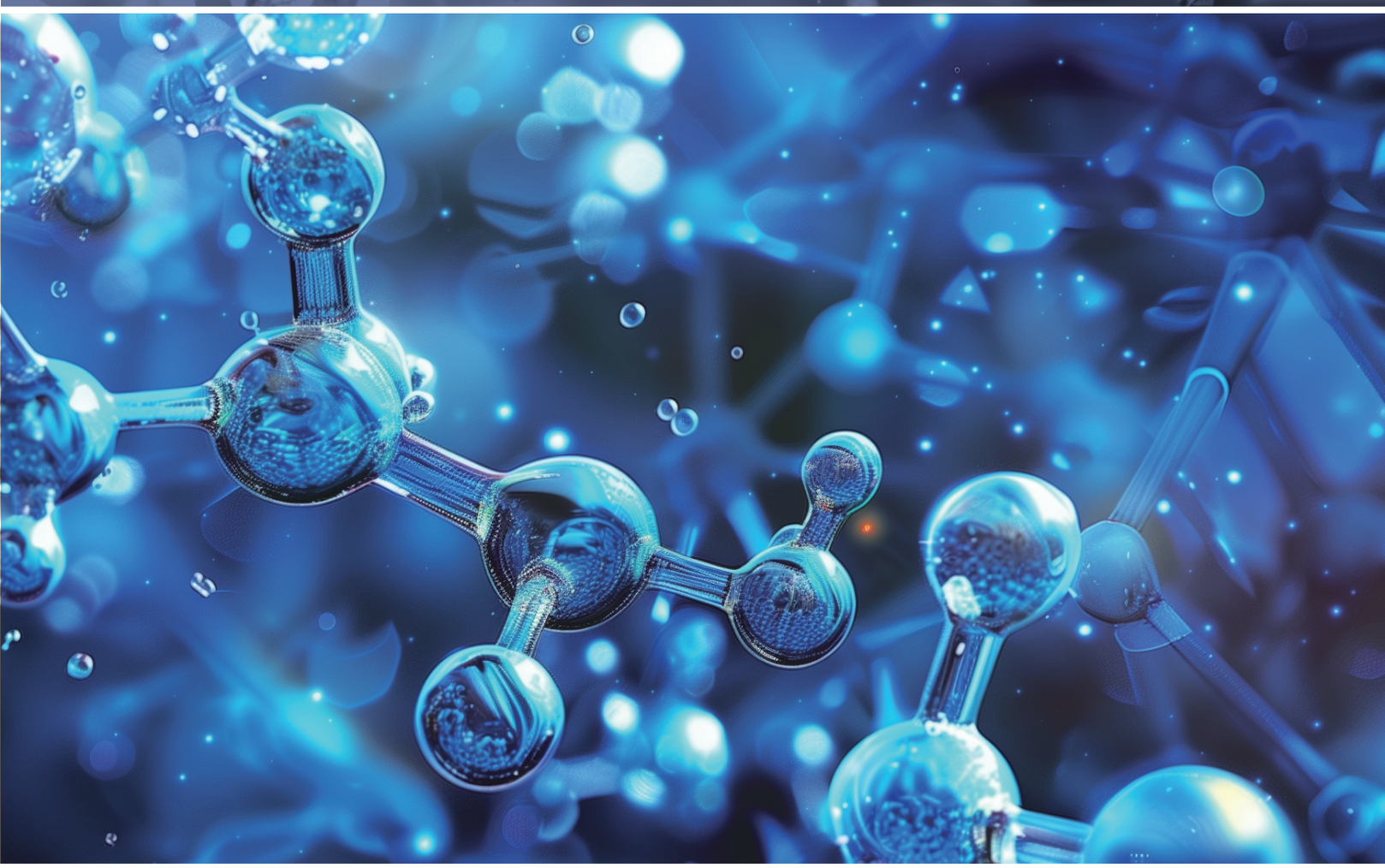
# Targeting the Untargetable: KRAS

## Unlocking New Avenues in Cancer Therapy



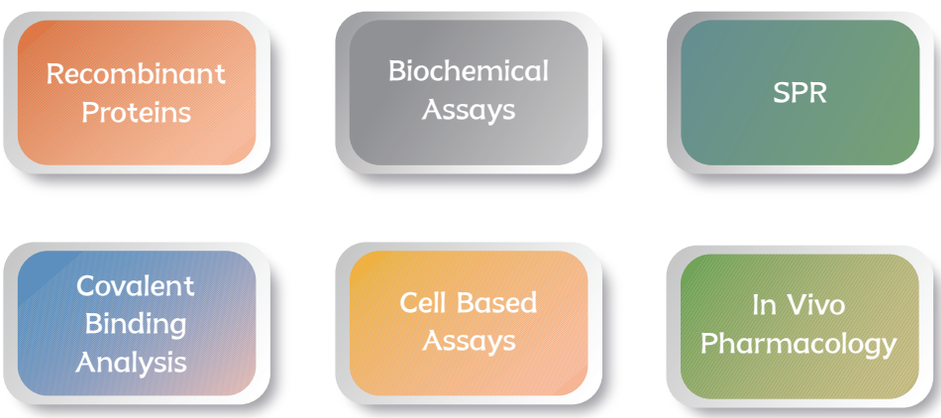
Innovative CRO+Explorer
ICE Bioscience

# Unlocking KRAS: A Journey of Discovery

For decades, KRAS was deemed an elusive target in cancer therapy, often described as 'undruggable' due to its high affinity for GTP/GDP and the lack of a suitable binding pocket for potential inhibitors. KRAS mutations are among the most prevalent in human cancers, driving tumor growth and survival in a significant number of patients, particularly those with pancreatic, colorectal, and lung cancers.

The challenge in targeting KRAS lies in its molecular structure and the pivotal role it plays in cell signaling pathways. Traditional small molecule approaches struggled to disrupt its functions effectively. However, recent breakthroughs have shifted this perspective dramatically. The advent of novel approaches such as covalent inhibitors that target specific KRAS mutants, like the G12C mutation, has opened new avenues in the treatment of KRAS-mutant cancers.

Current progress in the field of KRAS drug discovery is a testament to the power of perseverance and innovation in pharmaceutical research. With ongoing clinical trials and the FDA approval of the first targeted KRAS G12C inhibitor, the landscape of cancer treatment is undergoing a significant transformation. Looking forward, the focus is not only on discovering more selective drugs that target different KRAS mutations but also on understanding