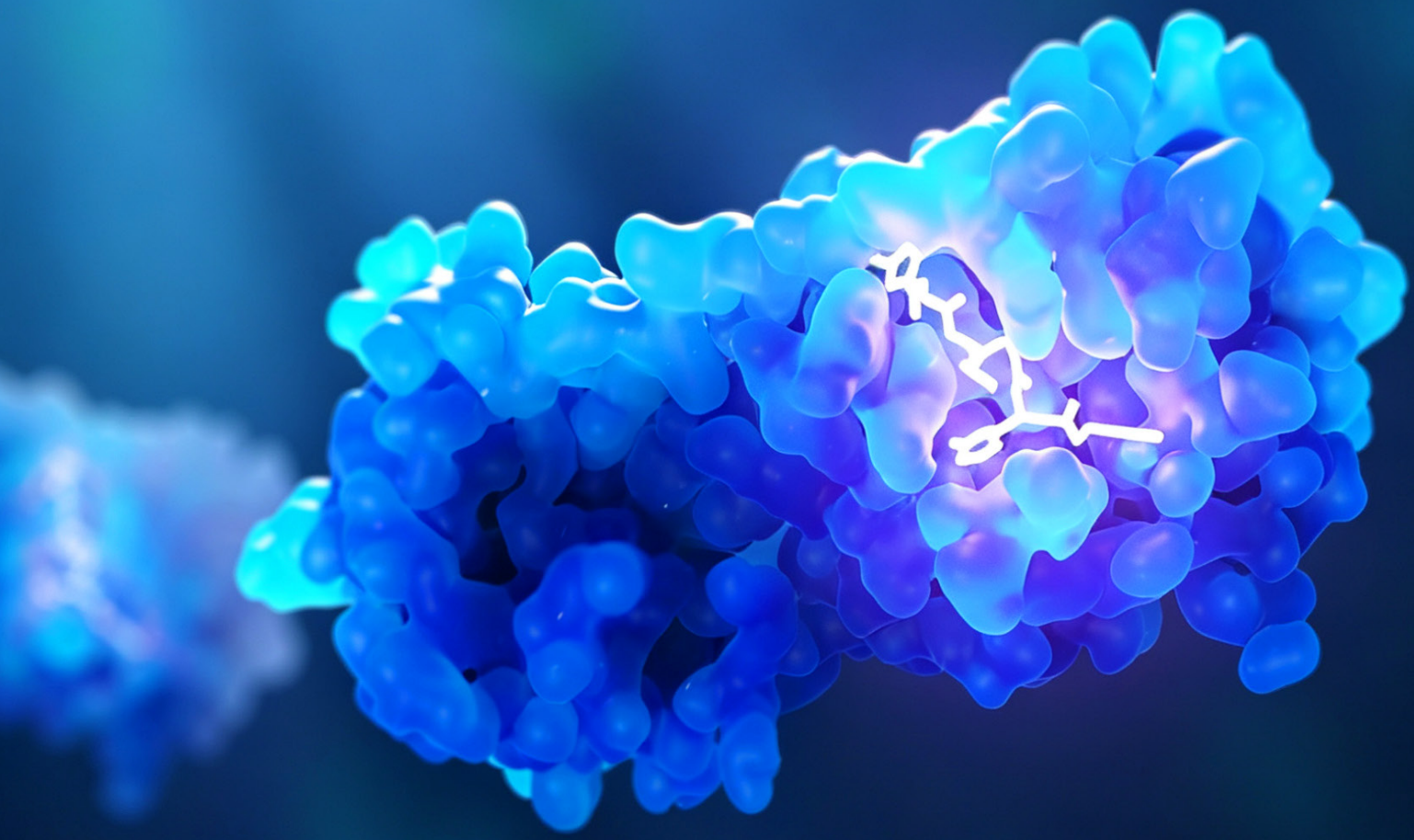
WuXi AppTec

Integrated Strategies for KRAS Inhibitor Development

Accelerating the R&D and Clinical Translation of Next-Generation Inhibitors



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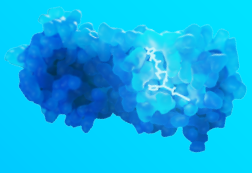


Introduction

As one of the most frequently mutated oncogenes in human cancers, KRAS was long deemed an "undruggable" target. The recent landmark approvals of KRAS G12C inhibitors have sparked rapid advancements in KRAS-targeted drug development. However, given the diverse array of KRAS mutational subtypes and the growing challenge of acquired clinical resistance, developing next-generation inhibitors that cover broader mutation profiles and effectively overcome resistance remains pivotal for maximizing the clinical value of KRAS-targeted therapies.

