A Cell Line Platform of Resistance to KRAS Inhibitors and Pan-RAS Molecular Glues

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Introduction

KRAS mutations are dominant oncogenic drivers in many solid tumors. Although KRAS G12C inhibitors and pan-RAS targeted agents have shown clinical promise, intrinsic and acquired resistance remains a major challenge.In this study, we established a well-validated panel of resistant cell lines against KRAS G12C inhibitors, pan-RAS molecular glues, and pan-RAS inhibitors. We performed multi-omics characterization, cross-resistance profiling, and genomic analysis to define key resistance features, including copy number alterations and secondary KRAS mutations. This platform provides a robust preclinical model for investigating resistance mechanisms and supporting the development of next-generation RAS-targeted therapies.

RAS Inhibitor Activity across a KRAS Mutant Cancer Cell Panel

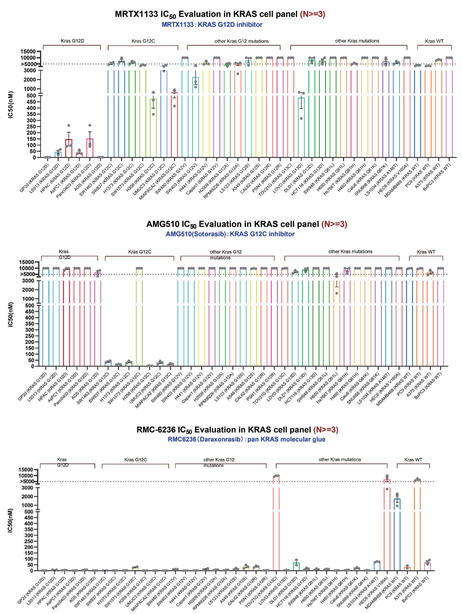


Figure1. Potency of KRAS inhibitors in 3D-cultured KRAS-mutant cancer cell lines. RMC-6236 (Daraxonrasib) shows broader inhibitory activity across diverse KRAS mutations compared with other small-molecule inhibitors.

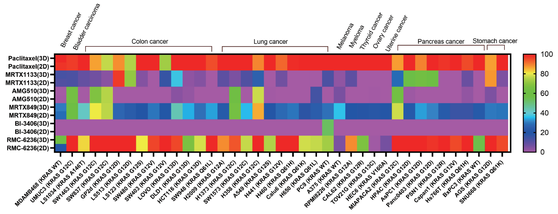
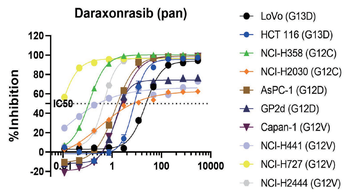
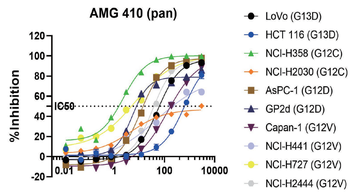


Figure2. Consistency of KRAS inhibitor potency in 2D vs. 3D culture systems. IC_{50} evaluation across a broad KRAS-mutant cell panel shows consistent inhibitor potency between 2D and 3D detection methods.



	Bottom	Top	IC50
LoVo (G13D)	2.88	93.78	22.45
HCT 116 (G13D)	-1.72	95.39	7.33
NCI-H358 (G12C)	1.68	100.29	0.11
NCI-H2030 (G12C)	-9.91	62.80	0.32
AsPC-1 (G12D)	-10.97	99.06	1.33
GP2d (G12D)	-11.61	74.21	1.30
Capan-1 (G12V)	-19.70	99.10	1.76
NCI-H441 (G12V)	-20.00	66.49	0.01
NCI-H727 (G12V)	-20.00	96.37	0.01
NCI-H2444 (G12V)	5.29	97.23	0.46



	Bottom	Top	IC50
LoVo (G13D)	-3.06	96.40	53.87
HCT 116 (G13D)	1.45	98.17	623.74
NCI-H358 (G12C)	15.68	100.25	2.24
NCI-H2030 (G12C)	2.92	46.96	4.64
AsPC-1 (G12D)	-8.19	97.57	11.25
GP2d (G12D)	1.12	78.93	5.08
Capan-1 (G12V)	-9.62	98.96	132.29
NCI-H441 (G12V)	-4.63	78.94	50.97
NCI-H727 (G12V)	8.26	100.40	6.73
NCI-H2444 (G12V)	-3.19	96.05	32.93

Figure 3. Dose–response curves of pan-RAS inhibitors Daraxonrasib and AMG410 in KRAS-mutant cancer cell lines.

KRAS G12C inhibitors and pan-RAS molecular glue/inhibitor Resistant Cell Line

Table 1. Summary of in vitro drug-induced resistant cell line generation and characterization.

