#0009: MRT-6160, a VAV1-directed molecular glue degrader, attenuates T and B cell effector functions and inhibits disease progression in a spontaneous autoimmune MRL-Fas^{lpr} mouse model

Cartwright ANR[2], Gyger L[2], Desai F[1], Vora S[1], Kostikova A[2], Wang X[1], Trenh P[1], May K[1], Nguyen S[1], Chris King[1], Lam D[1], Lucas X[2], Zlotosch M[1], Liardo E[2], Lamberto I[1], Demarco B[1], Bonenfant D[2], Townson S[1], Krishnan E[1], Janku F[1], Castle J[1], McAllister L[2], Paterson A[1], Peluso M[1]

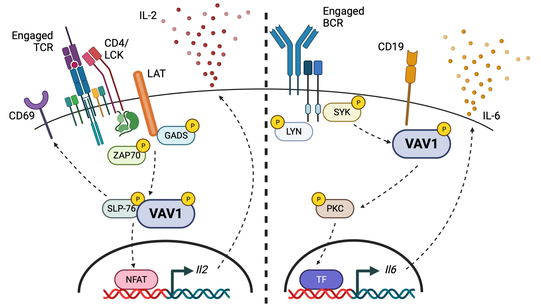
[1]*Monte Rosa Therapeutics Inc., 321 Harrison Ave, Boston, MA 02118, United States*

[2]*Monte Rosa Therapeutics AG, WKL-136.3, Klybeckstrasse 191, 4057 Basel, Switzerland*

VAV1 is a 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



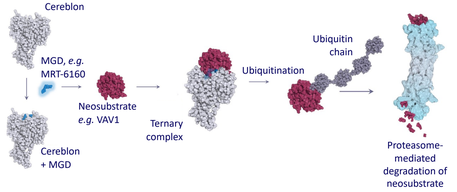
- VAV1 expression is highly restricted to immune cells
- VAV1 is required for antigen receptor-mediated signaling of T- and B-cells
- CRISPR-mediated[1] or genetic loss[2] of VAV1 is associated with decreased effector functions of both T and B cells

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

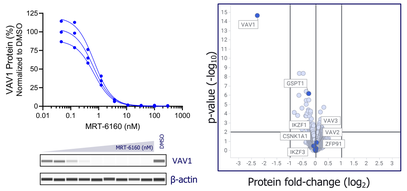
Molecular glue degraders (MGD) function to induce structural changes in ubiquitin ligases, such as cereblon, to drive the formation of ternary structures with a target protein.

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.

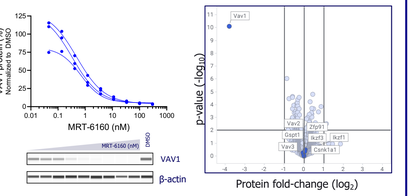
MGDs can induce degradation of otherwise 'undruggable' proteins as the mechanism does not require a classical binding pocket, contrary to conventional protein inhibitors, significantly increasing the target space and potential utility across a range of diseases.



