VAV1-Induced Proximity: Insights from Molecule Glue Profiling

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Background

Molecular glue degraders (MGDs) induce the proximity of target proteins to E3 ubiquitin ligases, leading to target protein ubiquitination and subsequent degradation by the proteasome. Unlike conventional inhibitors that target enzymatic activity, these molecules enable the degradation of proteins previously deemed "undruggable," thereby rendering them "druggable". With a first-in-class VAV1-directed MGDs (MRT6160) enters in clinical phase I, VAV1 degraders showed the remarkable potential in Immunology and inflammatory diseases such as rheumatoid arthritis and colitis. Current advancements highlight its ability to reduce proinflammatory cytokine production, inhibit pathogenic T cell polarization, and mitigate autoimmune responses.

Our integrated platform offers a comprehensive approach to exploring VAV1-targeting MGDs through a suite of state-of-the-art in vitro assays. These assays include the detection of binary and ternary complexes, intracellular protein interactions, and target degradation analysis using HiBiT assays. Additionally, the platform incorporates functional evaluations of T and B cell activities, cytokine profiling, and proteomics studies. Together, these components provide deep insights into the selectivity and potential off-target effects of these novel therapeutic agents.

Assay Summary Table

Binary/Ternary Complex Formation Assay		IC50 or Kd, nM		
HTRF biochemical assay	Binary	1217		
	Ternary	hVAV1	hVAV2	hVAV3
		18.35	>10,000	>10,000
		mouse/rat	dog	cyno
		18.04/14.28	14.08	17.89
Spectral shift biophysical assay		Binary: Kd=511 Ternary: Kd=16		
Cellular NanoBRET assay		IC50=18.45		

VAV1 degradation		DC50, nM			
Jurkat by HiBiT		8.98			
JESS analysis		Jurkat	T cell	B cell	PBMC
		3.79	0.65	0.94	0.99
Functional assays		**IC50,nM**			
T cells	Proliferation (Flow cytometry)	1.48			
	CD69 detection (Flow cytometry)	0.32			
	IL-2 secretion	0.35			
B cells	CD69 detection (Flow cytometry)	0.77			
	IL-6 secretion	0.75			
TH17 cell	IL-17A secretion by ELISA	0.08			

Summary

The common challenges of VAV1-like targets, such as complex ternary formation, stringent selectivity, and functional validation, are precisely the strengths of our platform. By addressing these commonalities through robust assay development and scalable workflows, we provide a universal solution for discovering and optimizing MGDs with broad therapeutic potential. Our platform doesn’ t just target VAV1—it enables researchers to uncover and exploit the shared principles of molecular glue activity, driving innovation across a wide range of targets.

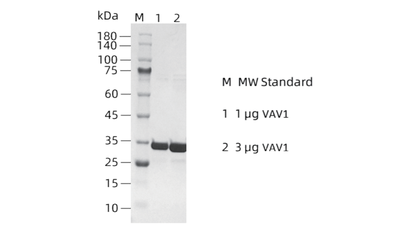
References

[1] Application of induced proximity for therapeutic discovery. Cacace, Angela et al. Cell Chemical Biology, Volume 31, Issue 6, 1036 – 1038.
[2]. Neurath, M.F. and Berg, L.J. (2024) ‘Vav1 as a putative therapeutic target in autoimmune and chronic inflammatory diseases’, Trends in Immunology, 45(8), pp. 580–596. doi:10.1016/j.it.2024.06.004.
[3]. A vav1-directed molecular glue degrader, MRT-6160, reduces joint inflammation in a collagen-induced arthritis autoimmune disease model (no date) ACR Meeting Abstracts. Available at: https://acrabstracts.org/abstract/a-vav1-directed-molecular-glue-degrader-mrt-6160-reduces-joint-inflammation-in-a-collagen-induced-arthritis-autoimmune-disease-model/ (Accessed: 22 January 2025).

