#W135: 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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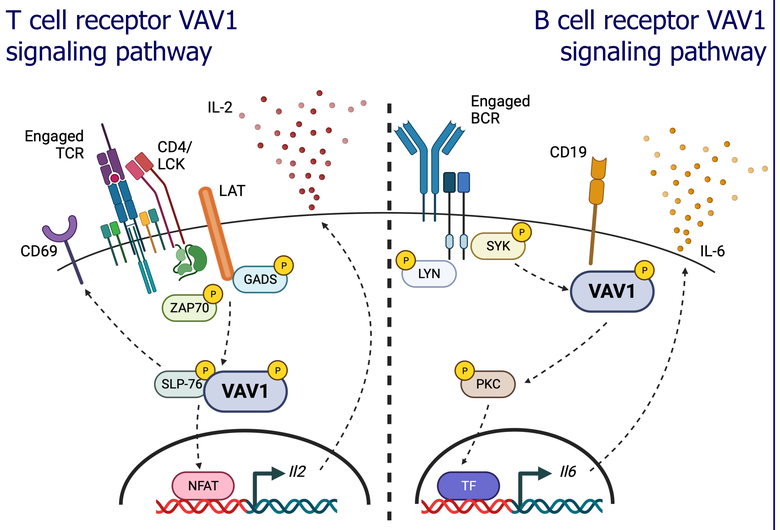
VAV1 is a guanine nucleotide exchange factor with a critical role in T- and B-cell receptor signaling and activity



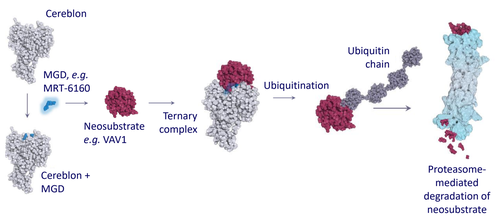
- VAV1 expression is highly restricted to immune cells
- VAV1 is required for antigen receptor-mediated signaling of T- and B-cells
- CRISPR-mediated[1] or genetic loss[2] of VAV1 is associated with decreased effector functions of both T and B cells

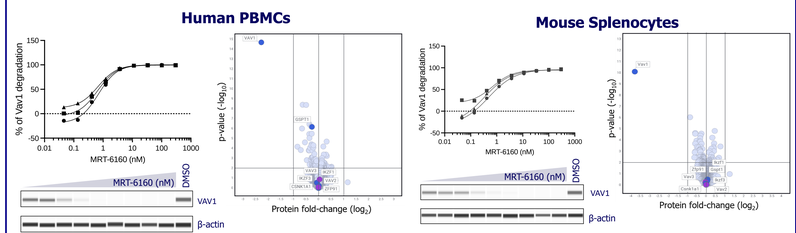
MRT-6160 is a rationally designed molecular glue degrader that selectively degrades VAV1 in human and mouse immune cells

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.

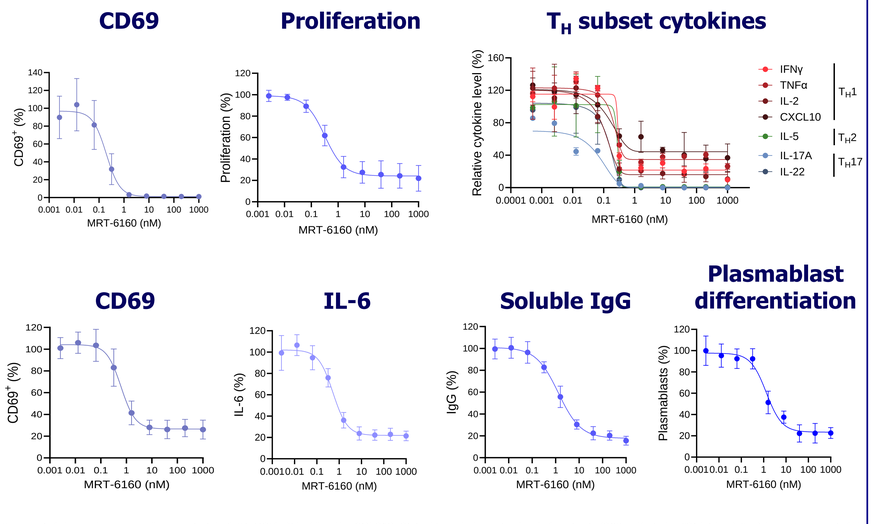
MGDs can induce degradation of otherwise 'undruggable' proteins as the mechanism does not require a classical binding pocket, contrary to conventional protein inhibitors, significantly increasing the target space and potential utility across a range of diseases.





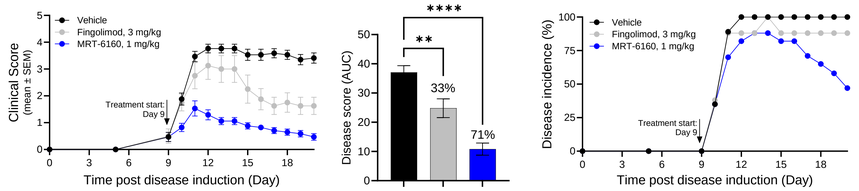
Human PBMCs and mouse splenocytes were treated overnight with a dose-range of MRT-6160, VAV1 protein levels were then assessed by JESS. Percentage (%) VAV1 degradation was calculated by normalizing VAV1 expression to β-actin loading control and shown as relative to DMSO control. Data from N = 3 biological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



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 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 or 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 normalized to respective stimulation DMSO control. N = 3 donors.

