse splenocytes were treated for 24 hrs with 10 μM MRT-6160 then assessed by quantitative tandem mass tag proteomics. The y-axis represents p-value [$-\log_{10}$]; the x-axis represents protein fold change [$\log_2$] relative to DMSO (0.1%) control samples. Dark blue circles represent CRBN neosubstrates including the target, VAV1, and other known cereblon neosubstrates; GSPT1, IKZF1, IKZF3, CSNK1A1 (CK1α), SALL4, and ZFP91. Purple circles represent VAV family members VAV2 and VAV3.

MRT-6160 inhibits TCR activity and CD4⁺ T_H17 polarization in a concentration-dependent manner



Purified primary human pan-T cells were pre-treated with MRT-6160 for 24 hrs followed by αCD3/αCD28 TCR stimulation and analyses after 24 hrs (CD69, flow cytometry, *upper left*), 48 hrs (cytokines, MSD, *upper center*) or 96 hrs (proliferation, flow cytometry, *upper right*). Data are normalized to TCR-stimulated DMSO control. N = 5 donors. Purified primary human CD4⁺ cells were pre-treated with MRT-6160 for 24 hrs, followed by αCD3/αCD28 TCR stimulation and polarization towards the T_H17 subtype as indicated. After 3 days, IL-17A levels (pg/mL) in the supernatant were assessed by AlphaLISA *(lower)*. Representative data from N = 2 donors.

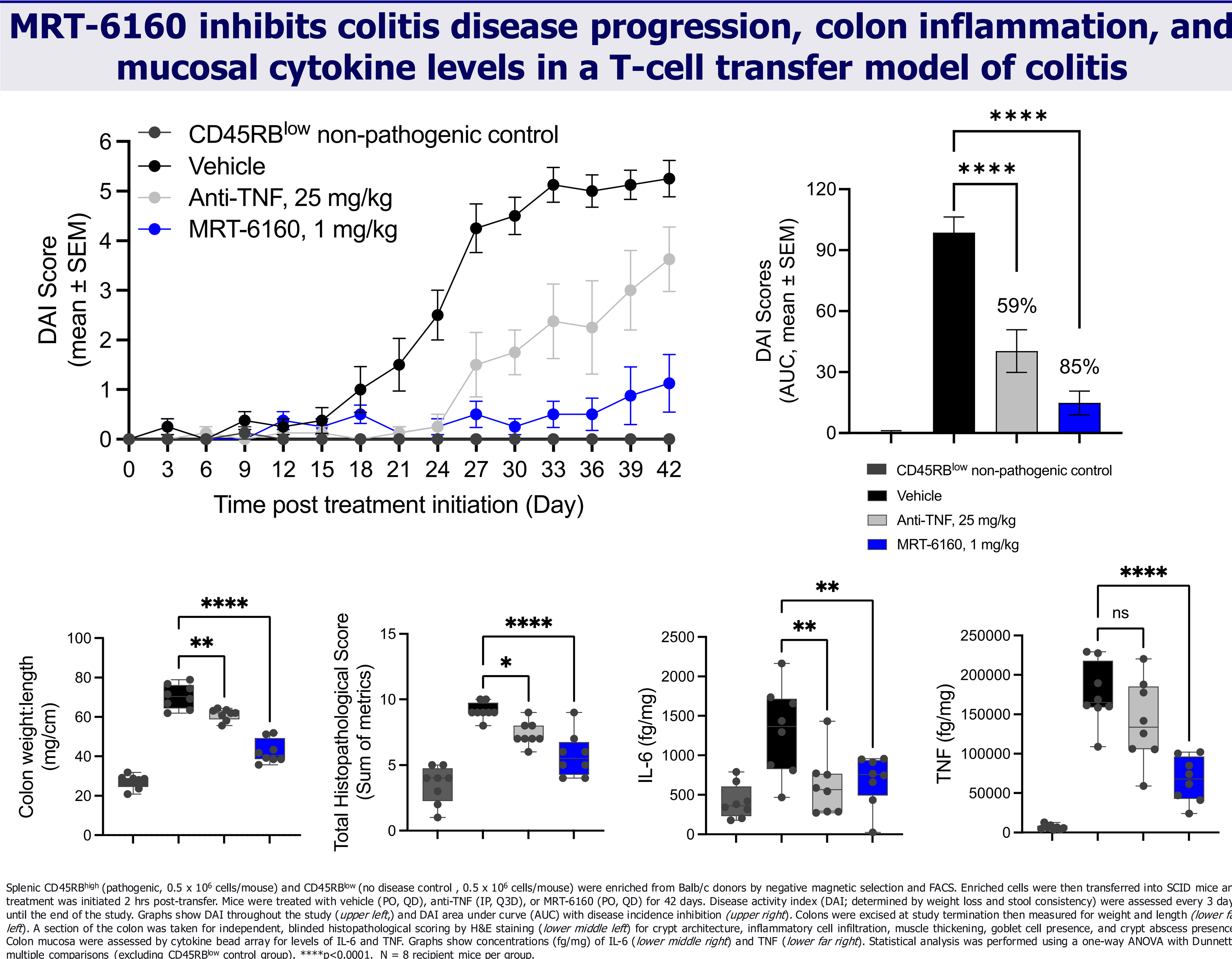
MRT-6160 reduces expression of inflammatory disease-associated T cell differentiation and chemokines and calprotectin subunit genes



