P809: MRT-6160, a VAV1-Directed Molecular Glue Degrader, Inhibits Disease Progression in a Preclinical Model of T/B-cell Mediated Experimental Autoimmune Encephalomyelitis

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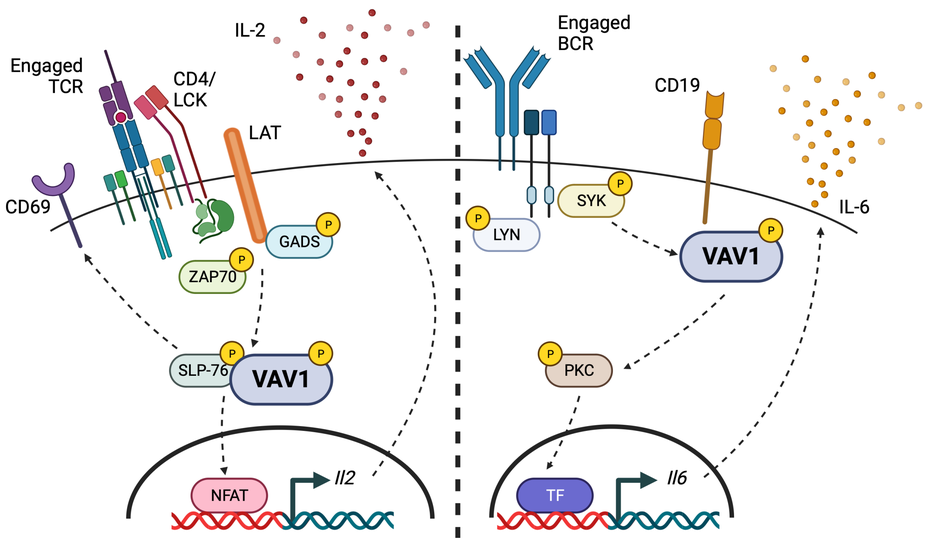
VAV1 is a guanine nucleotide exchange factor with a critical role in T- and B-cell receptor signaling and activity

T cell receptor VAV1 signaling pathway

B cell receptor VAV1 signaling pathway



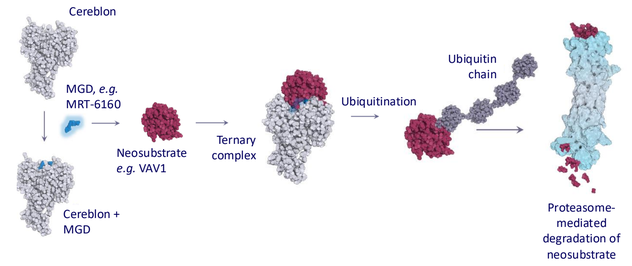
- VAV1 expression is highly restricted to immune cells
- VAV1 is required for antigen receptor-mediated signaling of T- and B-cells
- CRISPR-mediated[1] or genetic loss[2] of VAV1 is associated with decreased effector functions of T and B cells

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

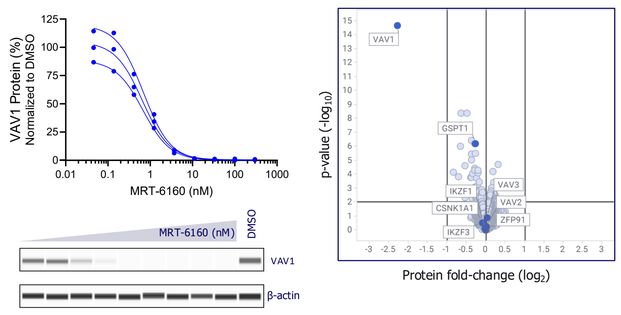
Molecular glue degraders (MGD) function to induce structural changes in ubiquitin ligases, such as cereblon, to drive the formation of ternary structures with a target protein.

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.

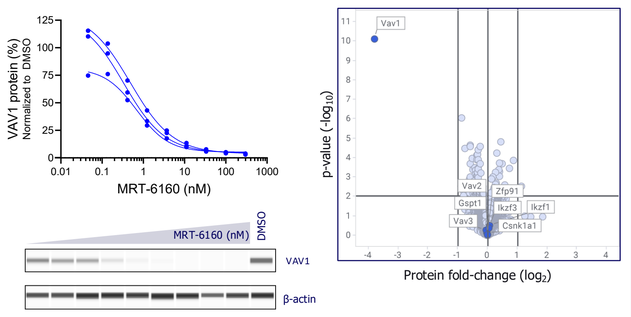
MGDs can induce degradation of otherwise 'undruggable' proteins as the mechanism does not require a classical binding pocket, contrary to conventional protein inhibitors, significantly increasing the target space and potential utility across a range of diseases.