The KRAS Signaling Pathway: From Molecular Switch to Tumor Driver

As a member of the small GTPase family, KRAS acts as a crucial molecular switch tethered to the inner cell membrane, cycling between an inactive GDP-bound state ("off") and an active GTP-bound state ("on"). Upon growth factor binding to surface receptors, guanine nucleotide exchange factors (GEFs), such as SOS1, are activated. These GEFs promote the exchange of GDP for GTP, switching KRAS "on." Once activated, KRAS triggers vital downstream signaling cascades, including the RAF-MEK-ERK and PI3K-AKT-mTOR pathways. Signal termination relies on GTPase-activating proteins (GAPs), which hydrolyze GTP back to GDP, returning KRAS to its resting state [1].

KRAS is the most frequently mutated isoform within the RAS family, responsible for approximately 80% of all RAS-driven cancers (Figure 1) [2]. Oncogenic mutations typically occur at hotspot residues such as G12, G13, and Q61. These mutations impair the protein's intrinsic GTPase activity and evade GAP-mediated down-regulation, perpetually locking KRAS in its active GTP-bound state. This relentless signaling drives uncontrolled tumor cell proliferation, survival, migration, and metabolic reprogramming. Epidemiological data indicate that KRAS mutations are present in approximately 90% of pancreatic ductal adenocarcinomas (PDAC), 30–44% of colorectal cancers (CRC), and nearly 30% of non-small cell lung cancers (NSCLC), serving as a major driver of poor clinical prognosis [3].

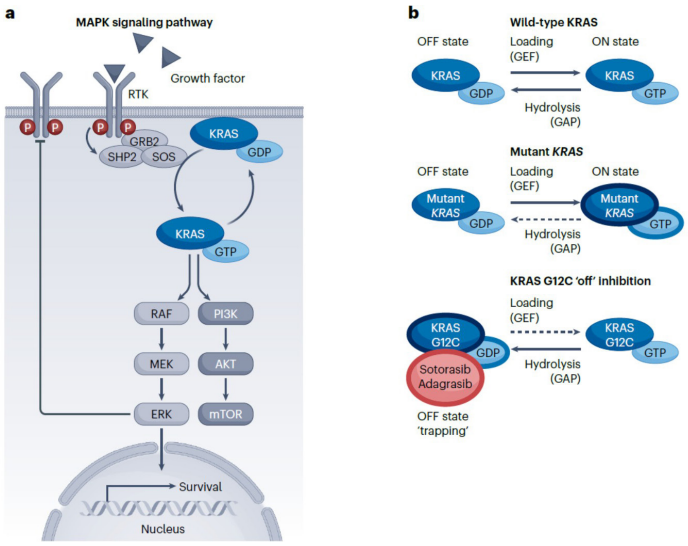


Figure 1. Regulation of KRAS and signal transduction pathways [2]

Advances in KRAS Inhibition: From G12C -target to Pan-Mutant Coverage

Historically, KRAS was deemed an "undruggable" target due to its relatively smooth surface and the absence of traditional deep binding pockets. A major breakthrough occurred in 2013 when Shokat et al. discovered a druggable pocket within the Switch-II region (S-IIP) of the KRAS G12C protein[4]. This revelation proved that selective targeting of mutant KRAS was possible, igniting a wave of drug discovery efforts.

Subsequently, Sotorasib (AMG 510) and Adagrasib (MRTX849) received FDA approval for the treatment of locally advanced or metastatic non-small cell lung cancer (NSCLC) harboring KRAS G12C mutations, bringing renewed hope to countless patients.