Binary/Ternary Complex Formation Assay

To comprehensively evaluate binary and ternary complex formation in molecular glue studies, we have developed and applied multiple complementary assays:

a)Protein Expression and Purification



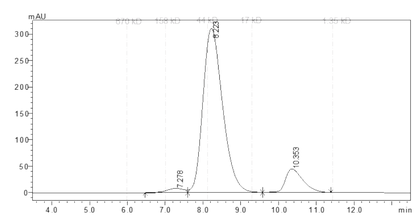


Figure 1: Diagram of VAV1 Structure and Analysis of Purified VAV1 Protein.

b) CRBN Binary/Ternary Formation Assay Using TR-FRET

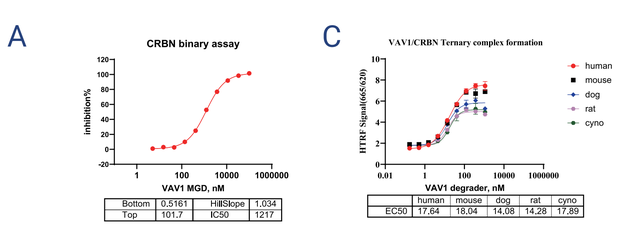


Figure 2: Example data of CRBN&MGD binding A) and CRBN/VAV1 ternary complex formation B).

c) Dianthus Spectral Shift Assay

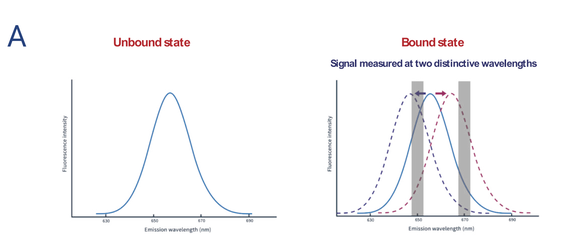


Figure 3. A) Mechanism of Spectral Shift assay; B) Example data of binary binding (CRBN and VAV1 degrader) and ternary complex formation(CRBN, VAV1 and VAV1 degrader).

d) NanoBRET PPI Assay

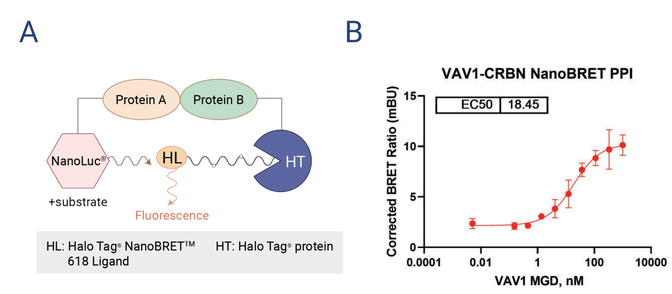


Figure 4: A) Mechanism of NanoBRET PPI assay. B) Example Data: NanoBRET PPI assay results for VAV1-CRBN interaction, demonstrating signal changes upon MGD treatment.

Degradation and Selectivity Analysis

a)VAV1 Degradation Analysis by HiBiT Assay and Jess system

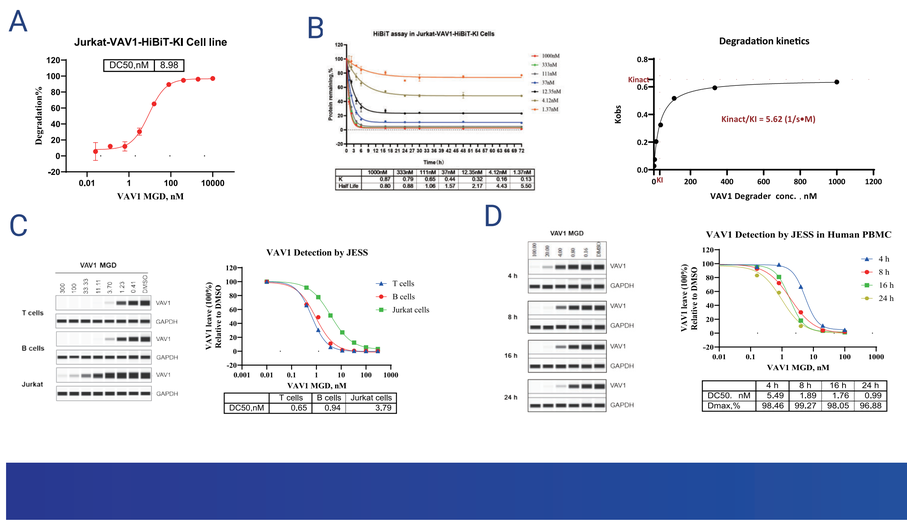
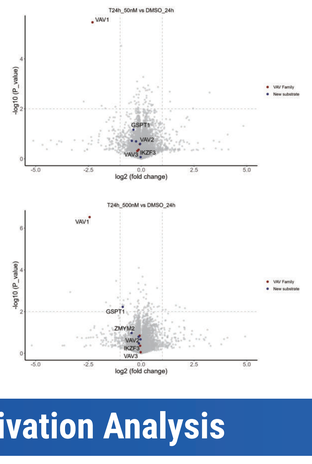


Figure 5: A) and B) VAV1 degradation data in Jurkat-VAV1-HiBiT-KI cells. C) VAV1 degradation assay in Jurkat, T and B cells. D) Time-dependent VAV1 degradation: data from PBMC cells.

b) Selectivity of VAV1 MGD Assessed by DIA Proteomics

Data-Independent Acquisition (DIA) proteomics was used to assess the selectivity of the test compound in a dose-dependent manner. DIA proteomics confirmed the molecule's strong target degradation activity and high selectivity.