Human PBMCs

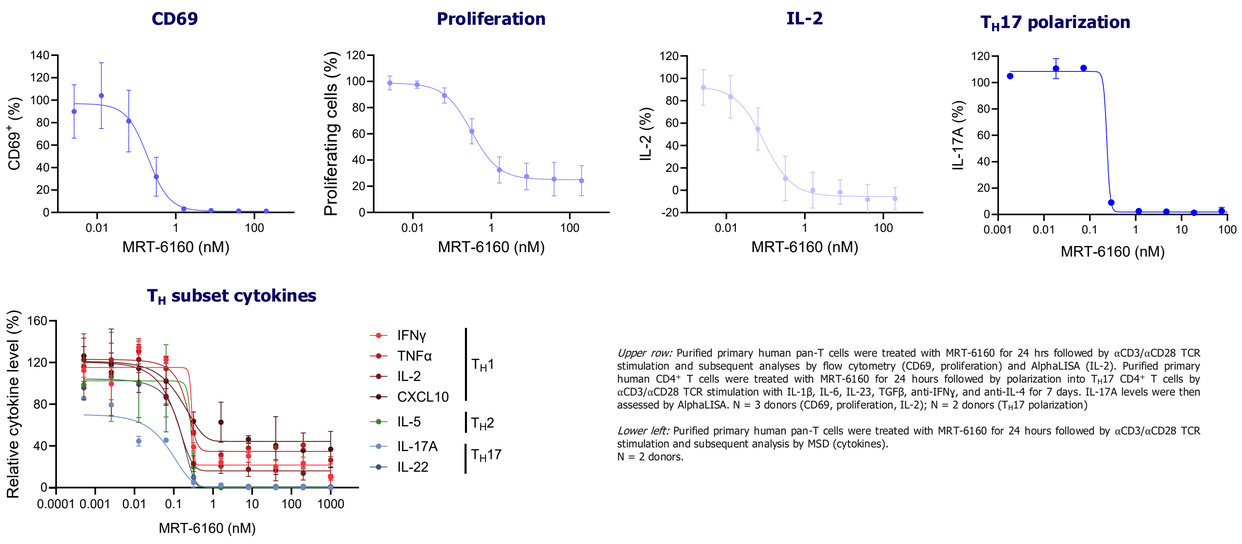


Mouse Splenocytes



Human PBMCs and mouse splenocytes were treated overnight with increasing concentrations of MRT-6160. VAV1 protein levels were then assessed by JESS. Percentage (%) VAV1 protein was calculated by normalising 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.

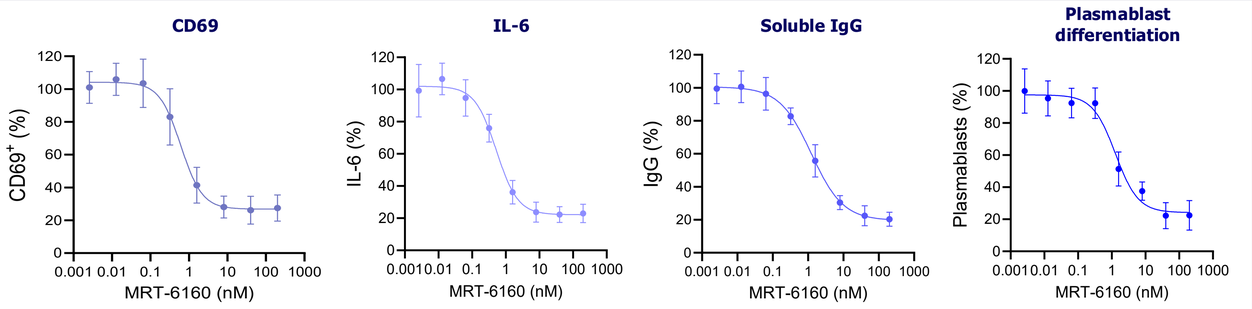
MRT-6160-induced degradation of VAV1 attenuates T cell activation and Th17 cytokine secretion



Upper row: Purified primary human pan-T cells were treated with MRT-6160 for 24 hrs followed by αCD3/αCD28 TCR stimulation and subsequent analyses by flow cytometry (CD69, proliferation) and AlphaLISA (IL-2). Purified primary human CD4+ T cells were treated with MRT-6160 for 24 hours followed by polarization into T_H17 CD4+ T cells by αCD3/αCD28 TCR stimulation with IL-1β, IL-6, IL-23, TGFβ, anti-IFNγ, and anti-IL-4 for 7 days. IL-17A levels were then assessed by AlphaLISA. N = 3 donors (CD69, proliferation, IL-2); N = 2 donors (T_H17 polarization)