the complex biology of KRAS inhibition to circumvent resistance mechanisms and improve patient outcomes. The era of direct KRAS targeting has just begun, and the potential for developing more effective cancer treatments is vast and inspiring.




ICE Bioscience offers an integrated suite of drug discovery services, encompassing the production of high-quality recombinant proteins, the execution of detailed biochemical assays, and precise binding analyses using Surface Plasmon Resonance (SPR). We specialize in covalent binding analysis to investigate the durability of drug-target interactions, alongside versatile cell-based assays to assess compound efficacy and toxicity. Complementing these in vitro techniques, our in vivo pharmacology capabilities provide critical insights into the pharmacokinetics and pharmacodynamics of candidate drugs within relevant models.

# Recombinant Proteins

Mutations in the KRAS gene represent some of the most prevalent genetic alterations in human tumors, with the majority occurring in codons 12 and 13. However, significant mutations in codons 61 and 146 have also been identified. At ICE Bioscience, we excel in producing high-quality KRAS recombinant proteins, encompassing key mutants such as G12C, G12D, G12R, G12V, G13C, G13D, and Q61H, available with various tags to meet diverse research needs. Beyond our extensive KRAS offerings, we provide a broad selection of RAS pathway-related proteins, including SOS1, SOS2, and cRAF, to support comprehensive research into their interactions and functions.

