#POS1200: MRT-6160, a VAV1-Directed Molecular Glue Degrader, Reduces Joint Inflammation and Autoantibody Production in a Collagen-Induced Arthritis Autoimmune Disease Model

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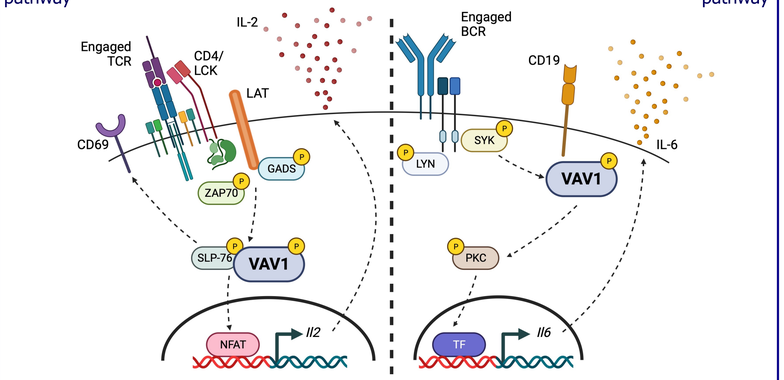
VAV1 is a guanine nucleotide exchange factor with a critical role in T- and B-cell receptor signaling and activity



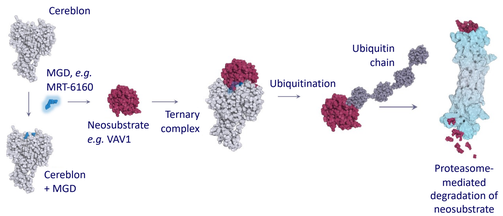
- VAV1 expression is highly restricted to immune cells
- VAV1 is required for antigen receptor-mediated signaling of T- and B-cells
- CRISPR-mediated[1] or genetic loss[2] of VAV1 is associated with decreased effector functions of both T and B cells

MRT-6160 is a rationally designed molecular glue degrader that selectively degrades VAV1 in human and mouse immune cells

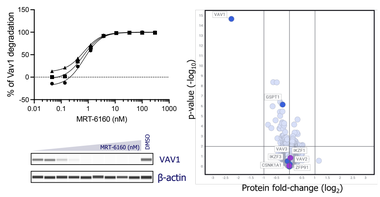
Molecular glue degraders (MGD) function to induce structural changes in ubiquitin ligases, such as cereblon, to drive the formation of ternary structures with a target protein.

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

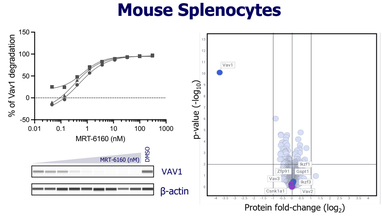
MGDs can induce degradation of otherwise 'undruggable' proteins as the mechanism does not require a classical binding pocket, contrary to conventional protein inhibitors, significantly increasing the target space and potential utility across a range of diseases.