Building on the clinical success of G12C inhibitors, current research and development are rapidly expanding to encompass broader mutation profiles and novel mechanisms of action:

- **Broad-Spectrum Inhibition:** A major focus is the development of pan-KRAS inhibitors designed to cover a diverse array of mutations beyond G12C, such as G12D, G12V, and G13D.
- **Innovative Modalities:** Concurrently, researchers are deploying next-generation strategies, including Proteolysis Targeting Chimeras (PROTACs) to induce targeted KRAS degradation, and molecular glues to stabilize specific protein-protein interactions[5,6]. These multi-dimensional approaches strive to comprehensively dismantle KRAS-driven malignancies.

KRAS Resistance: From Mechanistic Insights to Combination Strategies

The clinical utility of KRAS inhibitors is significantly hindered by both primary (intrinsic) and acquired resistance. Primary resistance is frequently associated with co-occurring mutations in genes such as KEAP1 and STK11. In contrast, acquired resistance involves a highly complex and varied landscape of mechanisms, which primarily manifest across three dimensions (Figure 2):

1. On-Target Alterations: Resistance often arises from secondary KRAS mutations (including Switch-II pocket mutations like R68S, H95D/G/N/R, and Y96C/D/H/N, as well as *de novo* activating mutations at hotspots G12, G13, and Q61) or through KRAS gene amplification.

2. Pathway Reactivation and Bypass Tracks: Core drivers at the signaling level include the feedback activation of upstream receptor tyrosine kinases (RTKs) such as EGFR and MET, the activation of alternative RAS isoforms (e.g., HRAS, NRAS), and the subsequent reactivation of downstream MAPK and PI3K cascades.

3. Phenotypic and Transcriptional Reprogramming: Cellular plasticity, including the epithelial-mesenchymal transition (EMT) and YAP/TAZ-driven transcriptional reprogramming, also plays a critical role in facilitating tumor evasion.

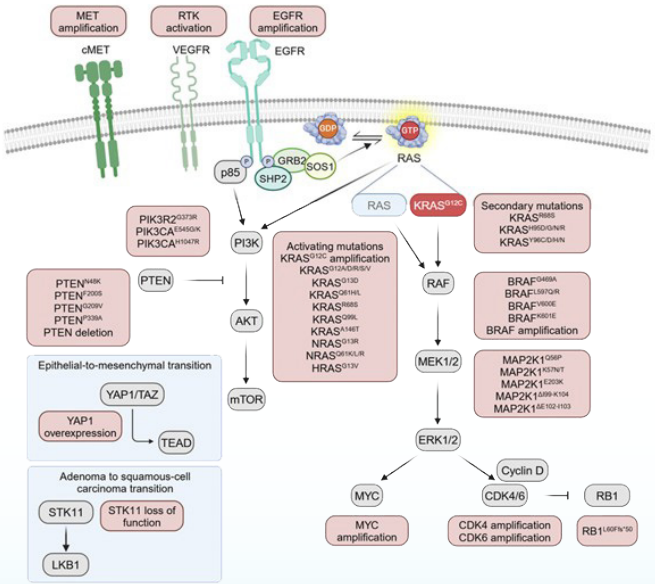
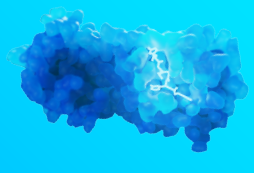


Figure 2. Resistance mechanisms to KRAS inhibition [6]



Given these multifaceted escape routes, rational combination therapy strategies have become an imperative choice to effectively overcome clinical resistance and maximize the efficacy of KRAS inhibitors. Currently, preclinical and clinical studies are actively investigating diverse rational combination strategies, which can be broadly categorized into five fundamental approaches (Figure 3) [6, 7] :

1. Upstream and Downstream Vertical Blockade: Blocking upstream receptor tyrosine kinase (RTK) signaling with SHP2 or SOS1 inhibitors effectively prevents signal transmission to RAS. Meanwhile, combinations with EGFR inhibitors or antibodies (e.g., afatinib, cetuximab) have demonstrated robust synergistic effects, particularly in colorectal cancer models. Targeting downstream nodes with MEK/ERK inhibitors can further suppress pathway output. Furthermore, highly aggressive "triple vertical combinations" (e.g., G12C inhibitor + MEK inhibitor + EGFR antibody) are yielding promising preliminary efficacy in early-stage clinical trials[8].