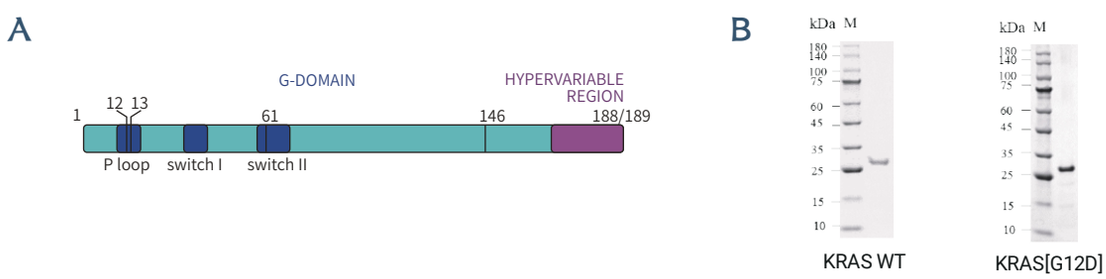


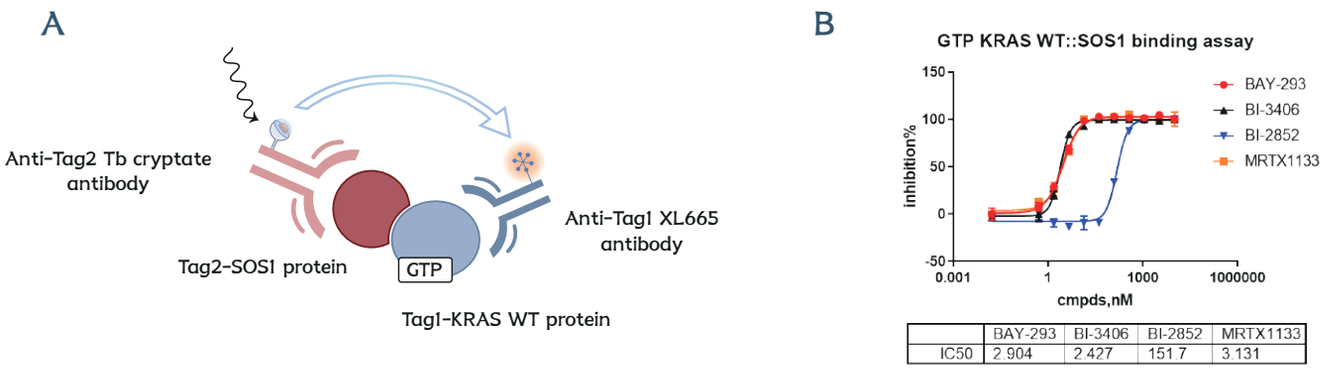

Figure 1. (A) The Kras structure. (B) Purified proteins of KRAS and KRAS mutant G12D at ICE Bioscience (SDS-PAGE data).

# Protein-Protein Interaction Assays

The γ-phosphate of GTP anchors two critical regions, Switch I and Switch II, into a compact conformation. This configuration facilitates interactions with downstream effectors such as cRAF, PI3Kα, and RALGDS, as well as with the allosteric sites of SOS1 and SOS2. The Protein SOS, a guanine nucleotide exchange factor, is recruited by activated growth factor receptors. It promotes nucleotide exchange by displacing GDP from KRAS, allowing GTP to bind to the nucleotide-binding pocket.

At ICE Bioscience, we leverage Homogeneous Time-Resolved Fluorescence (HTRF) technology for:

- The straightforward and quick characterization of compound and antibody blockers;
- Detailed characterization of interactions between KRAS/mutants and key proteins such as SOS1, SOS2, and cRAF, as well as HRAS and cRAF, NRAS and cRAF interactions.
- Facilitation of high-throughput screening to accelerate discovery processes and enhance research efficiency.



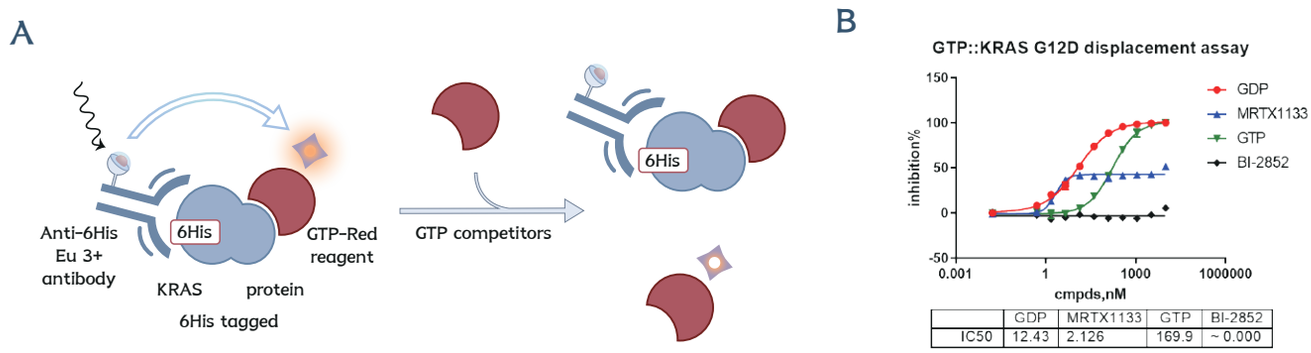

| | BAY-293 | BI-3406 | BI-2852 | MRTX1133 |
|---|---|---|---|---|
| IC50 | 2.904 | 2.427 | 151.7 | 3.131 |

Figure 2. (A) Principle of the HTRF KRAS WT/SOS1 protein-protein interaction assay. When the donor and acceptor antibodies are brought into close proximity due to SOS1 and KRAS binding, excitation of the donor antibody triggers fluorescent resonance energy transfer (FRET) towards the acceptor antibody, which in turn emits specifically at 665 nm. This specific signal is directly proportional to the extent of KRAS/SOS1 interaction. (B) Pharmacology data of GTP-KRAS interaction with the allosteric site of SOS1.

# KRAS GTP Displacement Assays

KRAS GTP displacement assay is designed to measure the displacement of guanosine triphosphate (GTP) from the KRAS protein, a process pivotal for the activation of KRAS and its downstream signaling cascades, which are often implicated in cancer progression and tumorigenesis.

