# PRECLINICAL BIOLOGICAL SCREENING AND EVALUATION OF KRAS MOLECULAR GLUES

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## Introduction

KRAS is a pivotal oncogene within the RAS family, encoding a small GTPase that plays a central role in regulating cell proliferation, survival, and differentiation. Mutations in KRAS—such as G12D, G12V, and G12C—result in a constitutively active GTP-bound state, driving oncogenesis in approximately 20% of human cancers, including pancreatic, colorectal, and non-small cell lung cancer. Historically, drug development targeting KRAS has been challenging due to the absence of a well-defined binding pocket on its protein surface. However, recent advances have introduced molecular glues as a promising new class of therapeutic agents capable of modulating previously undruggable targets.

KRAS/CYPA molecular glues bind to and reshape the intracellular chaperone protein CYPA (cyclophilin A), enabling it to form a stable ternary complex with the activated state of KRAS (such as KRAS G12C) (CYPA: molecular glue: KRAS). The formation of this complex can block the interaction between KRAS and downstream effector proteins (such as RAF and PI3K), thereby inhibiting the oncogenic signaling of KRAS.

Our platform integrates a comprehensive suite of cutting-edge in vitro assays and in vivo studies, seamlessly facilitating the investigation and screening of novel therapeutic agents from early discovery through to preclinical readiness.

## 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.

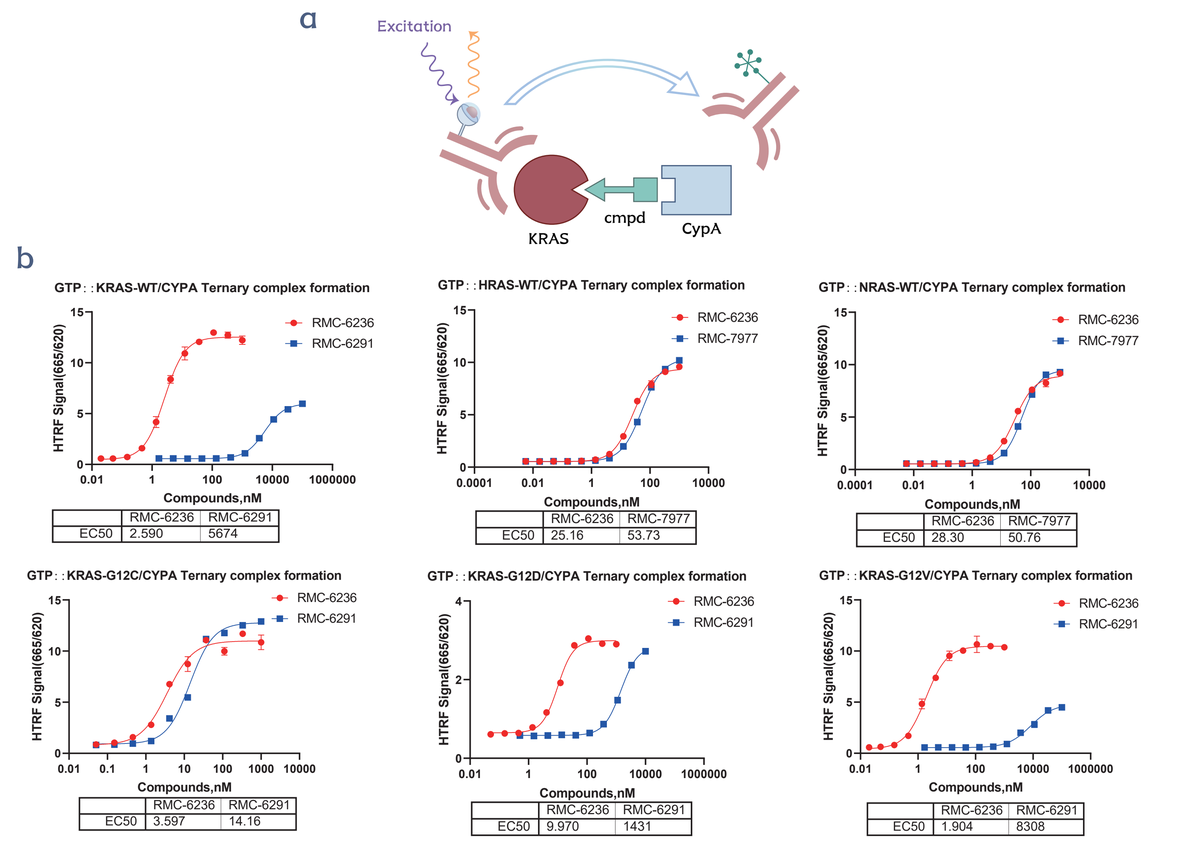


Figure 1: KRAS (ON)/CypA ternary complex formation by HTRF.
a) Schematic diagram of the principle of HTRF detection of KRAS (ON)/CypA ternary complex formation.
b) The formation of the KRAS/MG/CYPA ternary complex, including KRAS WT,KRAS G12 mutations, HRAS,NRAS was quantitatively assessed using HTRF.