2. Combination with Immunotherapy: Because oncogenic RAS signaling stabilizes PD-L1 mRNA and upregulates its expression, combining KRAS inhibitors with PD-1/PD-L1 immune checkpoint inhibitors holds significant therapeutic promise. Notably, triplet regimens (G12C inhibitor + SHP2 inhibitor + anti-PD-1) have been shown to actively remodel the tumor microenvironment (TME) and significantly bolster systemic anti-tumor immune responses [9].

3. Combination with Standard Chemo/Radiotherapy: Given that chemotherapy continues to be a cornerstone of oncology, both preclinical and clinical data confirm that pairing G12C inhibitors with standard chemotherapeutic agents, such as carboplatin or gemcitabine, can substantially prolong the duration of tumor regression and enhance overall treatment efficacy[10].

4. Focusing on newly identified targets: Novel dependencies frequently emerge post-resistance. Inhibitors targeting these newly identified vulnerabilities—such as the pivotal autophagy kinase ULK1, tissue factor (TF), or farnesyltransferase (FTase)—are demonstrating early translational potential in overcoming acquired resistance to G12C inhibitors[11].

5. Next-Generation RAS Modalities: Beyond combinations, a new wave of innovative RAS-targeting monotherapies is rapidly advancing. Assets such as pan-RAS inhibitors (e.g., RMC-6236), RAS(ON) state-specific inhibitors (e.g., RMC-9805), and targeted PROTAC degraders (e.g., ASP3082) have already exhibited potent anti-tumor activity in preclinical evaluations [12,13].

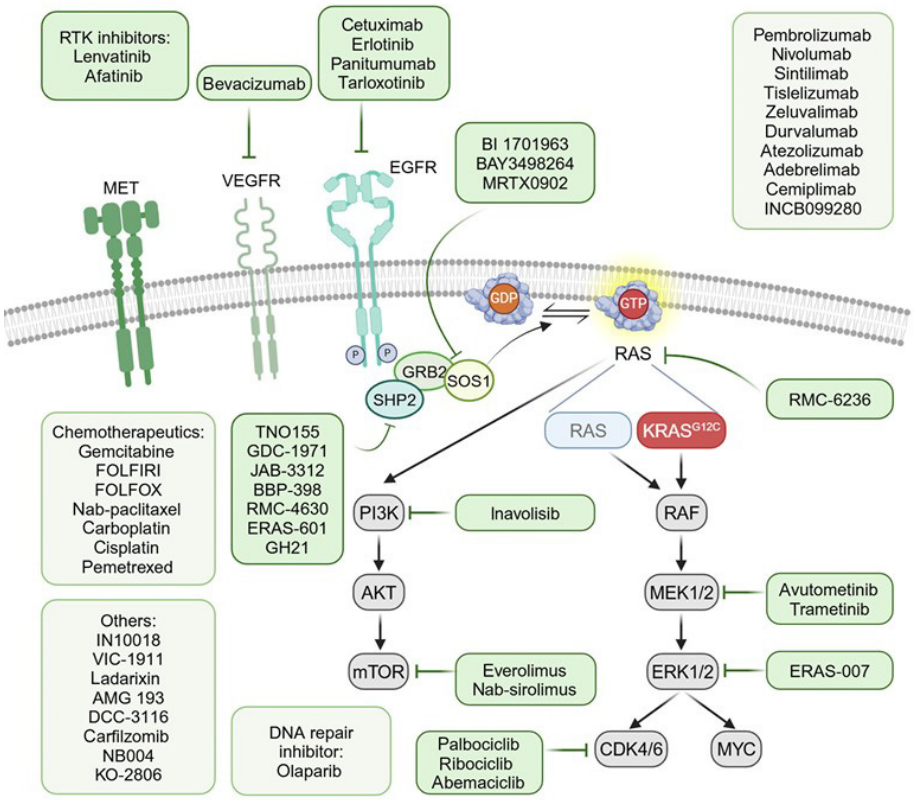


Figure 3. Combinatorial strategies under clinical investigation to enhance KRAS inhibitor efficacy [6]

Leveraging our deep expertise in KRAS biology and the technical demands of translational drug discovery, WuXi Biology has established a comprehensive, end-to-end service platform for KRAS related assays. Our robust capabilities encompass *in vitro* biochemical assays, highly translational cellular and animal models, and rigorous *in vivo* efficacy evaluations within complex drug-resistant models, covering a broad spectrum of KRAS mutations. WuXi Biology is dedicated to empowering our global partners to accelerate the discovery and clinical translation of next-generation KRAS-targeted therapies.