- **High Specificity:** Precise targeting of GTP competitors, including key KRAS mutations.
- **Quantitative Data:** Detailed efficacy measurement for compound ranking.
- **Fast Results:** Optimized protocols ensure rapid turnaround.

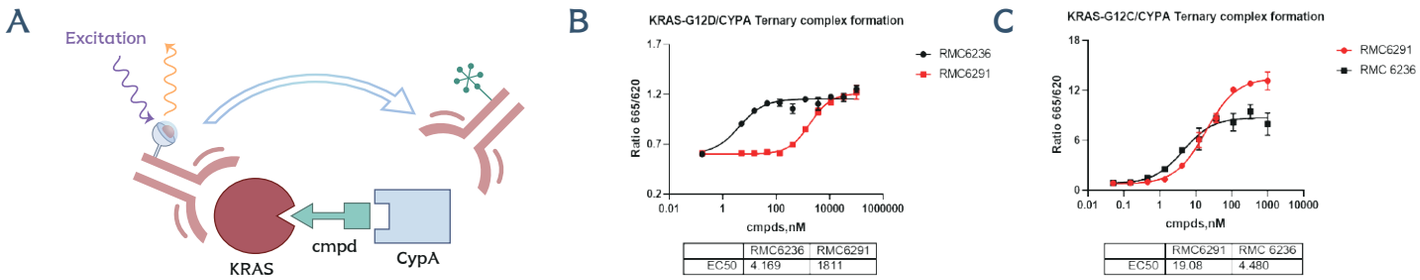



| | GDP | MRTX1133 | GTP | BI-2852 |
|---|---|---|---|---|
| IC50 | 12.43 | 2.126 | 169.9 | ~ 0.000 |

Figure 3. (A) The assay utilizes a labeled GTP analog to monitor the binding and displacement of GTP on the KRAS protein. When a compound successfully displaces the GTP from KRAS, it indicates potential inhibitory effects on KRAS activation. This displacement is quantitatively measured, providing valuable insights into the compound's efficacy in inhibiting the KRAS pathway. (B) Assessment of the inhibitory effect of compounds on the binding of GTP with KRAS G12D.

# KRAS/CYPA

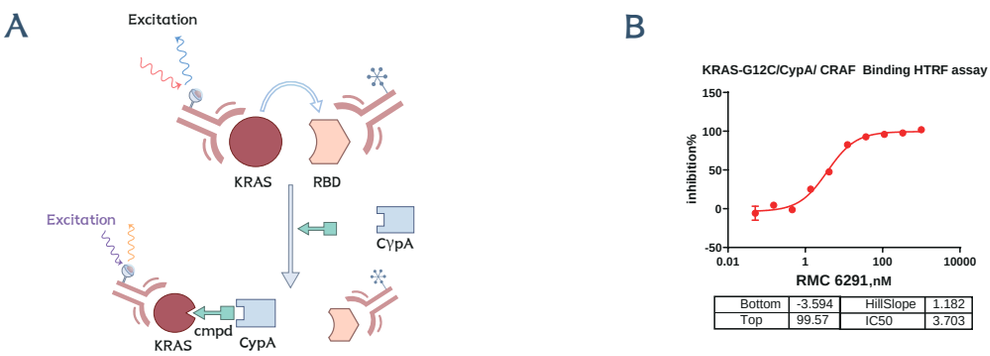
The research on molecular glues targeting KRAS, particularly in combination with cyclophilin A (CYPA), has shown promising progress. Scientists have developed small molecules that can remodel CYPA to interact with the active, GTP-bound state of KRAS. This interaction forms a ternary complex that inactivates oncogenic signaling, which has led to tumor regression in human cancer models.




| | RMC6236 | RMC6291 |
|---|---|---|
| EC50 | 4.169 | 1811 |

| | RMC6291 | RMC 6236 |
|---|---|---|
| EC50 | 19.08 | 4.480 |

Figure 4. (A) TR-FRET ternary complex formation assay for molecular glues. (B, C) The graphical data illustrate the interaction of two compounds, RMC6236 and RMC6291, with the KRAS-G12D/CYPA and KRAS-G12C/CYPA complex.



