# Preclinical Biological Screening and Evaluation of KRAS Molecular Glues

**Zhaoxia Yin, Lili Chai, Xian Wu, Xiaojian Wang and Tiejun Bing**

ICE Bioscience, INC. Building 14, Yard 18, Kechuang 13th Street, Beijing, China 100176
Email: bingtj@ice-biosci.com

Innovative CRO+Explorer
ICE Bioscience

Abstract Number:387

## intrroduction

The KRAS protein is a signaling molecule associated with various types of cancer. Due to its high mutation rate and critical role in tumor development, KRAS has become an important target for anticancer drug development. However, because the KRAS protein lacks traditional small-molecule binding pockets, it has long been considered an "undruggable" target. In recent years, Molecular Glue technology has provided a new approach for targeting KRAS by inducing or stabilizing protein-protein interactions (PPIs).

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.

| Ternary Complex Formation Assay | RMC-6236, IC50 or Kd, nM | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| KRAS mutation | WT | G12V | G12C | G12D | G12S | G13C | G13D | Q61H | HRAS | NRAS |
| KRAS (ON)/CypA Ternary assay by HTRF | 2.568 | 1.86 | 3.60 | 3.60 | - | - | - | - | - | - |
| KRAS(ON)/CypA /cRAF binding assay by HTRF | - | 6.54 | 4.00 | 24.34 | 5.03 | 6.01 | - | - | 8.74 | 14.12 |
| Ternary assay by SPR | | | 9.97e-02 | | | | | | | |
| 3D cell proliferation | 55.89 | 0.96 | 0.50 | 4.07 | - | - | 23.90 | 6.83 | - | - |
| p-ERK detection | 8.69 | 0.24 | 0.17 | 1.47 | - | - | 1.13 | 0.73 | | |

## 1. 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.

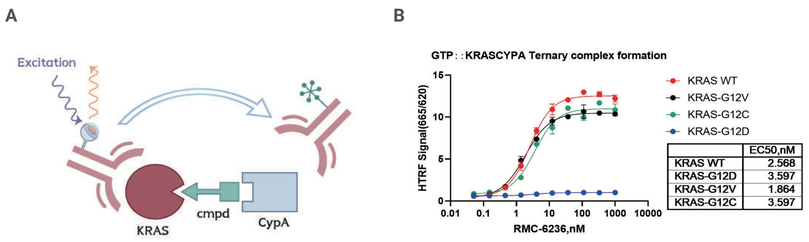


**Figure 1: KRAS (ON)/CypA ternary complex formation by HTRF.**

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 both KRAS wt and KRAS G12 mutations, was quantitatively assessed using HTRF.

## 2. 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.

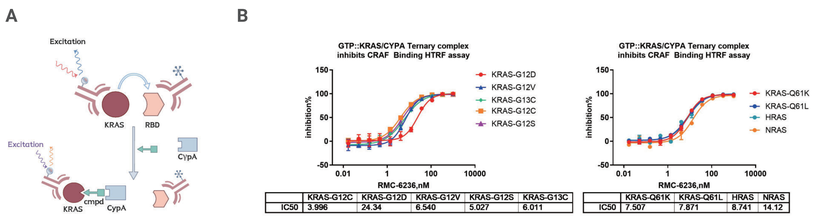


**Figure 2: KRAS(ON)/CypA/cRAF binding assay by HTRF.**

a.The mechanism of KRAS(ON)/CypA/cRAF binding assay .
b.MRC-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.

## 3. KRAS MG/CypA/KRAS binary and ternary assay by SPR

Surface Plasmon Resonance (SPR) is an optical method that enables the real-time measurement of molecular interactions and determination of the equilibrium dissociation constant (KD), quantifying the strength of molecular binding affinity.

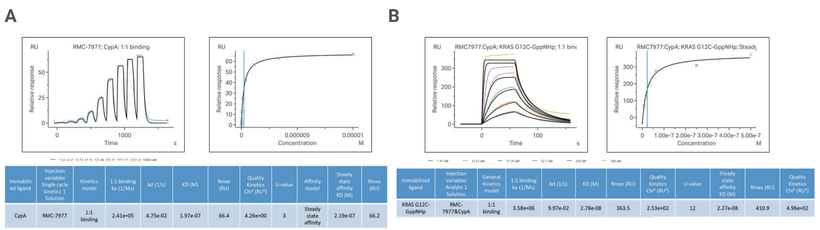


**Figure 3: KRAS MG and CypA binding assay by SPR.**

a.The binding affinity between the KRAS molecular glue RMC-7977 and CYPA was analyzed through SPR.
b.RMC-7977:CypA:KRAS G12C ternary complex formation is analysised by SPR.