Case Study 1: *In Vitro* Biochemical Assays and Common RAS R&D Strategies

WuXi Biology has established a comprehensive *in vitro* biochemical assay platform covering diverse KRAS mutations. This platform integrates multiple functional evaluations—including RAS-RAF protein-protein interaction (PPI) analysis, KRAS- cyclophilin A (CYPA) molecular glue binding assays, and nucleotide exchange inhibition assays—providing high-quality, robust data to support KRAS-targeted drug discovery (Figure 4).

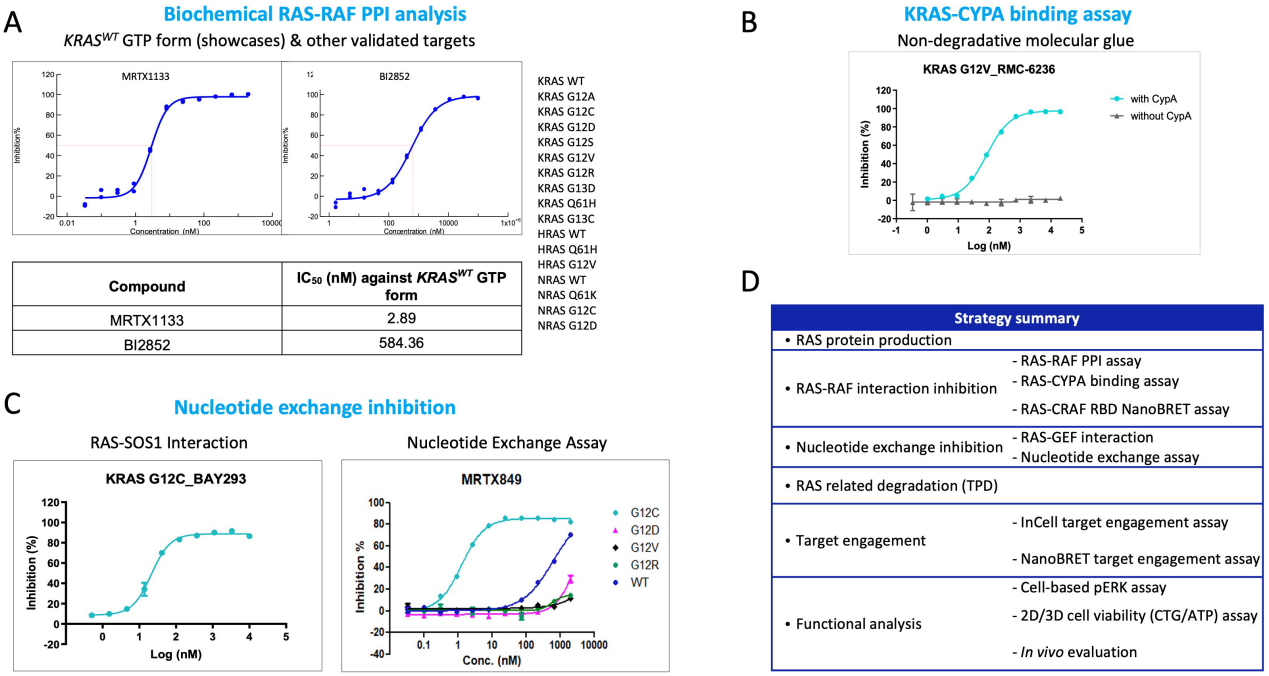
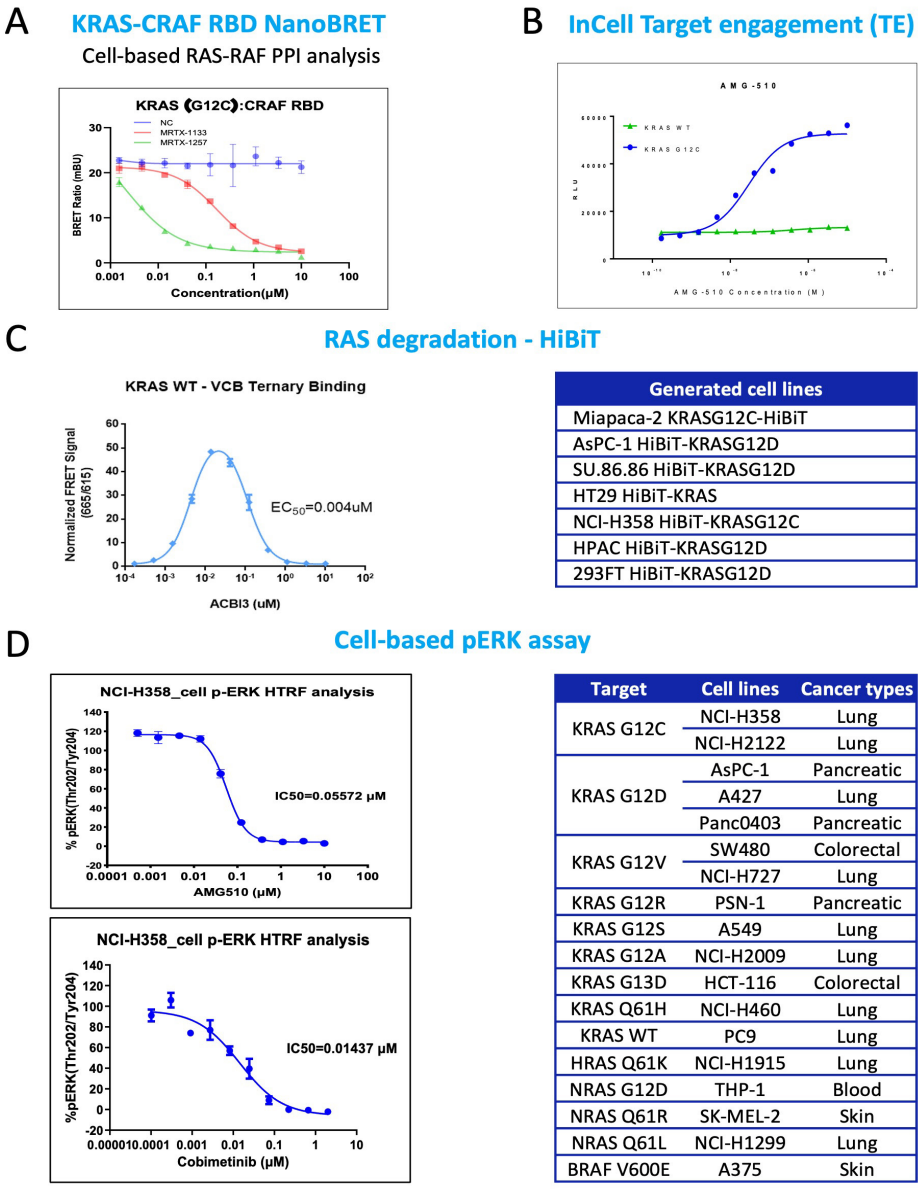