## KRAS Protein

| Target | WT | G12A | G12C | G12D | G12V | G12R | G12S | G13C | G13D | Q61H | Q61L | Q61K | Q61R | A146T | KRAS [G12C/ Y64H] | KRAS [G12D/ Y64H] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TR-FRET | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ |
| SPR | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | √ | N.D | N.D |

Table 1. TR-FRET and SPR assays have been developed for KRAS mutation sites.

## 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.

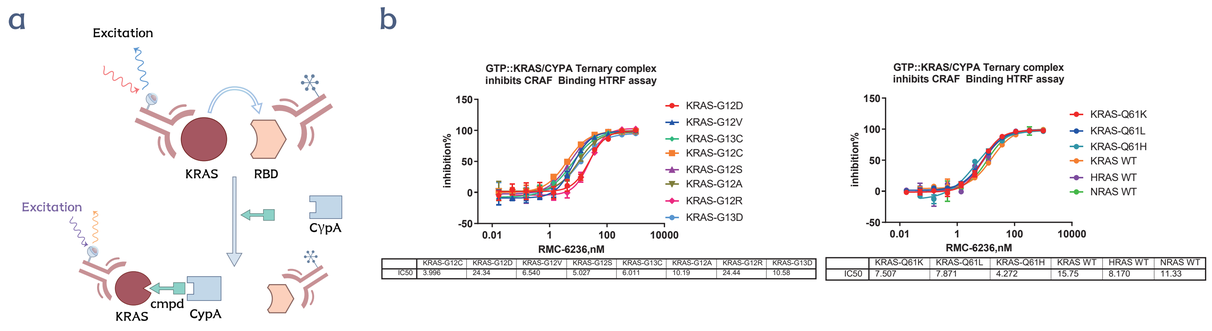


Figure 2: KRAS(ON)/CypA/cRAF binding assay by HTRF.
a) The mechanism of KRAS(ON)/CypA/cRAF binding assay .
b) RMC-6236 induces the formation of a ternary complex between KRAS and CYPA, thereby inhibiting the binding of cRAF to KRAS, including KRAS WT, mutants, HRAS, and NRAS.

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

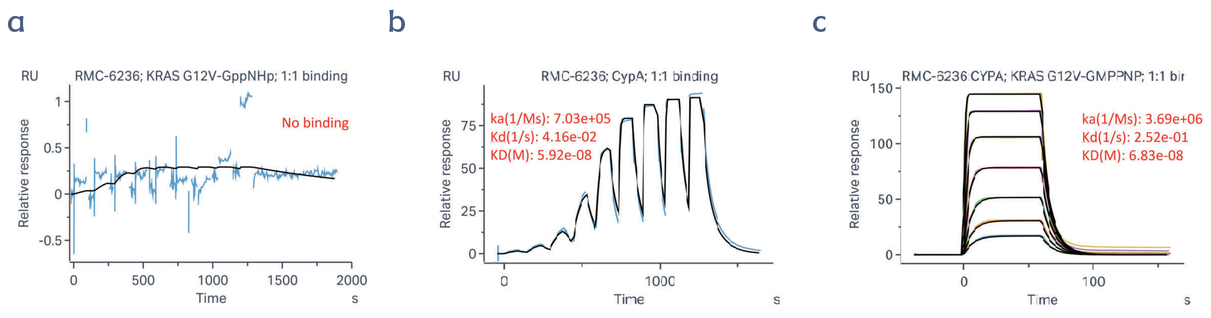


Figure 3. Schematic and binding data illustrating our SPR approach for measuring binding kinetics and determining cooperativity (α) for RMC-6236 binary and ternary complex formation. a. RMC-6236 shows no direct binding to KRAS[G12V]. b. To measure the kinetics of RMC-6236 binding to CypA. c. Representative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

## Cell Based Assay

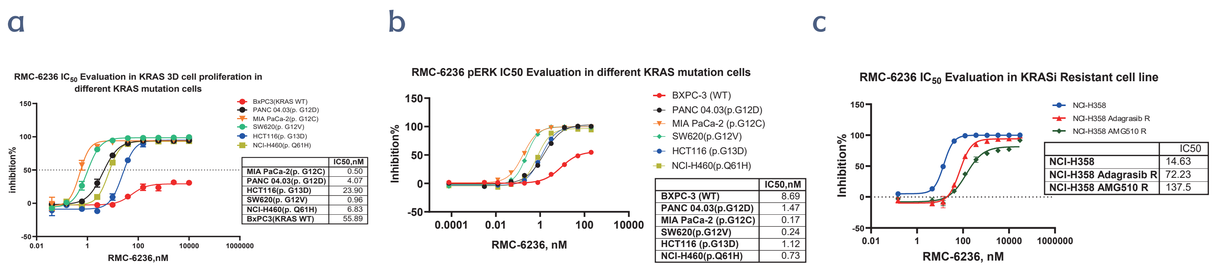


Figure 4. Cell proliferation assay for the IC50 evaluation of RMC-6236 in resistant cell lines(a) and different KRAS mutation cell lines(b), and pERK detection in different KRAS mutation cell lines(c). The results suggests that the compound does not exhibit significant resistance, and can inhibit a broader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

