PRECLINICAL BIOLOGICAL SCREENING AND EVALUATION OF KRAS MOLECULAR GLUES

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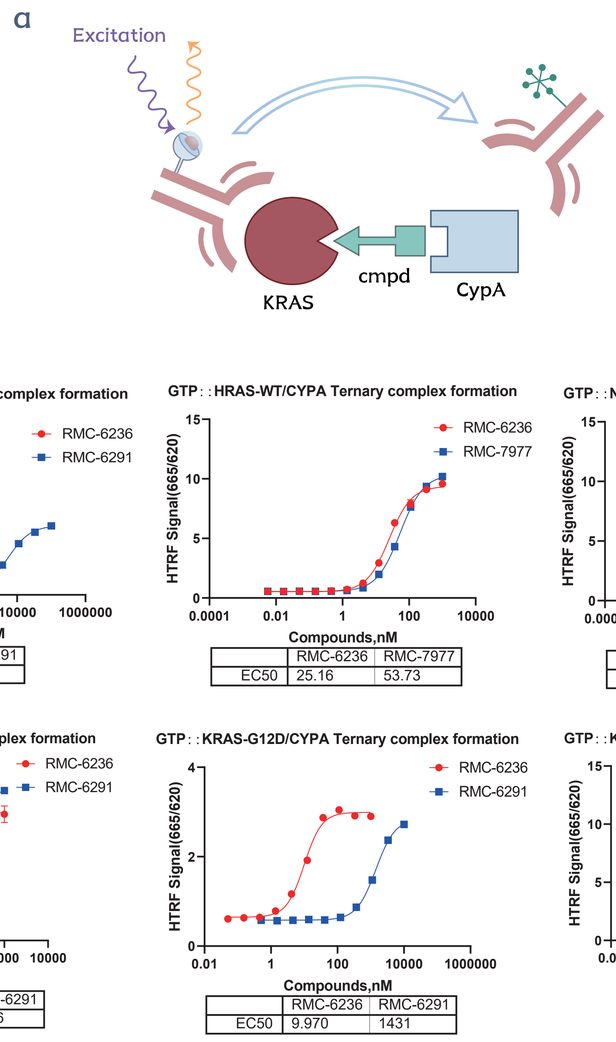
Introduction

KRAS molecular glue is an innovative small-molecule drug designed to target the KRAS mutant protein. It works by binding to cyclophilin A (CYPA) within the cell, thereby reshaping the surface of CYPA. This modification enables CYPA to bind with KRAS-GTP, forming a stable ternary complex. The formation of this complex effectively disrupts the interaction between KRAS-GTP and its downstream effector proteins, ultimately inhibiting the oncogenic signaling pathways driven by KRAS.

ICE has developed a comprehensive screening and evaluation platform for KRAS molecular glues, which includes a series of biochemical and cellular-level assays. For example, the platform utilizes biochemical and biophysical methods for the screening of POI binders, detection of KRAS(ON)/CypA/cRAF binary complexes, and KRAS(ON)/CypA ternary complexes. It also incorporates cell-based functional assays, such as 2D/3D cell proliferation and detection of ERK phosphorylation, a marker associated with signaling pathways. In addition, the platform offers p-ERK and 2D/3D panel assay. The table below summarizes and presents some of the data from ICE.

KRAS (ON)/CypA Ternary Assay by HTRF

Molecular glues(MG) can induce the formation of a ternary complex between KRAS and CYPA. The detection of this ternary complex formation using the HTRF method facilitates high-throughput screening for KRAS molecular glues.



	RMC-6236	RMC-6291
EC50	2.590	5674

	RMC-6236	RMC-7977
EC50	25.16	53.73

	RMC-6236	RMC-7977
EC50	28.30	50.76

	RMC-6236	RMC-6291
EC50	3.597	14.16

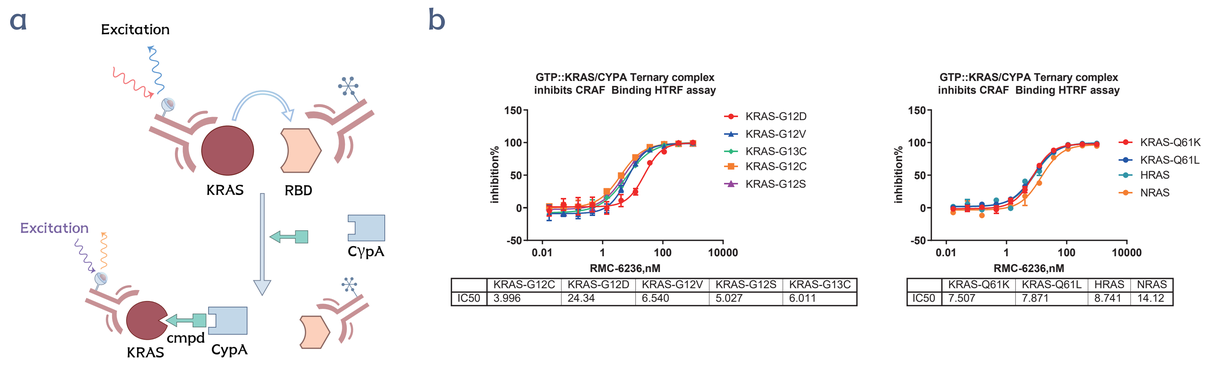
	RMC-6236	RMC-6291
EC50	9.970	1431

	RMC-6236	RMC-6291
EC50	1.904	8308

Figure 1: KRAS (ON)/CypA ternary complex formation by HTRF.
a) Schematic diagram of the principle of HTRF detection of KRAS (ON)/CypA ternary complex formation.
b) The formation of the KRAS/MG/CYPA ternary complex, including KRAS WT,KRAS G12 mutations, HRAS,NRAS was quantitatively assessed using HTRF.