Figure 4. *In vitro* biochemical protein-level assays and common RAS R&D strategies

Case Study 2: Cellular Functional Assay Platform for KRAS

At the cellular level, WuXi Biology systematically evaluates the efficacy of KRAS pathway-targeted agents. Our platform encompasses NanoBRET-based KRAS-CRAF interaction assays, intracellular target engagement, RAS protein degradation (HiBiT system), and downstream pERK signaling functional readouts (Figure 5). By integrating varied KRAS-mutant tumor models, this platform significantly accelerates the optimization and progression of KRAS-targeted compounds.



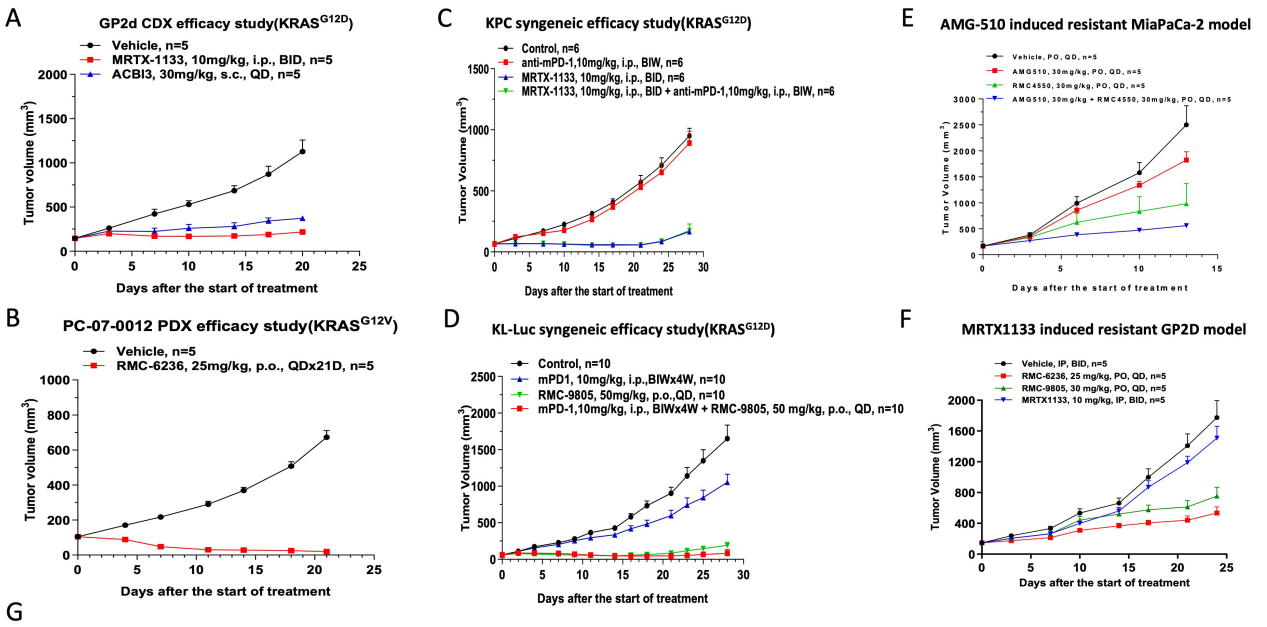
Generated cell lines
Miapaca-2 KRASG12C-HiBiT
AsPC-1 HiBiT-KRASG12D
SU.86.86 HiBiT-KRASG12D
HT29 HiBiT-KRAS
NCI-H358 HiBiT-KRASG12C
HPAC HiBiT-KRASG12D
293FT HiBiT-KRASG12D

Target	Cell lines	Cancer types
KRAS G12C	NCI-H358	Lung
	NCI-H2122	Lung
KRAS G12D	AsPC-1	Pancreatic
	A427	Lung
	Panc0403	Pancreatic
KRAS G12V	SW480	Colorectal
	NCI-H727	Lung
KRAS G12R	PSN-1	Pancreatic
KRAS G12S	A549	Lung
KRAS G12A	NCI-H2009	Lung
KRAS G13D	HCT-116	Colorectal
KRAS Q61H	NCI-H460	Lung
KRAS WT	PC9	Lung
HRAS Q61K	NCI-H1915	Lung
NRAS G12D	THP-1	Blood
NRAS Q61R	SK-MEL-2	Skin
NRAS Q61L	NCI-H1299	Lung
BRAF V600E	A375	Skin

Figure 5. Cellular functional assay platform for KRAS

Case Study 3: KRAS-Related *In Vivo* Efficacy Platform

WuXi Biology offers an extensive array of *in vivo* models—including CDX, PDX, syngeneic, and drug-resistant models—covering a broad spectrum of KRAS mutations to support the preclinical efficacy evaluation of KRAS inhibitors across varying mechanisms of action (Figure 6). Utilizing these models, we have systematically validated the *in vivo* anti-tumor activity of multiple flagship KRAS-targeted agents (e.g., MRTX1133, AMG 510, and RMC-6236).



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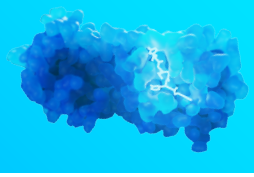
CDX (various KRAS mutants)	PDX (various KRAS mutants)	Syngeneic	Ba/F3 engineered models
122	182	10	19

Figure 6. *In vivo* efficacy models for KRAS inhibitors

Furthermore, through long-term *in vitro* or *in vivo* dosing induction, WuXi Biology has successfully developed a robust panel of KRAS inhibitor-resistant cell lines and *in vivo* resistance models. These critical models are instrumental for elucidating underlying resistance mechanisms (e.g., secondary on-target mutations and bypass signaling activation) and evaluating the anti-tumor efficacy of next-generation inhibitors or rational combination strategies in an acquired resistance setting. They provide a crucial preclinical foundation for overcoming resistance in KRAS-targeted therapies.

