HARNESSING INDUCED PROXIMITY STRATEGIES FOR THERAPEUTIC DISCOVERY TARGETING VAV1

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Abstract

Induced proximity, which utilizes small molecules to facilitate an interaction between two proteins to harness natural biological pathways, represents a revolution in synthetic chemistry and drug discovery [1]. This area has gained significant clinical traction, with targeted protein degradation leading the way. One example is Molecular glue degraders (MGDs), which induce the proximity of target protein to E3 ubiquitin ligases, leading to target ubiquitination and degradation. The mechanism is different from traditional inhibitors in drug discovery and broaden the approach, especially for the target used to be considered “undruggable” . MRT-6160, a first-in-class VAV1-directed MGDs currently in clinical phase I, showed the remarkable potential in Immunology and inflammatory diseases such as rheumatoid arthritis and colitis [2][3]. 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.

CRBN Binary/Ternary Formation Analysis Using Biochemical, Biophysical and Cellular Based Assay

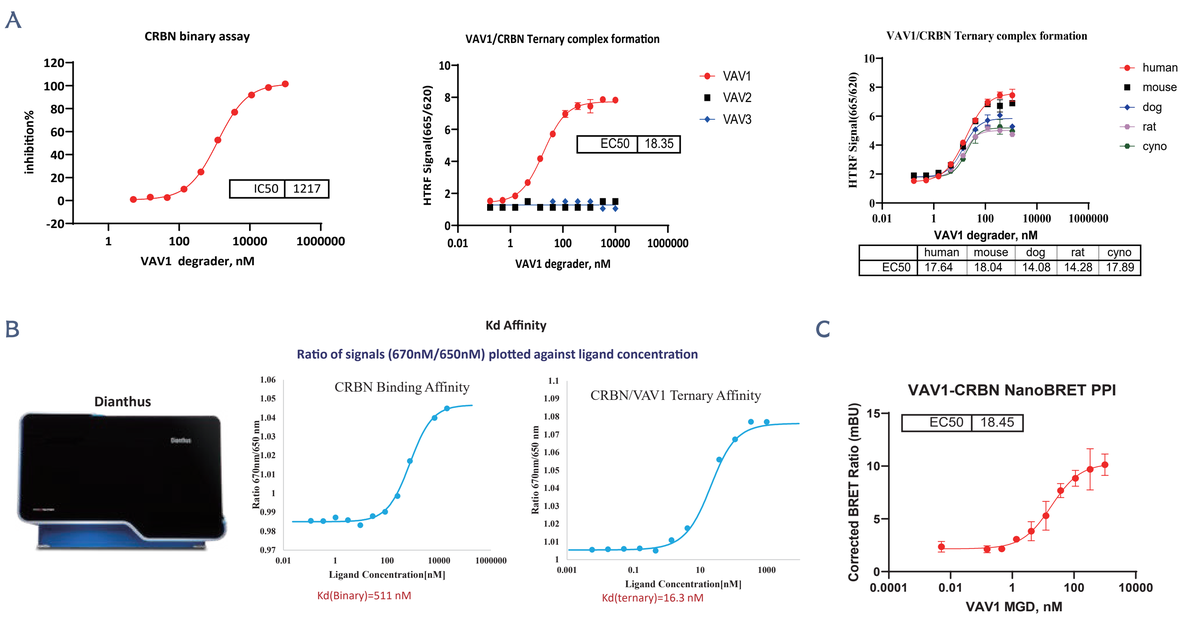

Figure 1: CRBN&MGD binary binding and CRBN/VAV1 ternary complex formation using TR-FRET A) ,Dianthus spectral shift assay B) and cellular NanoBRET assay C).