Oral dosing of MRT-6160 attenuates disease progression and reduces disease incidence in an experimental autoimmune encephalomyelitis disease model

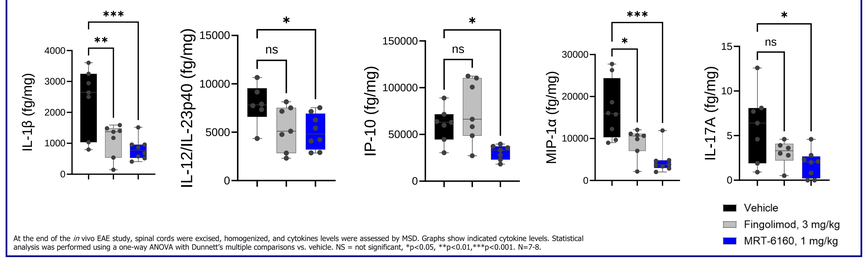


C57BL/6 mice were immunized with myelin oligodendrocyte protein (aa1-125) on Day 0 and injected intraperitoneally with pertussis toxin on Days 0 and 2. Upon onset of disease (Day 9), mice were treated PO QD with vehicle, fingolimod (3 mg/kg), or MRT-6160 (1 mg/kg) and assessed daily for disease scores (0-5, progressive weaking from tail to front paws). Graphs show disease scores (*left*), disease score area under the curve (AUC) (*center*), and disease incidence (*right*). Statistical analysis was performed using a one-way ANOVA with Dunnett's multiple comparisons vs. vehicle. **p<0.01, ****p<0.0001. N=17 (vehicle and MRT-6160) or 8 (fingolimod).

Summary and Future Development

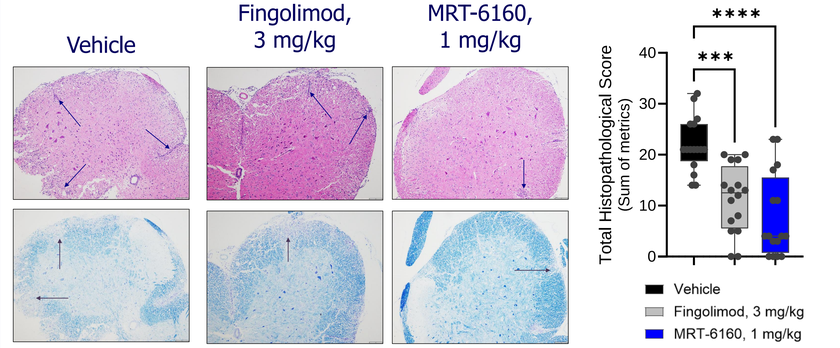
- MRT-6160 is a first-in-class selective VAV1 molecular glue degrader with oral bioavailability.
- Degradation of VAV1 inhibits T and B cell activation and cytokine production and inhibits B cell differentiation into plasmablasts and subsequent IgG production.
- Oral administration of MRT-6160 inhibits EAE disease progression, severity, and incidence concomitant with reduced pro-inflammatory cytokine levels in the spinal cord and reduced spinal cord immune cell infiltration and demyelination.
- MRT-6160 reduces expression of T cell activation and pro-inflammatory genes in the spinal cords of EAE mice.
- Given its *in vivo* efficacy and MOA profile, these data suggest that MRT-6160 has strong potential to alleviate disease symptoms in lymphocyte-mediated autoimmune and inflammatory diseases including inflammatory bowel disease, rheumatoid arthritis, multiple sclerosis, and psoriasis.
- MRT-6160 is currently being tested in Healthy Subjects (NCT06597799). Monte Rosa Therapeutics has a global exclusive license agreement with Novartis to advance VAV1 MGDs including MRT-6160.

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



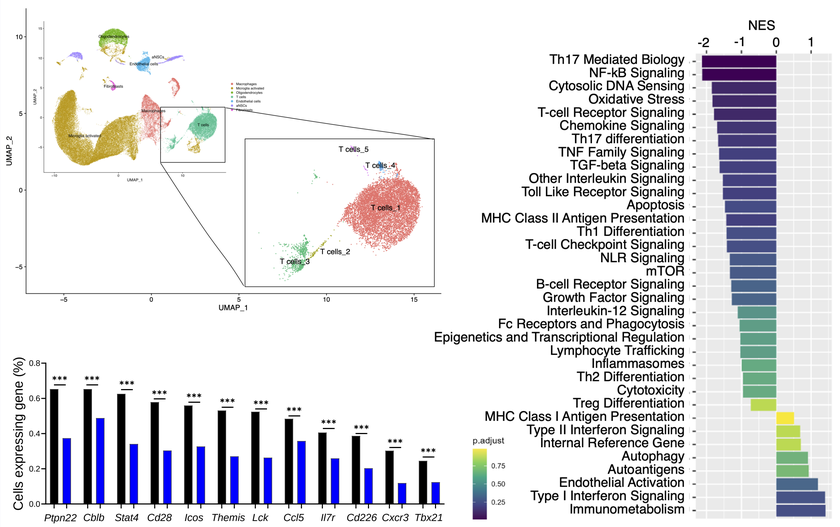
At the end of the *in* vivo EAE study, spinal cords were excised, homogenized, and cytokines levels were assessed by MSD. Graphs show indicated cytokine levels. Statistical analysis was performed using a one-way ANOVA with Dunnett's multiple comparisons vs. vehicle. NS = not significant, *p<0.05, **p<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*) 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). Analysis revealed downregulation of key genes involved in T-cell signalling in the MRT-6160-treated subgroup compared to the vehicle group, particularly within the T-cell subset. UMAP clustering was filtered on CD3-expressing cells (*upper* left). Graph showing 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*).

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

All authors are employees of Monte Rosa Therapeutics