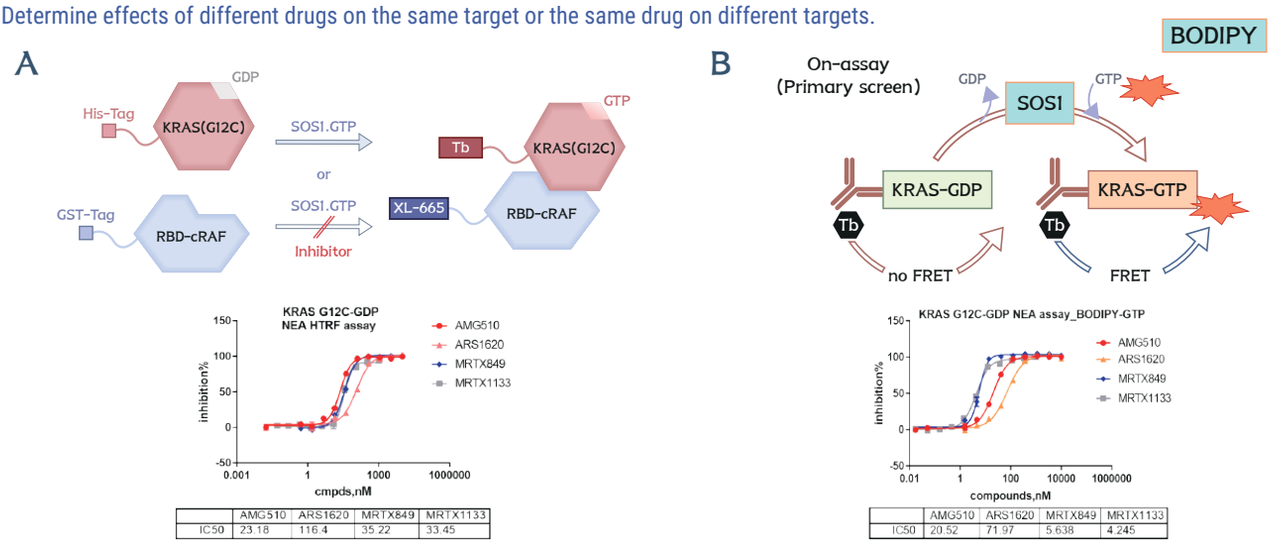

| | | | |
|---|---|---|---|
| Bottom | -3.594 | HillSlope | 1.182 |
| Top | 99.57 | IC50 | 3.703 |

Figure 5. KRAS-G12C/CypA/cRAF RBD Interaction Assay. (A) This assay demonstrates the binding interaction between KRAS and cRAF RBD, indicated by the presence of a HTRF signal. Upon the addition of a molecular glue compound and CypA, a significant decrease in the HTRF signal is observed (B).

# Nucleotide Exchange Assays

GDP-loaded KRAS is inactive and does not interact with downstream effector cRAF. The Nucleotide Exchange Assay(NEA) takes advantage of SOS-mediated nucleotide exchange to activate KRAS(G12C) bound to GDP. The main application of the assay is to identify compounds that lock KRAS in inactive “OFF” state by preventing GTP binding. Assay uses HTRF based detection of interaction.

- Two types of assays with different principles to choose from ;
- Evaluate various modes of nucleotide exchange inhibition;
- Determine effects of different drugs on the same target or the same drug on different targets.




| | AMG510 | ARS1620 | MRTX849 | MRTX1133 |
|---|---|---|---|---|
| IC50 | 23.18 | 116.4 | 35.22 | 33.45 |

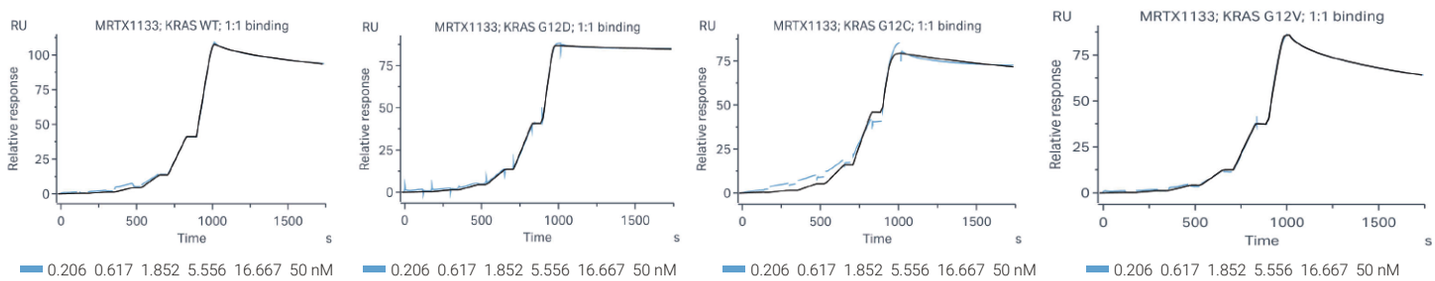
| | AMG510 | ARS1620 | MRTX849 | MRTX1133 |
|---|---|---|---|---|
| IC50 | 20.52 | 71.97 | 5.638 | 4.245 |

Figure 6. (A) Principle and experimental results of HTRF based detection of inhibition of KRAS G12C nucleotide exchange by various control compounds. (B) An alternative assay designed for the screening and profiling of KRAS inhibitors using fluorescent BODIPY-GDP to monitor nucleotide exchange.

