Molecule Glue Profiling Unveils the Dynamics of VAV1-Induced Proximity

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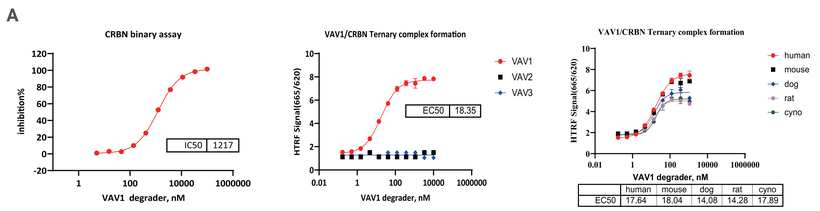
Abstract Number:1665

Abstract

Molecular glue degraders (MGDs) promote the proximity of target proteins to E3 ubiquitin ligases, enabling degradation of previously "undruggable" proteins. VAV1-targeting MGDs, like MRT6160, show promise in treating immunological diseases by reducing inflammation and autoimmune responses. Our integrated platform uses advanced in vitro assays to explore these MGDs, including complex detection, intracellular protein interaction analysis, and degradation studies. It also evaluates T and B cell activities and cytokine profiles. By addressing challenges such as complex formation and selectivity, our platform provides a universal solution for discovering and optimizing MGDs with broad therapeutic potential.

CRBN Binary/Ternary Formation Analysis Using Biochemical, Biophysical and Cellular based assay



	human	mouse	dog	rat	cyno
EC50	17.64	18.04	14.08	14.28	17.89

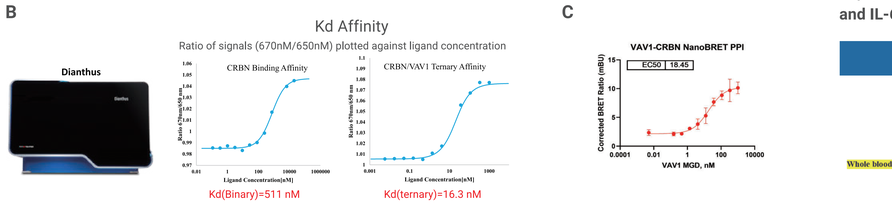
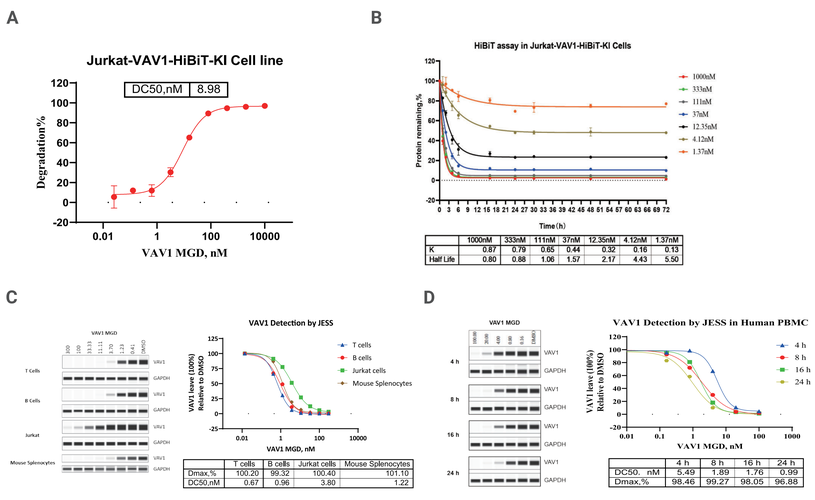


Figure 1: CRBN&MGD binary binding and CRBN/VAV1 ternary complex formation using TR-FRET A) ,Dianthus spectral shift assay B) and cellular NanoBRET assay C).

VAV1 Degradation Analysis by HiBiT Assay and Jess system



	1000nM	333nM	111nM	37nM	12.35nM	4.12nM	1.37nM
K	0.87	0.79	0.65	0.44	0.32	0.16	0.13
Half Life	0.80	0.88	1.06	1.57	2.17	4.43	5.50

	T cells	B cells	Jurkat cells	Mouse Splenocytes
Dmax,%	100.20	99.32	100.40	101.10
DC50,nM	0.67	0.96	3.80	1.22

	4 h	8 h	16 h	24 h
DC50, nM	5.49	1.89	1.76	0.99
Dmax,%	98.46	99.27	98.05	96.88

Figure 2: A) and B) VAV1 degradation data in Jurkat-VAV1-HiBiT-KI cells. C) VAV1 degradation assay in Jurkat, T,B and mouse splenocytes. D) Time-dependent VAV1 degradation in PBMC cells.