KRAS(ON)/CypA/cRAF Binding Assay by HTRF

Molecular glues facilitate the formation of a ternary complex between KRAS and CYPA, which in turn inhibits the binding of cRAF to KRAS. The mutual binding between cRAF and KRAS can be detected by HTRF.



	KRAS-G12C	KRAS-G12D	KRAS-G12V	KRAS-G12S	KRAS-G13C
IC50	3.996	24.34	6.540	5.027	6.011

	KRAS-Q61K	KRAS-Q61L	HRAS	NRAS
IC50	7.507	7.871	8.741	14.12

Figure 2: KRAS(ON)/CypA/cRAF binding assay by HTRF.
a) The mechanism of KRAS(ON)/CypA/cRAF binding assay .
b) RMC-6236 induces the formation of a ternary complex between KRAS and CYPA, thereby inhibiting the binding of cRAF to KRAS, including KRAS WT, mutants, HRAS, and NRAS.

KRAS(ON)/RMC-6236/CypA Binding Assay by SPR

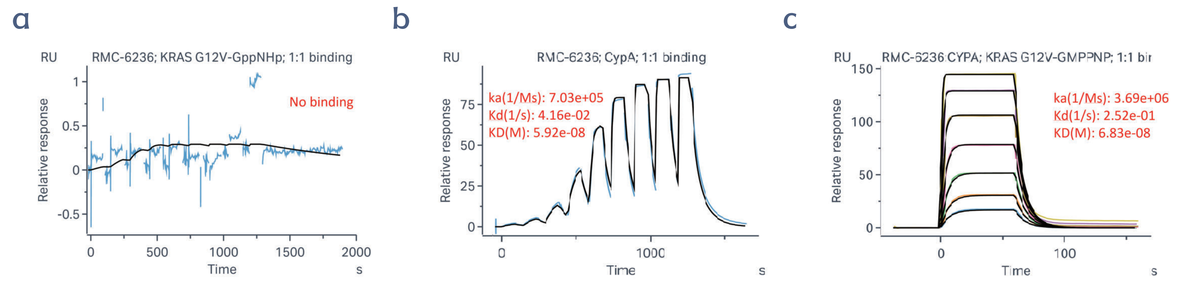
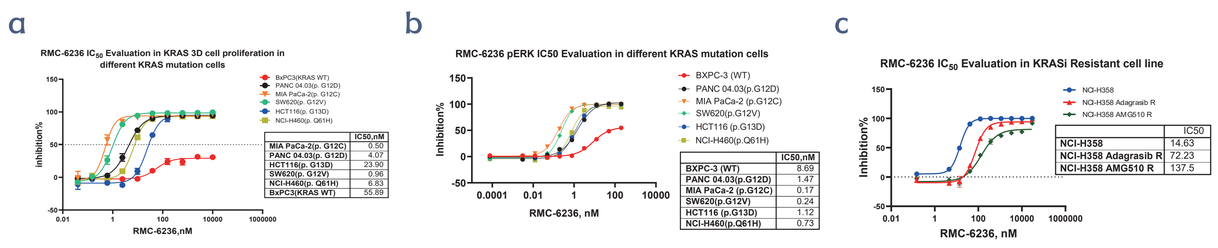


Figure 3. Schematic and binding data illustrating our SPR approach for measuring binding kinetics and determining cooperativity (α) for RMC-6236 binary and ternary complex formation. a. RMC-6236 shows no direct binding to KRAS[G12V]. b. To measure the kinetics of RMC-6236 binding to CypA. c. Representative SPR binding data is shown using this assay for the RMC-6236:CypA complex binding to immobilized KRAS[G12V].

Cell Based Assay



	IC50,nM
MIA PaCa-2(p. G12C)	0.50
PANC 04.03(p. G12D)	4.07
HCT116(p. G13D)	23.90
SW620(p. G12V)	0.96
NCI-H460(p. Q61H)	6.83
BxPC3(KRAS WT)	55.89

	IC50,nM
BXPC-3 (WT)	8.69
PANC 04.03(p.G12D)	1.47
MIA PaCa-2 (p.G12C)	0.17
SW620(p.G12V)	0.24
HCT116 (p.G13D)	1.12
NCI-H460(p.Q61H)	0.73

	IC50
NCI-H358	14.63
NCI-H358 Adagrasib R	72.23
NCI-H358 AMG510 R	137.5

Figure 4. Cell proliferation assay for the IC50 evaluation of RMC-6236 in resistant cell lines(a) and different KRAS mutation cell lines(b), and pERK detection in different KRAS mutation cell lines(c). The results suggests that the compound does not exhibit significant resistance, and can inhibit a broader spectrum of KRAS-mutated tumor cells compared to other small molecule inhibitors.