# SPR

Surface plasmon resonance (SPR) is one of the commonly used technologies for detailed and quantitative studies of protein-protein, protein-molecule interactions and determination of their equilibrium and kinetic parameters.

- Evaluate binding kinetics of compound to HRAS, NRAS, wild type KRAS and mutants;
- Utilize single-cycle kinetics or multi-cycle kinetics;
- Data was collected for wild type KRAS and mutants G12C, G12D, and G12V in a GDP-bound state, GTP-bound state, GMPPNP-bound state or GppNHp-bound state.
- Determine the binding selectivity of the peptide.



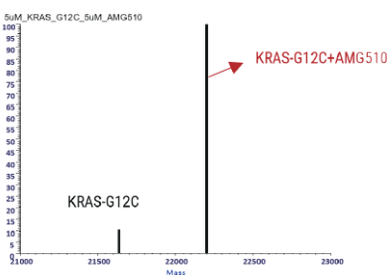

| Immobilized Ligand | Injection Variables Single Cycle Kinetics 1 Solution | General Kinetics Model | 1:1 Binding Ka (1/Ms) | Kd (1/S) | Kd (M) | Rmax (RU) | Quality Kinetics Chi² (RU²) | U-value |
|---|---|---|---|---|---|---|---|---|
| KRAS WT | MRTX1133 | 1:1 binding | 5.26e+06 | 7.75e-04 | 1.47e-10 | 108.4 | 1.05e+00 | 5 |
| KRAS G12D | MRTX1133 | 1:1 binding | 8.93e+06 | 5.83e-05 | 6.53e-12 | 86.9 | 5.99e-01 | 12 |
| KRAS G12C | MRTX1133 | 1:1 binding | 1.55e+06 | 1.93e-04 | 1.25e-10 | 79.8 | 7.54e+00 | 9 |
| KRAS G12V | MRTX1133 | 1:1 binding | 7.99e+06 | 3.42e-03 | 4.29e-10 | 86.5 | 2.48e-01 | 5 |

Figure 7. The data were collected in a GDP-bound state using single-cycle kinetics. The data were fitted using a 1:1 kinetic binding model. MRTX1133 binds with the highest affinity to KRAS mutant G12D.

# Covalent Binding Analysis

ICE Bioscience offers cutting-edge covalent binding analysis using mass spectrometry to detail modifications on KRAS mutants, essential for drug discovery. Our capability spans identifying intrinsic alterations in KRAS alone and discerning covalent interactions when combined with therapeutic compounds. This dual-focus approach allows precise evaluation of compound efficacy and specificity towards KRAS mutations, advancing the development of targeted cancer therapies.

- **Own Protein Library:** A comprehensive repository of proteins available for a broad range of assays.
- **Assay Establishment for New Proteins:** The ability to develop and validate assays for emerging protein targets.
- **Optimized Assay Conditions:** Tailored assay conditions to ensure maximum efficiency and accuracy.
- **High Throughput:** Capacity to handle approximately 200 samples per day, facilitating rapid project advancement.



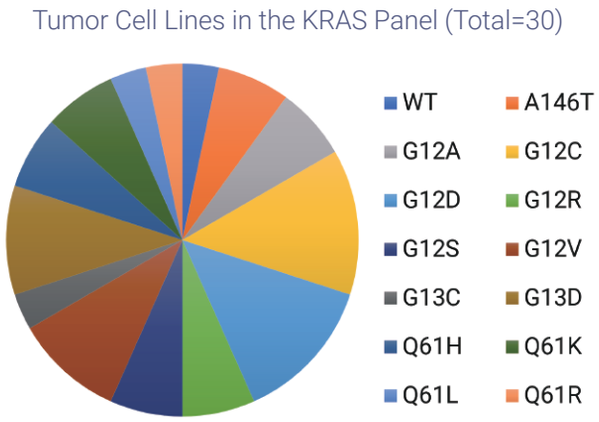

| Sequence Name | Modification | Average Mass | Theoretical Mass (Da) | Matched Mass Error (Da) | Intensity | Modification ratio |
|---|---|---|---|---|---|---|
| KRAS_G12C | 2xDisulfide bind,2xAcetylation | 21638.2998 | 21639.0809 | 0.7811 | 2.10E+09 | 91.10% |
| KRAS_G12C | 1xDisulfide bind,2xAcetylation, 1xAMG510 | 22198.9855 | 22199.6709 | 0.6854 | 2.05E+10 | |

Figure 8: Covalent Binding Analysis of Acetylated KRAS-G12C. The mass spectrometry profiles reveal acetylated KRAS-G12C both before and after covalent modification with a compound. The graph shows the mass of the acetylated KRAS-G12C with and without the compound, indicating the acetylated KRAS-G12C with the AMG510 compound covalently attached.