## 4. Cell based assay

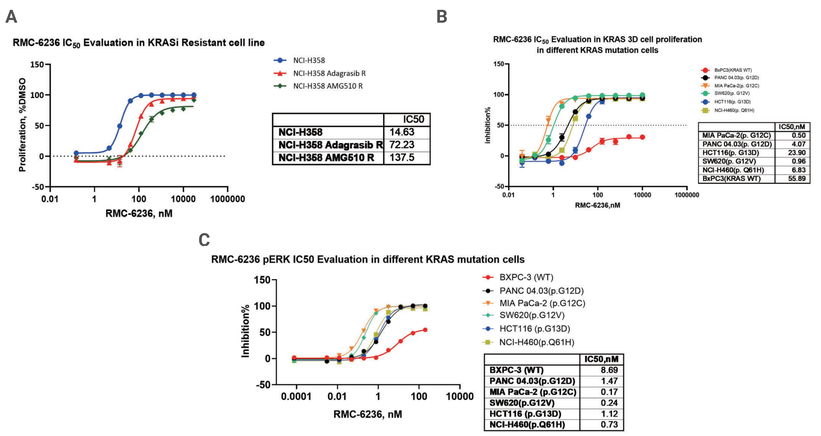


**Figure 4. Cell proliferation assay for the IC50 evaluation of RMC-6236 in resistant cell lines(a) and different KRAS mutation cell lines(b), and pERK detection in different KRAS mutation cell lines(c), results suggests that the compound does not exhibit significant resistance, and can inhibit a broader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

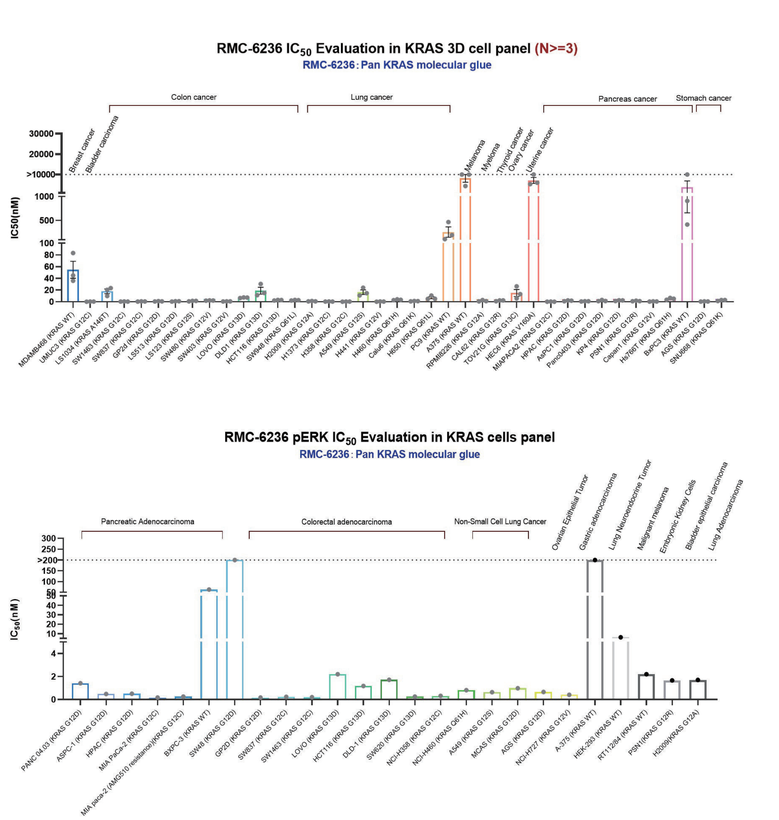


**Figure 5. The IC50 evaluation of KRAS MG across a panel of 3D cultured Pan KRAS cell lines(a) and pERK panel(b). The results indicate that RMC-6236, as a molecular glue, can inhibit a broader spectrum of KRAS-mutated tumor cells compared to other small molecule inhibitors.**

## Summary

- Utilizing the currently developed HTRF and SPR technologies, 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.

## References

Wenjing Su, Xuben Hou, et al. Targeting active RAS with molecular glue. Pharmaceutical Science Advances,Volume 2, December 2024, 100047.
James Cregg*Anne V. EdwardsStephanie ChangBianca J. Lee, et al. Discovery of Daraxonrasib (RMC-6236), a Potent and Orally Bioavailable RAS(ON) Multi-selective, Noncovalent Tri-complex Inhibitor for the Treatment of Patients with Multiple RAS-Addicted Cancers. Journal of Medicinal Chemistry, February 11, 2025.