Human PBMCs

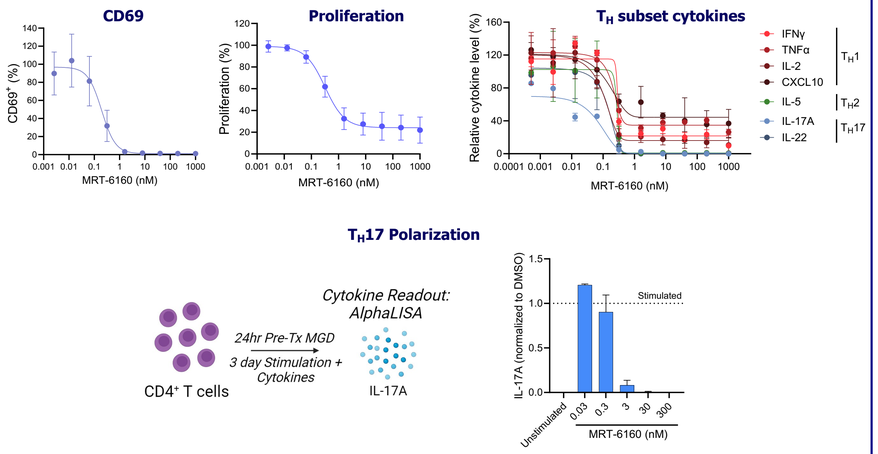


Mouse Splenocytes



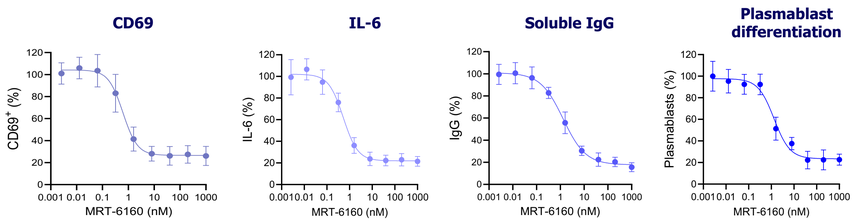
Human PBMCs and mouse splenocytes were treated overnight with dose-range of MRT-6160, after which VAV1 protein levels were assessed by JESS. Percentage (%) VAV1 degradation was calculated by normalizing VAV1 expression to β-actin loading control and shown as relative to DMSO control. Data from N = 3 biological replicates. 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-induced degradation of VAV1 attenuates T cell activation and effector functions



Upper row: Purified primary human pan-T cells were pre-treated with MRT-6160 for 24 hrs followed by αCD3/αCD28 TCR stimulation and subsequent analyses by flow cytometry (CD69, proliferation) or MSD (cytokines). N = 3 donors. *Lower row:* Purified primary human CD4+ T cells were treated with MRT-6160 for 24 hrs prior to polarization to a T_H17 phenotype by αCD3/αCD28 stimulation in the presence of IL-1β, IL-6, IL-23, TGFβ, αIFNγ, and αIL-4. IL-17A levels (pg/mL) in the supernatant were assessed by AlphaLISA after 3 days. N = 3 donors.

MRT-6160-induced degradation of VAV1 attenuates B cell activation and effector functions

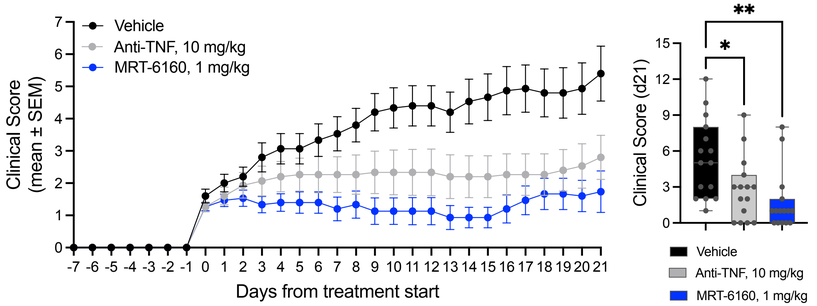


Purified primary human B-cells were pre-treated with MRT-6160 for 24 hrs followed by stimulation with anti-IgM and IL-4 and analysis of CD69 expression and IL-6 secretion 24 hrs post stimulation or stimulation with anti-IgM, sCD40L, IL-21, IL-2, and BAFF and analysis of soluble IgG and plasmablast differentiation on day 5 post stimulation. Data are normalized to respective stimulation DMSO control. N = 3 donors.