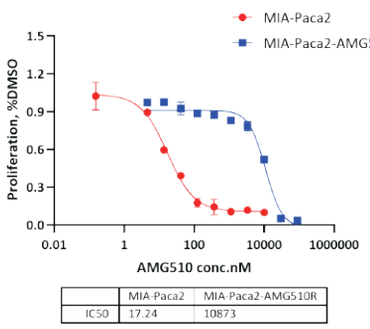
# Cell Based Assays and KRAS Cell Panel Screening

ICE Bioscience excels in the application of In-Cell Western (ICW) assays and KRAS Cell Panel Screening, offering precise analysis of compound interactions with KRAS in a cellular environment. Our ICW assays provide a powerful tool for quantifying protein levels and post-translational modifications within cells, allowing for detailed investigation into the mechanisms of action and efficacy of potential therapeutic compounds.

Our KRAS Cell Panel Screening leverages a curated selection of cell lines with distinct KRAS mutations, facilitating the evaluation of compound specificity and effectiveness across varied genetic contexts:




- Our KRAS panel includes 30 Ba/F3 and 30 tumor cell lines, with options to customize from 40+ Ba/F3 and 100+ KRAS cancer cell lines.
- Optional drug-resistant cell lines screening;
- Offers assay formats that encompass both 2D and 3D cellular models, including CellTiter-Glo (CTG) for cell viability and clonogenic assays to assess the proliferative capacity of cells.
- Assessment of the effects of individual compound or the comprehensive effects of multiple compounds.




| | MIA-Paca2 | MIA-Paca2-AMG510R |
|---|---|---|
| IC50 | 17.24 | 10873 |

Culture Medium:
DMEM+10%FBS+1%PS+25 μM AMG510
Build cycle: 8 months
Duration of action: 7 days
Confluence: 80%
Starting concentration:
MIA-Paca2 WT 10 μM
MIA-Paca2/AMG510 R 90 μM

Figure 9: AMG510 Response in KRAS G12C MIA-Paca2 Cell Line. The blue curve shows the drug-resistant KRAS G12C MIA-Paca2 cell line, which maintains higher viability despite AMG510 treatment, reflecting its resistance. In contrast, the red curve indicates the wild-type KRAS G12C MIA-Paca2 cells, exhibiting reduced viability with increasing concentrations of AMG510, denoting a sensitive response to the drug.

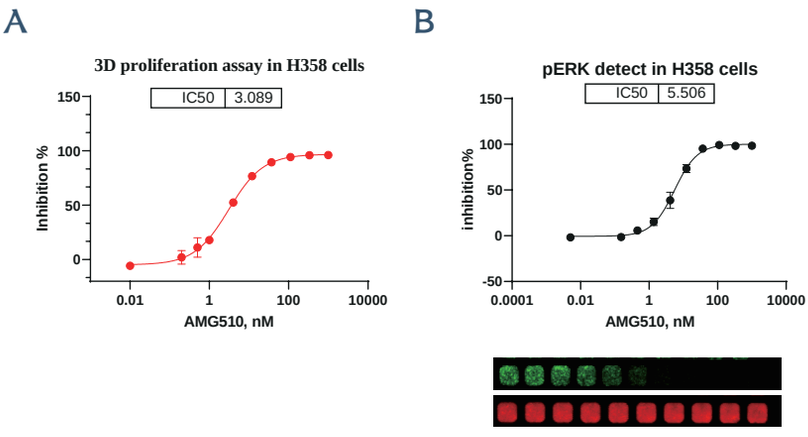
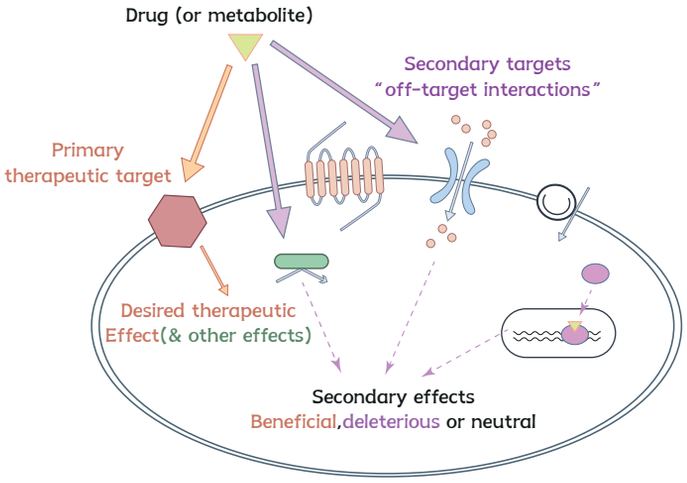



Figure 10: Efficacy of AMG510 on H358 Cell Line. (A) Demonstrates the inhibitory effect of AMG510 on 3D proliferation of H358 cells, which possess the KRAS G12C mutation. (B) Shows quantification of pERK protein levels, a downstream effector of KRAS, in H358 cells following treatment with AMG510, as measured by In-Cell Western assay.