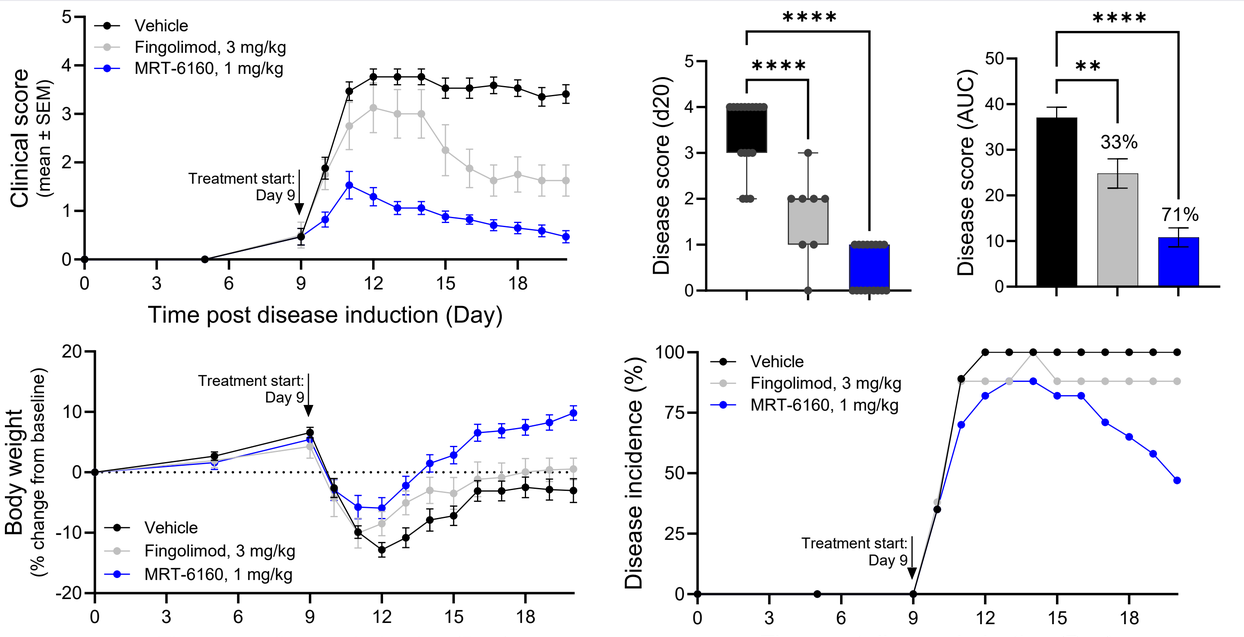
Lower left: Purified primary human pan-T cells were treated with MRT-6160 for 24 hours followed by αCD3/αCD28 TCR stimulation and subsequent analysis by MSD (cytokines).
N = 2 donors.

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



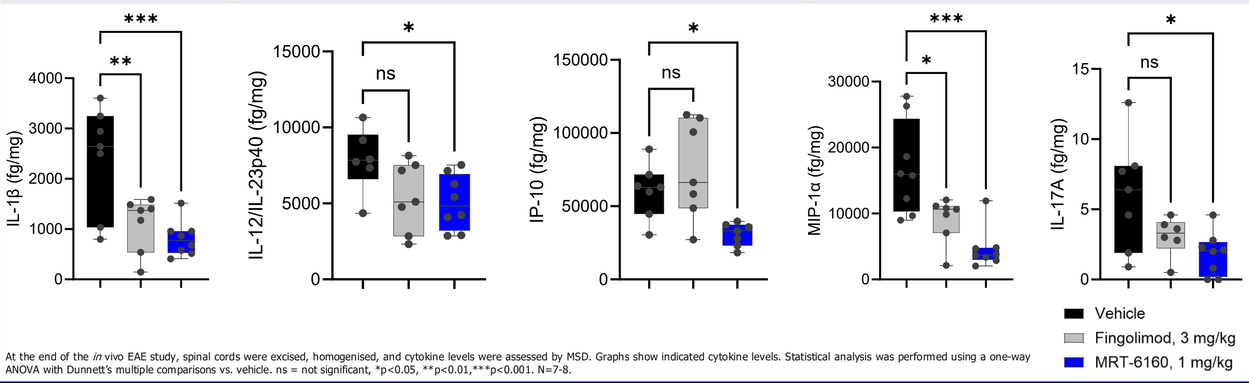
CD69 and IL-6: Purified primary human B-cells were pre-treated with MRT-6160 for 24 hrs followed by stimulation with anti-IgM and IL-4 and analyses of CD69 expression (flow cytometry) and IL-6 secretion (AlphaLISA) 24 hrs post stimulation. *Soluble IgG and plasmablast differentiation:* Purified primary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