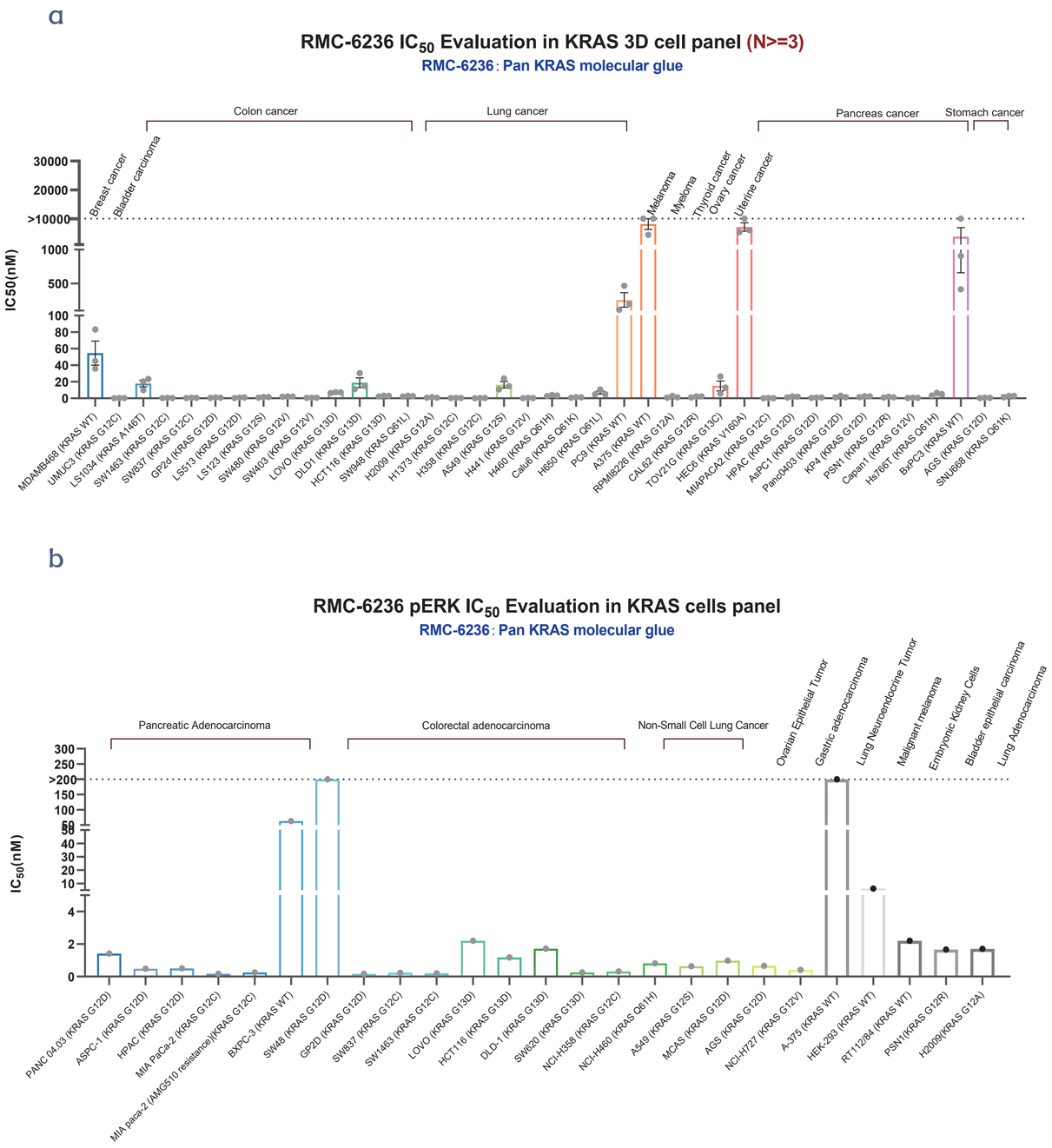


Figure 5. The IC50 evaluation of KRAS MG across a panel of 3D cultured Pan KRAS cell lines(a) and pERK panel(b). The results indicate that RMC-6236, as a molecular glue, can inhibit a broader spectrum of KRAS-mutated tumor cells compared to other small molecule inhibitors.

## 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.