Automated Platform OT2:
For Magnetic Nanomaterials Enzymatic Digestion

- High Stability
- High Sensitivity
- High Throughput – 96 samples in 6 hours

Most Advanced MS Platform Orbitrap Astral:
For High-throughput DIA Acquisition

- High Sensitivity (single cell)
- High Throughput (60~180 Samples per day, SPD)
- High Resolution (80000)
- In-depth Coverage – more than 5000 genes per run

- Faster
- Lower cost
- Proteomics at early stage

Figure 6: Human PBMCs were treated with 50 nM or 500 nM VAV1 MGD or DMSO control for 24 hours.

Immune Cell Activation Analysis

VAV1 associates with TCR and BCR signaling pathways and its degradation attenuates immune cell activation, proliferation, and cytokine production.

a)Degradation of VAV1 inhibits T/B cell activation and effector functions

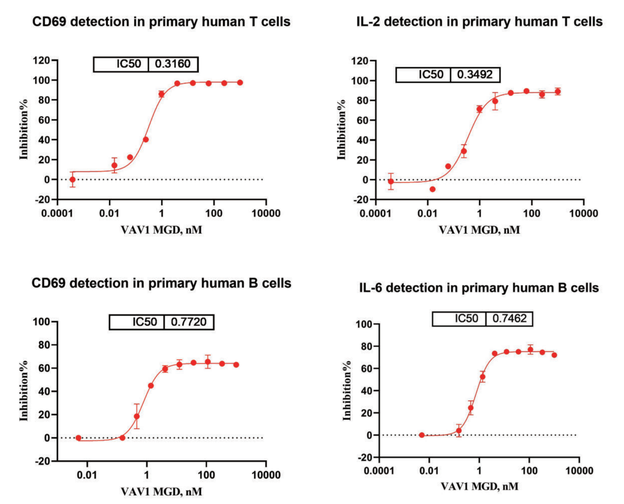


Figure 7: Primary human pan-T cells and B-cells were pre-treated with compound for 24 hrs. T cells were then stimulated with CD3/CD28 for analyses at 24 hrs (A. CD69 expression), 48 hrs (B. IL-2 secretion), and 96 hrs (C. cell proliferation). B cells were stimulated with anti-IgM + IL-4 for analyses at 24 hrs (D. CD69 expression and E. IL-6 secretion).

b) Degradation of VAV1 inhibits CD4+ TH17 polarization

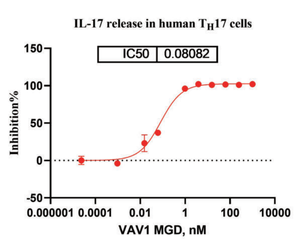
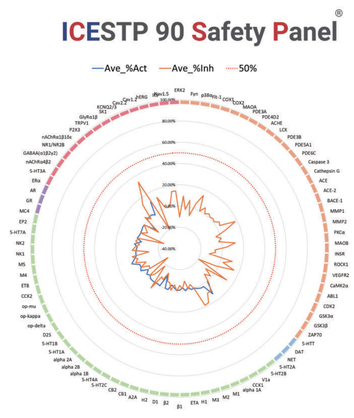


Figure 8: Primary human CD4+ T cells were pre-treated with compound for 24 hrs, followed by CD3/CD28 TCR stimulation and polarization towards the TH17 subtype as indicated (IL-6, TGF β1, IL-1β, IL-21, IL-23, IL-4 and IFN-γ). After 5 days, IL-17A levels (pg/mL) in the supernatant were assessed by ELISA.

c) Off-target profiling by 90 Safety panel



- All Functional assay
- Both agonist and antagonist modes are available

Targets	SafetyOne-44	SafetyMAX-90
GPCR	24	37
Ion Channel	8	15
Enzyme	7	32
Nuclear Receptor	2	3
Transporter	3	3
Total Functional Assays	74	138
TAT	3 weeks	6 weeks

Figure 9: Example of VAV1 degrader that showed no off-target effects i ICESTP 90 Safety Panel®