# In Vitro Safety Pharmacology

Challenges related to safety concerns in drug development persist as a significant hurdle for the pharmaceutical sector. Safety Pharmacology, as outlined by ICH S7A (2001), places a critical emphasis on the evaluation of drugs for off-target effects. In vitro Pharmacological profiling is now more frequently employed at the initial stages of drug discovery to detect unfavorable off-target activity profiles and predict clinical adverse effects to reduce unwarranted attrition.



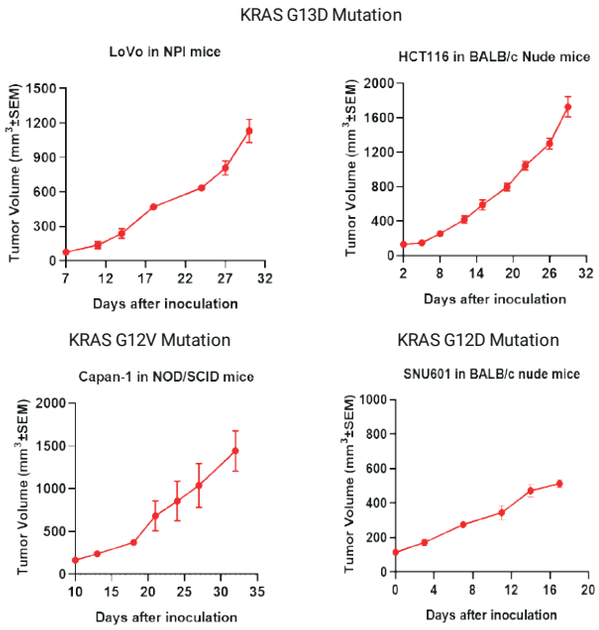

| Targets | SafetyOne44 | SafetyIMax90 |
|---|---|---|
| GPCR | 24 | 37 |
| Ion Channel | 8 | 15 |
| Enzyme | 7 | 32 |
| Nuclear Receptors | 2 | 3 |
| Transporter | 3 | 3 |
| No. of Targets | 44 | 90 |
| No. of Assays | 74 | 138 |

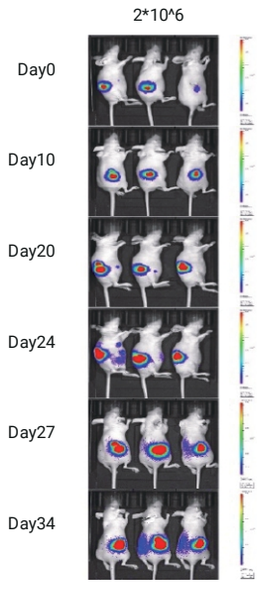
- **State-of-the-art Functional Safety Panels:** ICESTP™ SafetyOne44 is designed for 44 specific targets, while ICESTP™ SafetyMax90 stands as the most comprehensive functional safety panel available, encompassing up to 90 targets.
- **Specialized Functional Panels:** Tailored to specific research areas. Compared to binding assays, our functional assays offer the advantage of expertise in distinguishing between different modes of action.

# In Vivo Pharmacology

ICE Bioscience's in vivo pharmacology services specialize in the evaluation of anti-cancer agents targeting KRAS mutations, utilizing Cell-Derived Xenograft (CDX) models. Our CDX models are specifically developed by implanting human cancer cell lines with known KRAS mutations into immunocompromised mice.

We offer state-of-the-art in vivo imaging services to support cancer research, providing real-time visualization of tumor progression and metastasis in live animal models. Our advanced imaging techniques are crucial for evaluating the in vivo activity of potential therapeutic agents targeting KRAS mutations. The accompanying image displays an orthotopic ovarian cancer model, showcasing the precision and clarity our imaging technology brings to monitoring disease states.




**Orthotopic Ovarian cancer Model**

ICE Bioscience
Innovative CRO+ Explorer

ICE Bioscience was founded in 2010 as an Innovative CRO+ Explorer company. We specialize in early drug discovery services, spanning from target validation to the identification of pre-clinical candidates. We stand out for our collaborative spirit and expertise in boldly exploring new therapeutic target research. Our commitment to drug discovery services, delivered with enthusiasm and professionalism, empowers clients to overcome challenges, address scientific puzzles, and fulfill our promises to clients, communities, the environment, and global health.


To learn more, please visit: en.ice-biosci.com