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

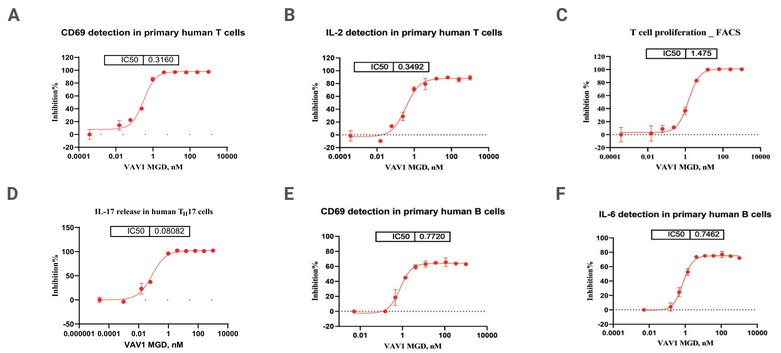


Figure 3: Primary human pan-T cells, CD4+ T cells, and B cells were pre-treated with compound for 24 hrs. T cells were stimulated with CD3/CD28 for analyses of CD69 expression (24 hrs, A), IL-2 secretion (48 hrs, B), and cell proliferation (96 hrs, C). CD4+ T cells were stimulated with CD3/CD28 and polarized to the TH17 subtype. After 5 days, IL-17A levels were measured by ELISA (D). B cells were stimulated with anti-IgM + IL-4 for CD69 expression and IL-6 secretion (24 hrs)(E and F).

In vivo assay of mVAV1 Degradation

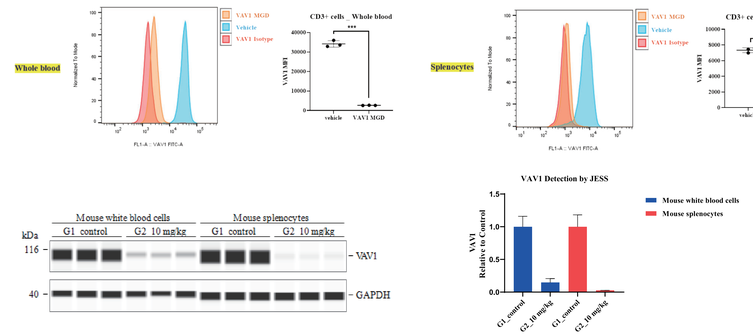


Figure 4: Oral dosing of VAV1 MGD in C57BL/6J mice, whole blood and spleen were sampling 24h later. mVAV1 degradation were analyzed by flow cytometry and Jess.

Selectivity analysis using proteomics and Off-target profiling by 90 Safety panel

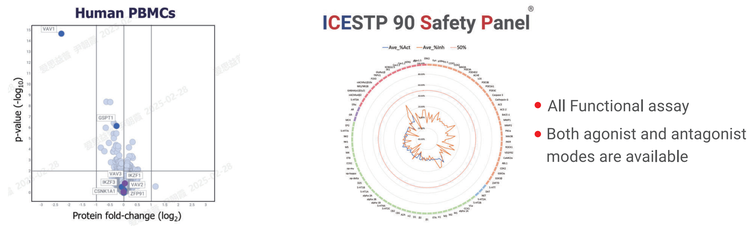
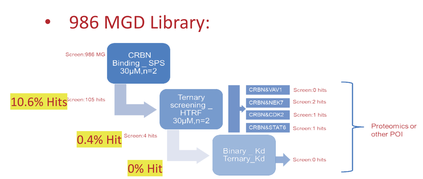


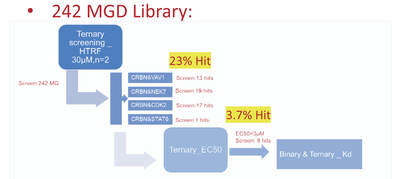
Figure 5: Selectivity by DIA based proteomics analysis and off-target profiling by ICESTP 90 Safety Panel®

CRBN related Molecular Glue library

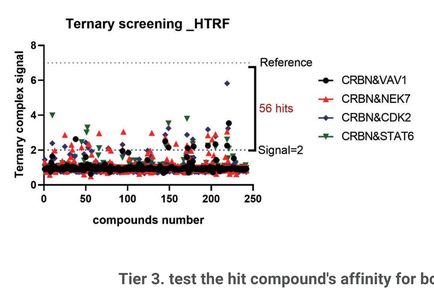
● ~1,000 compounds ● High diversity covered more scaffolds ● Powder or solution(384 plate)

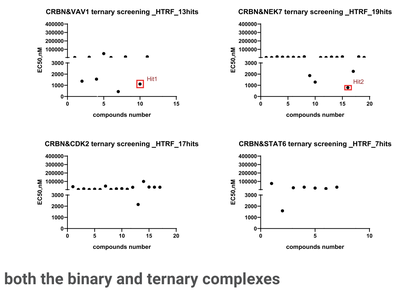


Tier 1. Ternary point screening_SPS:56hits

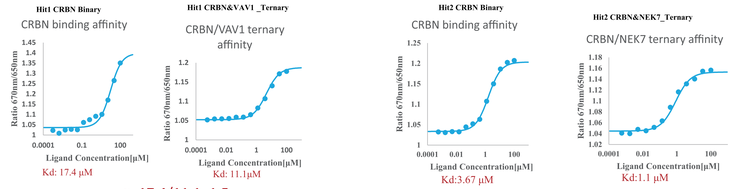


Tier 2. Ternary DRC _HTRF:10 hits





Tier 3. test the hit compound's affinity for both the binary and ternary complexes



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			