VAV1 Degradation Analysis by HiBiT Assay and Jess System

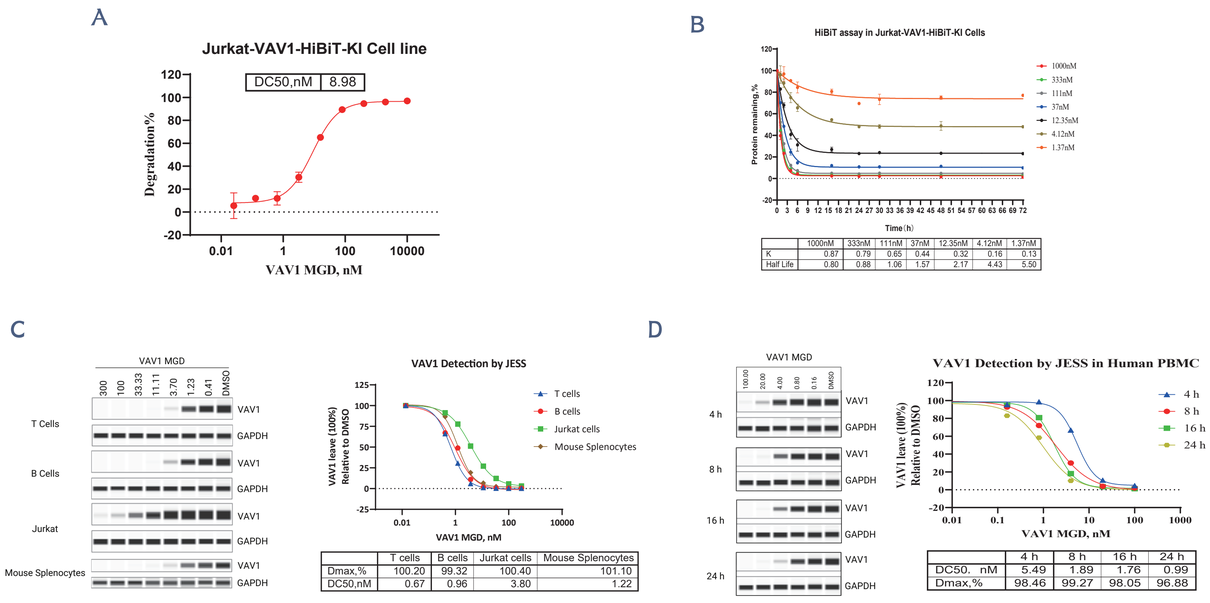

Figure 2: A) and B) VAV1 degradation data in Jurkat-VAV1-HiBiT-KI cells. C) VAV1 degradation assay in Jurkat, T,B and mouse splenocytes. D) Time-dependent VAV1 degradation in PBMC cells.

Degradation of VAV1 Inhibits T/B Cell Activation and Effector Functions

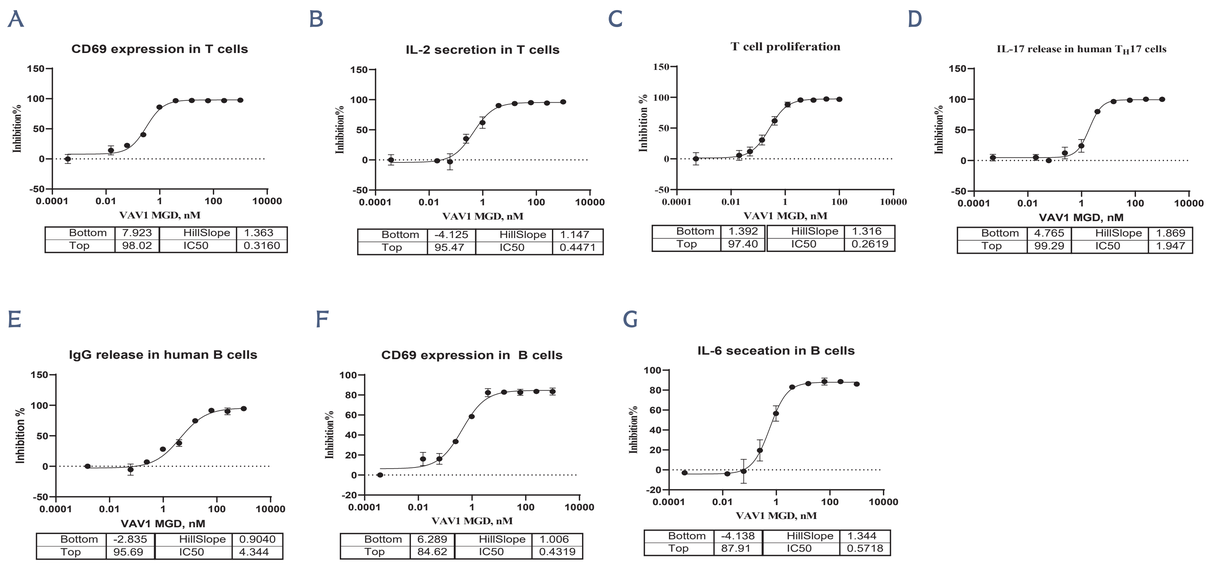

Figure 3: Primary human pan-T cells, CD4+ T cells, and B cells were pre-treated with compound for 24 hrs. T cells were stimulated with CD3/CD28 for analyses of CD69 expression (24 hrs, A), IL-2 secretion (48 hrs, B), and cell proliferation (96 hrs, C). CD4+ T cells were stimulated with CD3/CD28 and polarized 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 IgG release, CD69 expression and IL-6 secretion (24 hrs)(E-G).

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
		3.80	0.67	0.96 / 0.99
Functional assays		IC50,nM		
T cells	Proliferation (Flow cytometry)	0.26		
	CD69 detection (Flow cytometry)	0.32		
	IL-2 secretion	0.45		
B cells	CD69 detection (Flow cytometry)	0.43		
	IL-6 secretion	0.57		
	IgG release	4.34		
TH17 cell	IL-17A secretion by ELISA	1.95		