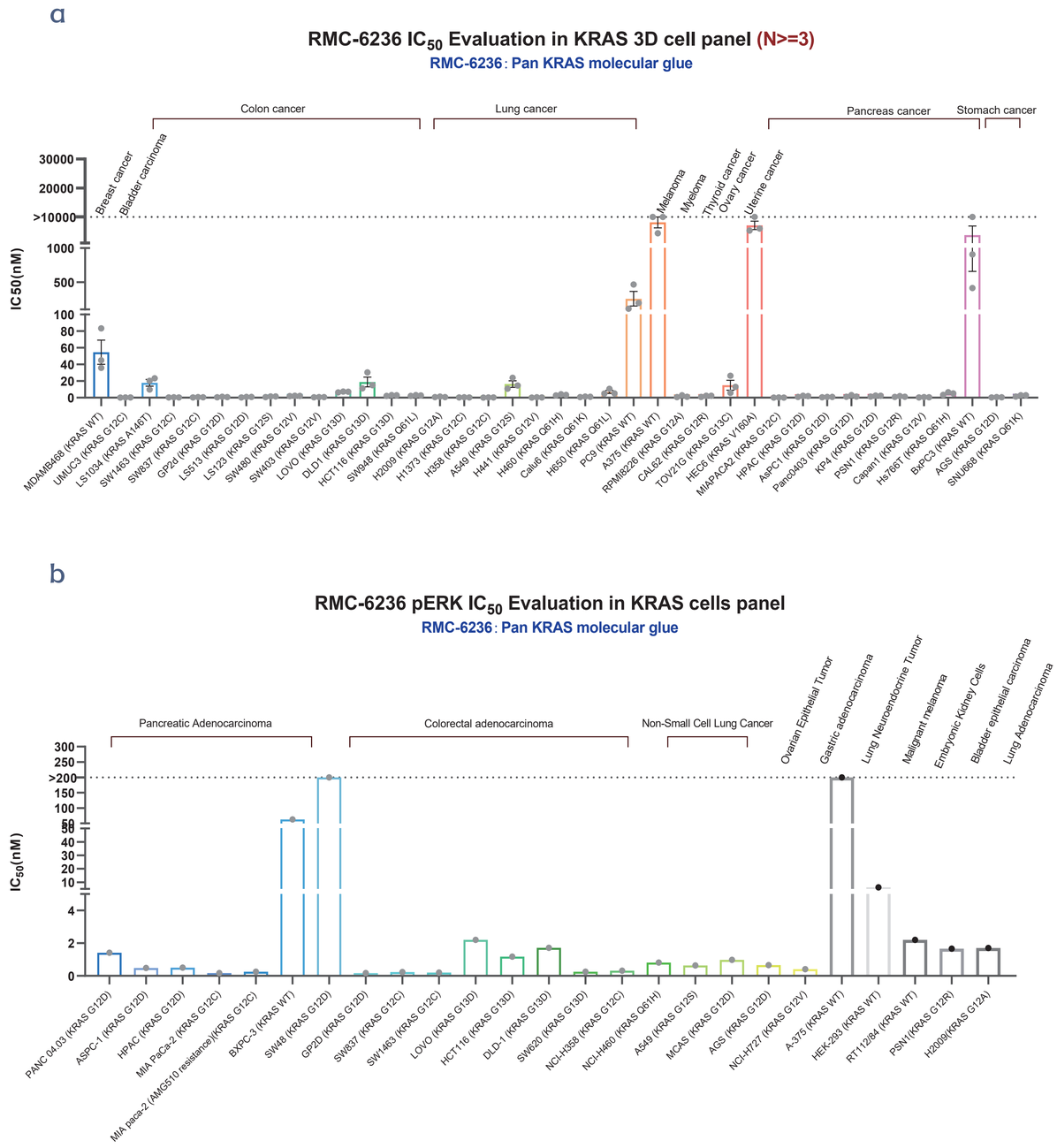


Figure 5. The IC50 evaluation of KRAS MG across a panel of 3D cultured Pan KRAS cell lines(a) and pERK panel(b). The results indicate that RMC-6236, as a molecular glue, can inhibit a broader spectrum of KRAS-mutated tumor cells compared to other small molecule inhibitors.

Summary

- Utilizing the currently developed HTRF, high-throughput screening of KRAS molecular glues can be achieved by detecting the formation of binary and ternary complexes.
- In cellular assays, both 2D/3D cell proliferation and ERK phosphorylation tests can be utilized for the screening and evaluation of the in vitro activity of KRAS molecular glues.
- For cell panel, we have established various panel types, such as Pan KRAS, KRAS G12D, KRAS G12C, KRAS G12V, HRAS panel, and NRAS panel, among others.