Oral dosing of MRT-6160 attenuates disease progression and disease incidence in the MOG EAE disease model



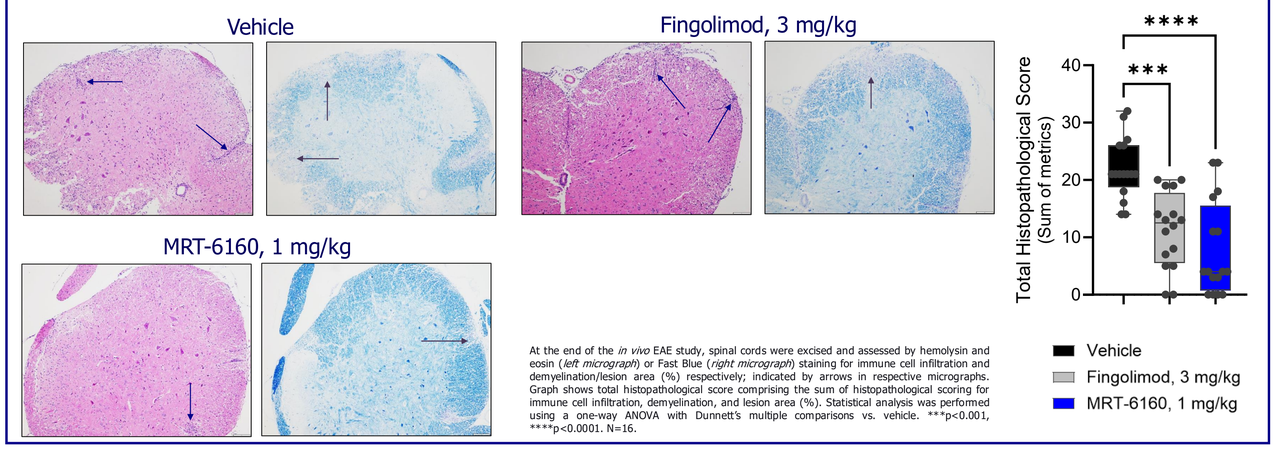
C57BL/6 mice were immunised with myelin oligodendrocyte protein (aa1-125) on Day 0 and injected intraperitoneally with pertussis toxin on Days 0 and 2. Upon onset of disease (Day 9), mice were treated PO QD with vehicle, fingolimod (3 mg/kg), or MRT-6160 (1 mg/kg) and assessed daily for disease scores (0-5, progressive weaking from tail to front paws). Graphs show disease scores over time (*upper left*), disease scores on Day 20 (*upper middle*), disease score area under the curve (AUC) (*upper right*), body weight change over time (*lower* left), and disease incidence (*lower right*). Statistical analysis was performed using a one-way ANOVA with Dunnett's multiple comparisons vs. vehicle. **p<0.01, ****p<0.0001.
N=16 (vehicle and MRT-6160) or N=8 (fingolimod).

MRT-6160 reduces spinal cord levels of pro-inflammatory cytokines associated with multiple sclerosis