VAV1 MGD In Vivo Efficacy Test on CIA Mouse

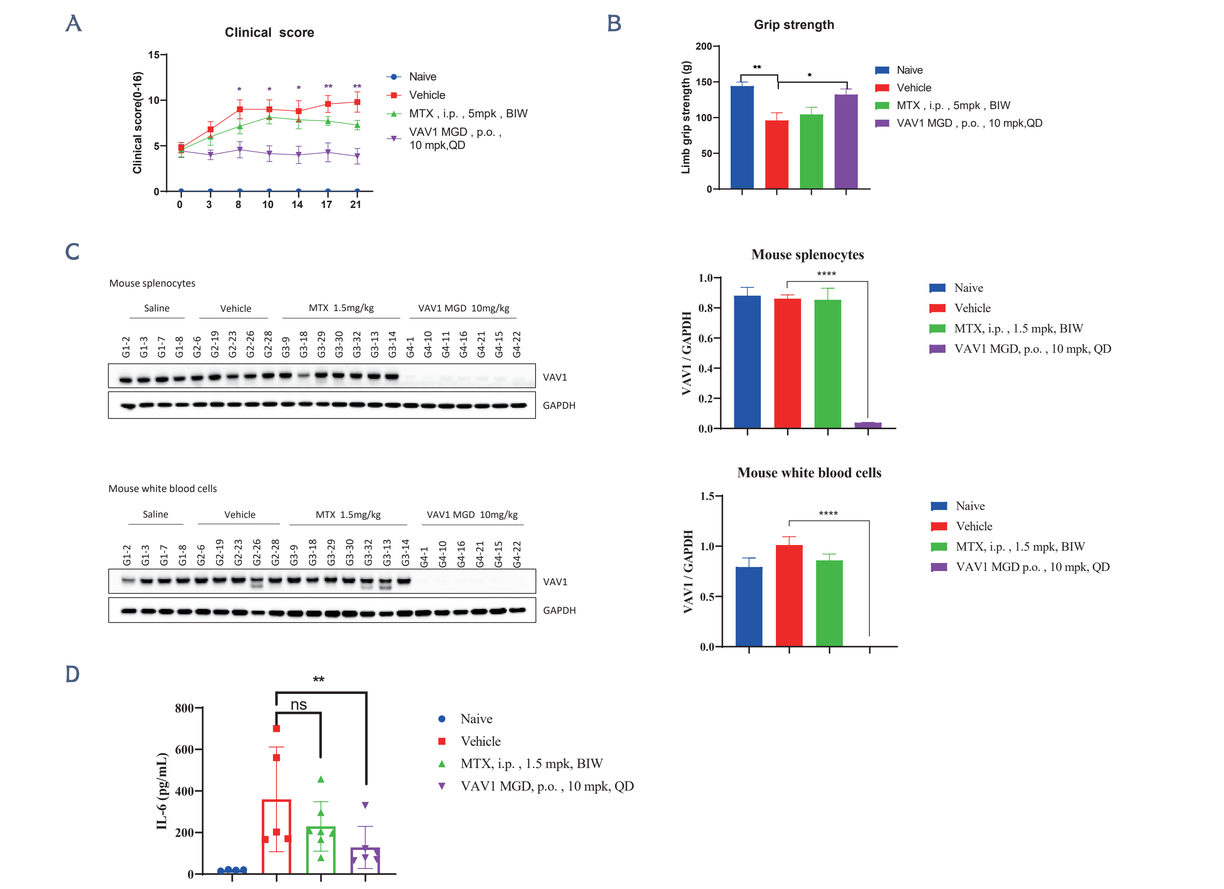

Figure 4 A. After group administration to the endpoint(Day21), the joint clinical score of the mouse. B. Mouse grip test 20 days after dosing.Each group compared with Vehicle group,*p＜0.05，**p＜0.01.
C. Oral dosing of VAV1 MGD in CIA mouse for 21 days lead to nearly completely degradation of VAV1.
D. The level of pro-inflammatory IL-6 in serum samples was significantly reduced in VAV1 MGD treated group. **P＜0.01

Selectivity Analysis Using Proteomics and Off-Target Profiling by 90 Safety Panel

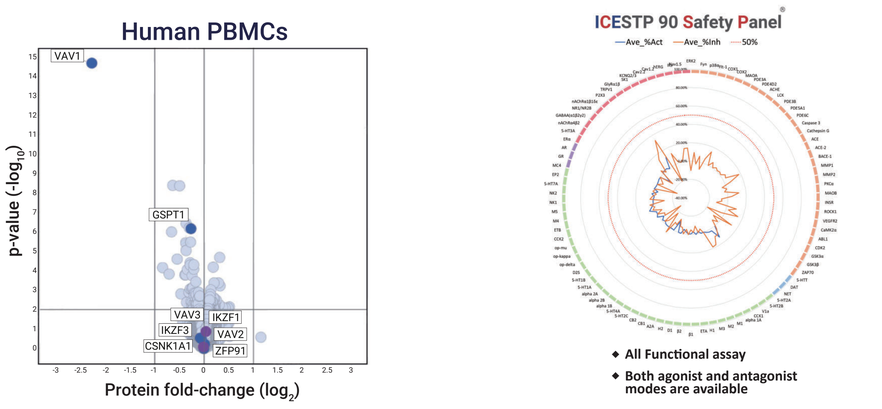

Figure 5: Selectivity by DIA based proteomics analysis and off-target profiling by ICESTP 90 Safety Panel®

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