Human PBMCs

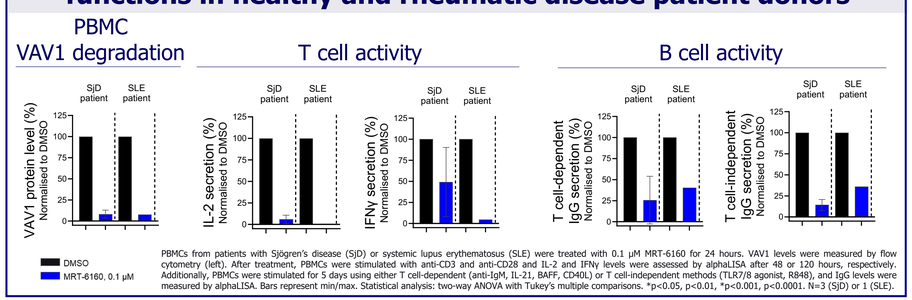


Mouse Splenocytes



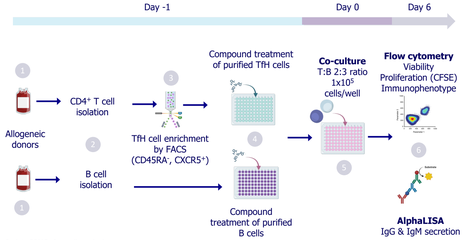
Human PBMCs and mouse splenocytes were treated overnight with dose-range of MRT-6160, after which VAV1 protein levels were assessed by JESS. Percentage (%) VAV1 degradation was calculated by normalizing VAV1 expression to β-actin loading control and shown as relative to DMSO control. Data from N = 3 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 or VAV family members; VAV2 and VAV3.

MRT-6160 degrades VAV1 and attenuates T and B cell effector functions in healthy and rheumatic disease patient donors

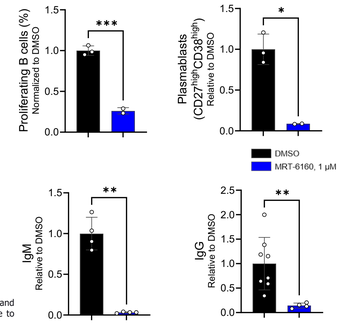


PBMCs from patients with Sjögren's disease (SjD) or systemic lupus erythematosus (SLE) were treated with 0.1 μM MRT-6160 for 24 hours. VAV1 levels were measured by flow cytometry (left). After treatment, PBMCs were stimulated with anti-CD3 and anti-CD28 and IL-2 and IFNγ levels were assessed by alphaLISA after 48 or 120 hours, respectively. Additionally, PBMCs were stimulated for 5 days using either T cell-dependent (anti-IgM, IL-21, BAFF, CD40L) or T cell-independent methods (TLR7/8 agonist, R848), and IgG levels were measured by alphaLISA. Bars represent min/max. Statistical analysis: two-way ANOVA with Tukey's multiple comparisons. *p<0.05, p<0.01, *p<0.001, p<0.0001. N=3 (SjD) or 1 (SLE).

MRT-6160 decreases TfH cell-mediated B cell activation, differentiation, and immunoglobulin secretion



1. Isolate PBMCs from allogenic donors
2. Purify CD4+ T cells or B cells from PBMCs using negative magnetic separation
3. Label CD4+ T cells with conjugated antibodies for FACS sorting of CD4, TCR, CXCR5 positive and CD45RA negative TfH cells
4. Seed and treat enriched TfH and B cells with 1 μM MRT-6160 individually for ~16 hours
5. Co-culture TfH and B cells in a 2:3 ratio while maintaining MRT-6160 in medium
6. Assess viability, proliferation and immunophenotype by flow cytometry, and secreted immunoglobulins and cytokines by AlphaLISA

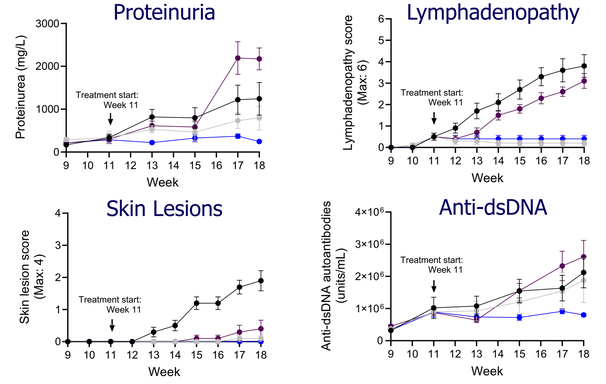


Cells were assessed by flow cytometry (*B cells: Viable CD45$^+$CD3$^-$CD19$^+$CD38$^-$; plasmablasts: Viable CD45$^+$CD3$^-$CD19$^+$CD38$^+$CD27$^+$*) and supernatants were assessed for IgM and IgG levels by alphaLISA. Graphs show cell frequencies or antibody levels (ng/mL) relative to DMSO. Bars show mean±SD. Statistical analysis was performed using a two-tailed unpaired t-test. *p<0.05, **p<0.01.