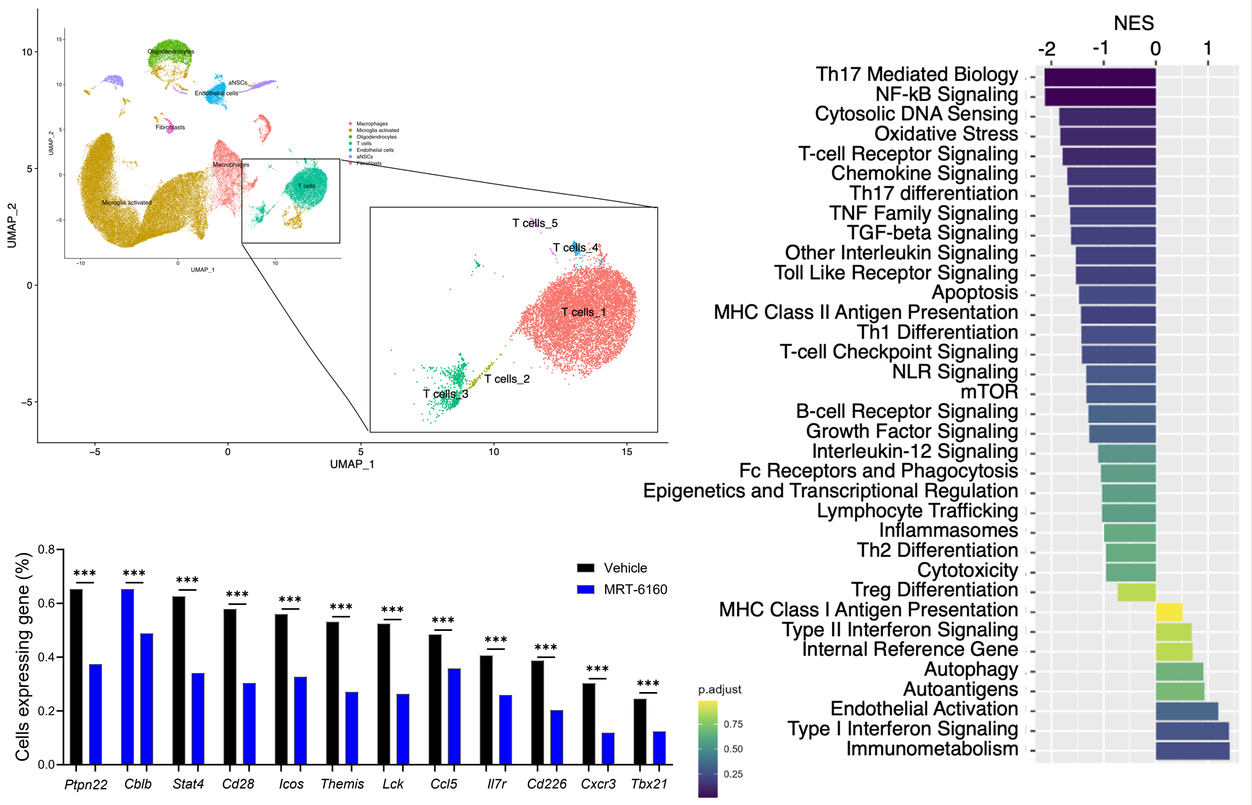
At the end of the *in* vivo EAE study, spinal cords were excised, homogenised, and cytokine levels were assessed by MSD. Graphs show indicated cytokine levels. Statistical analysis was performed using a one-way ANOVA with Dunnett's multiple comparisons vs. vehicle. ns = not significant, *p<0.05, **p<0.01,***p<0.001. N=7-8.

MRT-6160 reduces spinal cord immune cell infiltration, demyelination, and lesion area percentage



At the end of the *in vivo* EAE study, spinal cords were excised and assessed by hemolysin and eosin (*left micrograph*) or Fast Blue (*right micrograph*) staining for immune cell infiltration and demyelination/lesion area (%) respectively; indicated by arrows in respective micrographs. Graph shows total histopathological score comprising the sum of histopathological scoring for immune cell infiltration, demyelination, and lesion area (%). Statistical analysis was performed using a one-way ANOVA with Dunnett's multiple comparisons vs. vehicle. ***p<0.001, ****p<0.0001. N=16.

MRT-6160 reduces expression of T cell activation and differentiation gene sets in the spinal cord



At the end of the *in* vivo EAE study, spinal cords were excised, digested, and assessed by single-cell RNA sequencing (scRNA-seq). UMAP clustering was filtered on CD3-expressing cells (*upper* left). Graph shows percentage of cells expressing indicated genes across T cells in vehicle and MRT-6160-treated groups (*lower left*). Pathway enrichment analysis showing top pathways up- or down-modulated by MRT-6160 (*right*).

Summary and Future Development

- MRT-6160 is a first-in-class selective VAV1 molecular glue degrader with oral bioavailability
- Degradation of VAV1 inhibits T and B cell activation and effector functions
- Oral administration of MRT-6160 inhibits EAE disease progression, severity, and incidence
- Concomitant with disease scores, MRT-6160 reduced T cell activation and pro-inflammatory gene expression in the spinal cords of EAE mice
- MRT-6160-treated EAE mice showed reduced pro-inflammatory cytokine levels, immune cell infiltration, and demyelination within the spinal cord
- These data demonstrate that MRT-6160 inhibits EAE disease progression through reduction of T and B cell function
- Preclinical *in vivo* efficacy and MOA data suggest that MRT-6160 has the potential to treat multiple lymphocyte-mediated autoimmune and inflammatory diseases including multiple sclerosis, inflammatory bowel disease, rheumatoid arthritis, psoriasis, among others
- MRT-6160 has completed testing in Healthy Subjects (NCT06597799)
- Monte Rosa Therapeutics has a global exclusive license agreement with Novartis to advance VAV1 MGDs including MRT-6160

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

All authors are employees of Monte Rosa Therapeutics