Table 1. KRAS Inhibitor-Induced Resistance Models

Model ID	Cancer type	Target	Status	
			In vitro	*In vivo*
AMG510-R-Mia PaCa-2	Pancreas cancer	KRAS G12C	Ready	Ready
AMG510-R-LU-01-0462	NSCLC	KRAS G12C	/	Ready
AMG510-R-CO-04-0315	Colorectal cancer	KRAS G12C	/	Ready
MRTX-849-R-Mia PaCa-2	Pancreas cancer	KRAS G12C	Ready	Ready
MRTX1133-R-GP2D	Colorectal cancer	KRAS G12D	Ready	Ready
MRTX1133-R-KPC	Pancreatic cancer	KRAS G12D	Ready	Ready
MRTX1133-R-HPAC	Pancreatic cancer	KRAS G12D	Ready	Ongoing
RMC-6236-R-GP2D	Colorectal cancer	RAS(ON) multi	Ready	Ongoing
RMC-6236-R-MIA PaCa-2	Pancreatic cancer	RAS(ON) multi	Ready	Ongoing



References

1. Shi Y, Zheng H, Wang T, Zhou S, Zhao S, Li M, Cao B. Targeting KRAS: from metabolic regulation to cancer treatment. Mol Cancer. 2025 Jan 11;24(1):9.

2. Singhal A, Li BT, O'Reilly EM. Targeting KRAS in cancer. Nat Med. 2024 Apr;30(4):969-983.

3. Kasper S, Sebastian M. The Pharmacologic Inhibition of KRAS Mutants as a Treatment for Cancer: Therapeutic Principles and Clinical Results. Dtsch Arztebl Int. 2025 Mar 21;122(6):163-167.

4. Ostrem JM, Peters U, Sos ML, Wells JA, and Shokat KM (2013). K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions. Nature 503, 548–551.

5. Faryal B, Ul Abideen Z, Irfan M, Ahmed H, Jalilov F, Abduraximova L, Ashraf GA. Targeted Protein Degradation in Cancer: PROTACs, New Targets, and Clinical Mechanisms. Biomolecules. 2026 Feb 19; 16(2):325.

6. Konstantinidou M, Arkin MR. Molecular glues for protein-protein interactions: Progressing toward a new dream. Cell Chem Biol. 2024 Jun 20;31(6):1064-1088.7. Isermann T, Sers C, Der CJ, Papke B. KRAS inhibitors: resistance drivers and combinatorial strategies. Trends Cancer. 2025 Feb;11(2):91-116.

8. Liu, B., Zhou, H., Tan, L. et al. Exploring treatment options in cancer: tumor treatment strategies. Sig Transduct Target Ther 9, 175 (2024).

9. Fedele C, Li S, Teng KW, et al. SHP2 inhibition diminishes KRASG12C cycling and promotes tumor microenvironment remodeling. J Exp Med. 2021 Jan 4;218(1):e20201414.

10. Miyashita H, Kato S, Hong DS. KRAS G12C inhibitor combination therapies: current evidence and challenge. Front Oncol. 2024 May 2;14:1380584.

11. Baranyi, M., Molnár, E., Hegedűs, L. et al. Farnesyl-transferase inhibitors show synergistic anticancer effects in combination with novel KRAS-G12C inhibitors. Br J Cancer 130, 1059–1072 (2024).

12. Arbour K, Punekar S, Garrido-Laguna I . et al. 652O Preliminary clinical activity of RMC-6236, a first-in-class, RAS-selective, tri-complex RAS-MULTI(ON) inhibitor in patients with KRAS mutant pancreatic ductal adenocarcinoma (PDAC) and non-small cell lung cancer (NSCLC), Annals of Oncology, 34S458.

13. Yoshinari, T., Nagashima, T., Ishioka, H. et al. Discovery of KRAS(G12D) selective degrader ASP3082. Commun Chem 8, 254 (2025).