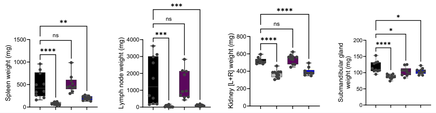
Summary and Future Development

- MRT-6160 is a first-in-class VAV1 MGD that **attenuates TCR/BCR activity** *in vitro* & *in vivo* attenuating T/B cell effector functions and differentiation.
- Oral administration of MRT-6160 **attenuates proteinuria, lymphadenopathy, skin lesion formation, autoantibody production, organomegaly, and histopathology** in an MRL-Fas^{lpr} mouse model.
- Given its *in vitro* and *in vivo* MOA profile, MRT-6160 has **strong potential to alleviate disease symptoms in multiple autoimmune and inflammatory diseases** including rheumatic diseases such as Sjögren's disease, SLE, rheumatoid arthritis and others.
- MRT-6160 is exclusively licenced by Novartis and has **completed assessment in a Phase I healthy volunteer study** (NCT06597799).

MRT-6160 inhibits key disease readouts in a spontaneous autoimmune MRL-Fas^{lpr} model

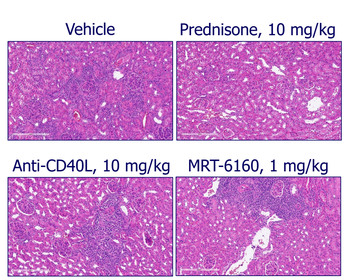


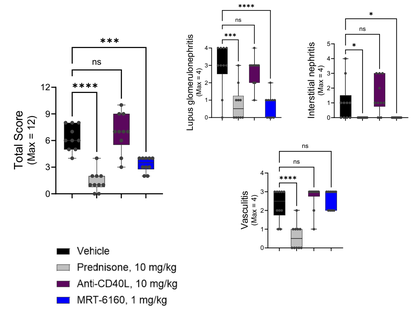
Terminal Organ Weights



MRL-Fas^{lpr} mice were treated with vehicle (PO QD), prednisone (10 mg/kg, PO QD), anti-CD40L (10 mg/kg, IP Q3D), or MRT-6160 (1 mg/kg, PO QD) from age 11 to 18 weeks. Mice were assessed from 9 weeks of age weekly for lymphadenopathy, skin lesions and fortnightly for proteinuria and serum anti-dsDNA levels. Lymph nodes, spleen, kidneys, and submandibular salivary glands were collected at study termination and weighed. Line graphs show in-life assessments where points represent mean±SEM. Min/max graphs show organ weights at study termination. 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 = 10 mice per group.

MRT-6160 reduces kidney glomerular and interstitial nephritis in MRL-Fas^{lpr} mice





MRL-Fas^{lpr} mice were treated with vehicle (PO QD), prednisone (10 mg/kg, PO QD), anti-CD40L (10 mg/kg, IP Q3D), or MRT-6160 (1 mg/kg, PO QD) from age 11 to 18 weeks. At termination of the study, kidneys were excised and assessed by histopathology using hemoxylin and eosin staining. Images are 200X magnification. Total histopathological scoring was determined by the sum of the metrics as follows. Lupus glomerulonephritis: 0, normal; 1, slight infiltration of mesangium; 2, increased infiltration of mesangium; 3, thickened basement membrane and lobular formation; 4, tubular casts, atrophy, glomerular crescent formation, and sclerosis. Interstitial nephritis: 0, normal with no infiltration; 1, occasional small pockets of mononuclear cell infiltration (<15 cells); 2, focal infiltration of mononuclear cells (≥16 cells); 3, multifocal and extensive mononuclear cell infiltration and extensive necrosis. Vasculitis: 0, normal with no infiltration; 1, occasional mononuclear cell perivascular infiltration; 2, several foci of mononuclear cells with no necrosis; 3, multifocal perivascular infiltration with/without necrosis; 4, multifocal and/or diffuse perivascular infiltration with extensive necrosis. Bars show min/max. 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.

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

All authors are employees of Monte Rosa Therapeutics