Summary and Future Development

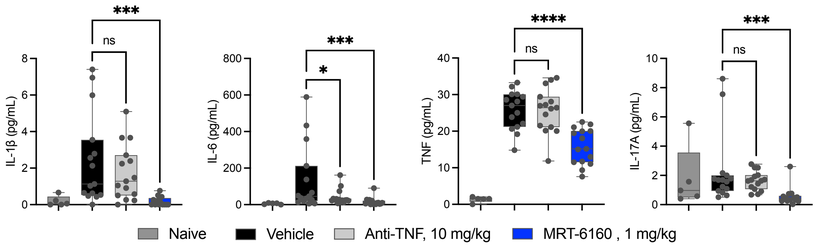
- MRT-6160 is a first-in-class VAV1 MGD that attenuates TCR- and BCR-mediated activity *in vitro* & *in vivo.*
- Degradation of VAV1 attenuates T/B cell activation, proliferation, effector functions, and differentiation.
- Therapeutic administration of MRT-6160-mediated degradation of VAV1 attenuates disease progression in a collagen-induced arthritis disease model.
- Degradation of VAV1 at disease onset attenuates serum pro-inflammatory cytokines and autoantibody production .
- Given the *in vitro* and *in vivo* MOA profile shown, MRT-6160 has strong potential to alleviate disease symptoms in multiple autoimmune and inflammatory diseases including rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, and psoriasis, amongst others.
- MRT-6160 is a development candidate with IND submission upcoming.

Oral dosing of MRT-6160 at disease onset attenuates disease progression in a collagen-induced arthritis (CIA) autoimmune murine disease model



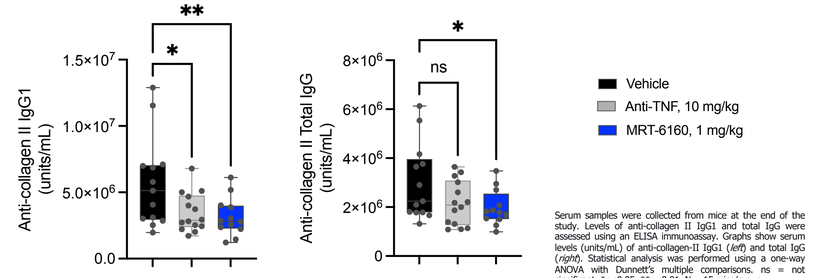
DBA/1 mice were immunized with bovine collagen-II emulsified in complete Freund's adjuvant on day 0 (intravenously), then boosted with chicken collagen-II emulsified in incomplete Freund's adjuvant on day 21 (subcutaneously). Mice were randomized into treatment groups following disease onset and treated with vehicle or MRT-6160 (PO QD) or anti-TNF (IP, TIW) for 21 days. Clinical scores (0–4) are the sum of individual paws from blinded assessment of inflammation and ankylosis. Graphs show longitudinal clinical scores (mean ± SEM, *left*) and clinical scores on day 21 (first to third quartile with min/max, *right*). Statistical analysis was performed using a one-way ANOVA with Dunnett's comparison. *p<0.05, **p<0.01. N = 15 mice/group.

MRT-6160 reduces serum levels of pro-inflammatory cytokines associated with rheumatoid arthritis



Serum samples were collected from mice at the end of the study. Pro-inflammatory cytokine levels were assessed by Mesoscale Discovery U-PLEX platform as per the manufacturer's instructions. Graphs show quantification of cytokines (pg/mL) for each indicated cytokine in naive, vehicle, anti-TNF, and MRT-6160 treatment groups. Naive group was excluded from statistical analysis and shown for comparison to diseased groups. Statistical analysis was performed using a one-way ANOVA with Dunnett's multiple comparisons. ns = not significant, *p>0.05, ***p<0.001, ****p<0.0001. N = 15 mice/group (diseased) or 5 mice/group (naive).

MRT-6160 reduces serum levels of T-cell-dependent anti-collagen II IgG1 and total IgG antibodies



Serum samples were collected from mice at the end of the study. Levels of anti-collagen II IgG1 and total IgG were assessed using an ELISA immunoassay. Graphs show serum levels (units/mL) of anti-collagen-II IgG1 (*left*) and total IgG (*right*). Statistical analysis was performed using a one-way ANOVA with Dunnett's multiple comparisons. ns = not significant, *p<0.05, **p<0.01. N = 15 mice/group.

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

All authors are employees of Monte Rosa Therapeutics