Gene	L2FC	Adj. p-value	Function
Clec4e	-3.46	1.2×10^{-30}	Induces *Il1b* expression and T_H17 differentiation
S100a9	-3.40	3.6×10^{-9}	Calprotectin subunit, induces inflammatory activation of endothelial cells
S100a8	-3.24	8.8×10^{-7}	Calprotectin subunit, induces inflammatory activation of endothelial cells
Cxcl5	-2.90	7.6×10^{-10}	Ligand for CXCR2, induces migration and chemotaxis of T cells
Mmp3	-2.88	9.7×10^{-10}	Cleaves extracellular matrix proteins and activates pre-cursors of TNFα and IL-1β
Selenbp1	2.11	1.7×10^{-10}	Selenium-binding protein, associated with long-term remission in UC patients

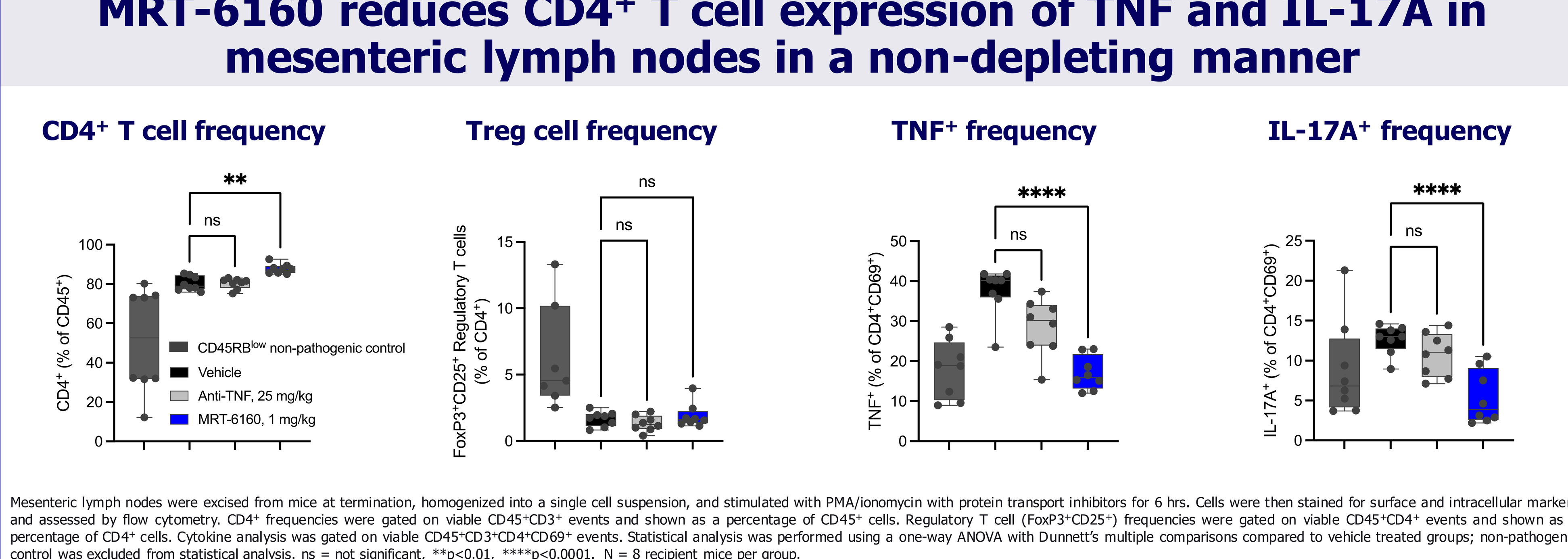
Colons were excised from mice at termination and stored in RNAlater. RNA was extracted from colon samples and quality was assessed by NanoDrop and Qubit. Gene counts were performing using the NanoString Autoimmune Profiling Panel and processed according to the manufacturer's instructions. Background signal was determined at twice the SD above the mean of the negative control. Data were normalized to 8 housekeeping genes and median of the ratio was used. Graphs show differentially expressed genes (DEG) in MRT-6160 vs vehicle treated mice. Table shows $\log_2$ fold change, p-value, and description of labelled genes in volcano plot.

MRT-6160 inhibits colitis disease progression, colon inflammation, and mucosal cytokine levels in a T-cell transfer model of colitis



Splenic $CD45RB^{high}$ (pathogenic, 0.5×10^6 cells/mouse) and $CD45RB^{low}$ (no disease control, 0.5×10^6 cells/mouse) were enriched from Balb/c donors by negative magnetic selection and FACS. Enriched cells were then transferred into SCID mice and treatment was initiated 2 hrs post-transfer. Mice were treated with vehicle (PO, QD), anti-TNF (IP, Q3D), or MRT-6160 (PO, QD) for 42 days. Disease activity index (DAI; determined by weight loss and stool consistency) were assessed every 3 days until the end of the study. Graphs show DAI throughout the study (*upper left*,) and DAI area under curve (AUC) with disease incidence inhibition *(upper right)*. Colons were excised at study termination then measured for weight and length *(lower far left)*. A section of the colon was taken for independent, blinded histopathological scoring by H&E staining (*lower middle left*) for crypt architecture, inflammatory cell infiltration, muscle thickening, goblet cell presence, and crypt abscess presence. Colon mucosa were assessed by cytokine bead array for levels of IL-6 and TNF. Graphs show concentrations (fg/mg) of IL-6 (*lower middle right*) and TNF (*lower far right*). Statistical analysis was performed using a one-way ANOVA with Dunnett's multiple comparisons (excluding $CD45RB^{low}$ control group). ****p<0.0001. N = 8 recipient mice per group.

MRT-6160 reduces CD4⁺ T cell expression of TNF and IL-17A in mesenteric lymph nodes in a non-depleting manner



Mesenteric lymph nodes were excised from mice at termination, homogenized into a single cell suspension, and stimulated with PMA/ionomycin with protein transport inhibitors for 6 hrs. Cells were then stained for surface and intracellular markers and assessed by flow cytometry. CD4⁺ frequencies were gated on viable CD45⁺CD3⁺ events and shown as a percentage of CD45⁺ cells. Regulatory T cell (FoxP3⁺CD25⁺) frequencies were gated on viable CD45⁺CD4⁺ events and shown as a percentage of CD4⁺ cells. Cytokine analysis was gated on viable CD45⁺CD3⁺CD4⁺CD69⁺ events. Statistical analysis was performed using a one-way ANOVA with Dunnett's multiple comparisons compared to vehicle treated groups; non-pathogenic control was excluded from statistical analysis. ns = not significant, **p<0.01, ****p<0.0001. N = 8 recipient mice per group.

Summary and Future Development

- MRT-6160 is a novel, potent, and selective VAV1 molecular glue degrader, which inhibits TCR-induced activation, cytokine production, proliferation, and T_H17 polarization.
- In a T-cell transfer model of colitis, MRT-6160 prevents disease progression, colon inflammation, and reduces pro-inflammatory cytokine production.
- Transcriptional analysis of colon tissue shows reduced expression of T_H17-associated, chemokine, and calprotectin subunit genes.
- These data suggest that MRT-6160 has strong potential to alleviate disease symptoms in multiple T-cell and/or T_H17 mediated autoimmune and inflammatory diseases including inflammatory bowel disease.
- MRT-6160 is a development candidate currently in IND-enabling studies.

References: 1. Schmidt *et al. Science* (2022); 2. Fujikawa *et al. J. Exp. Med.* (2003)

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All authors are employees of Monte Rosa Therapeutics