No.	Drug Resistant Cells	Status	RI#	STR	RNA-seq	WES	Drug	Drug Type	Target	Cancer Type	Method
1	MIA PaCa-2/Sotorasib R	In vitro validated	>100	√	√		Sotorasib	Targeted Small Molecule	KRAS G12C	Pancreatic	In Vitro Drug-Induced Resistance
2	NCI-H358/Adagrasib R	In vitro validated	>20	√	√	√	Adagrasib	Targeted Small Molecule	KRAS G12C	Lung	In Vitro Drug-Induced Resistance
3	NCI-H358/Sotorasib R	In vitro validated	>100	√	√	√	Sotorasib	Targeted Small Molecule	KRAS G12C	Lung	In Vitro Drug-Induced Resistance
4	LoVo/Daraxonrasib R	In vitro validated	>20	√	√	√	Daraxonrasib	Molecular Glue	pan-RAS	Colorectal	In Vitro Drug-Induced Resistance
5	HCT116/Daraxonrasib R	In vitro validated	>100	√	√	Ongoing	Daraxonrasib	Molecular Glue	pan-RAS	Colorectal	In Vitro Drug-Induced Resistance
6	GP2d/Daraxonrasib R	In vitro validated	>100	√	Ongoing	Ongoing	Daraxonrasib	Molecular Glue	pan-RAS	Colorectal	In Vitro Drug-Induced Resistance
7	GP2d/AMG410 R	In vitro validated	>100	√	Ongoing	Ongoing	AMG410	Targeted Small Molecule	pan-RAS	Colorectal	In Vitro Drug-Induced Resistance

Resistant cell lines were established by chronic exposure to KRAS G12C inhibitors or pan-RAS agents. RI, resistance index (fold change in IC_{50} of resistant vs. parental cells); STR, authenticated; WES, whole-exome sequencing; RNA-seq, transcriptome sequencing.

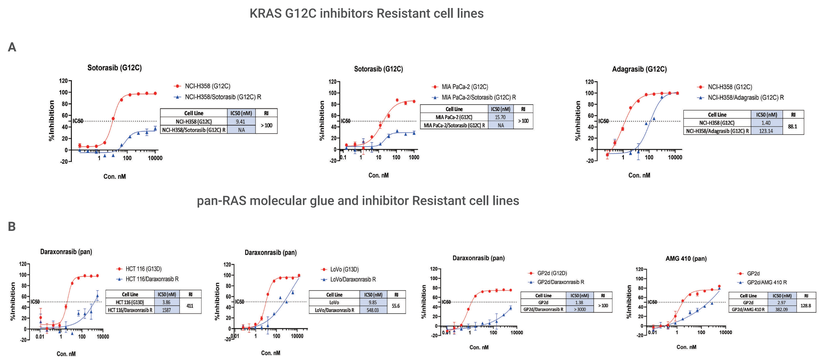


Figure 4. Dose–response curves from cell proliferation assays for KRAS G12C inhibitors (sotorasib, adagrasib)(A) and pan-RAS molecular glues/inhibitors (daraxonrasib, AMG410)(B).

Multi-Drug Cross-Resistance Profiling

Table 2. Cross-resistance profile of RAS inhibitor-resistant cell lines.

Resistance Index (RI)		RASi Resistant Cell Lines						
Drugs	Cells	NCI-H358 /Sotorasib R	MIA PaCa-2 /Sotorasib R	NCI-H358 /Adagrasib R	LoVo /Daraxonrasib R	HCT116 /Daraxonrasib R	GP2d /Daraxonrasib R	GP2d /AMG410 R
		G12C	G12C	G12C	G13D	G13D	G12D	G13D
Daraxonrasib(RMC-6236)	pan	> 10	ND#	1	> 20	> 100	> 100	> 100
AMG410	pan	ND#	ND#	ND#	ND#	Insensitive*	> 100	> 100
Zoldonrasib(RMC-9805)	G12D	ND#	ND#	ND#	Insensitive*	Insensitive*	> 100	> 100
MRTX1133	G12D	Insensitive*	ND#	Insensitive*	ND#	Insensitive*	> 100	> 100
Sotorasib(AMG-510)	G12C	> 100	> 10	ND#	ND#	Insensitive*	Insensitive*	Insensitive*
Adagrasib(MRTX849)	G12C	ND#	ND#	> 20	ND#	Insensitive*	Insensitive*	Insensitive*
Elironrasib(RMC-6291)	G12C	ND#	ND#	ND#	ND#	Insensitive*	Insensitive*	Insensitive*
RMC-5127	G12V	ND#	ND#	ND#	ND#	Insensitive*	Insensitive*	Insensitive*

Heatmap showing resistance index (RI) across a panel of KRAS-mutant (G12C/G12D/G13D) cell lines treated with pan-RAS and mutation-specific inhibitors. ND, not determined; Insensitive, no significant inhibition at maximum test concentration; RI >100, high-level resistance.

Bioinformatics Analysis for RAS Resistant Cell Line

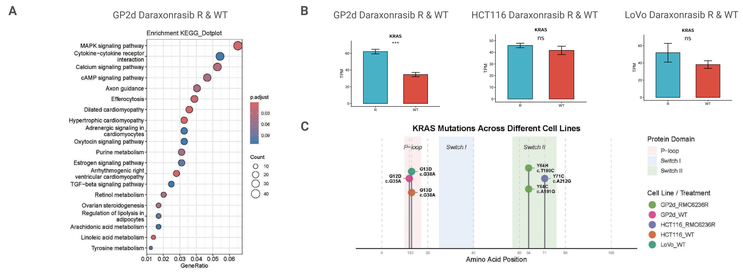


Figure 4. Genomic and transcriptomic analysis of Daraxonrasib resistance in KRAS G12D/G13D colorectal cancer cell lines. (A) KEGG pathway enrichment dot plot of differentially expressed genes in GP2d Daraxonrasib-resistant cells versus wild-type (WT) cell, highlighting the top enriched pathways (e.g., MAPK signaling). (B) KRAS transcript expression (TPM) in WT and Daraxonrasib-resistant (R) GP2d, HCT116, and LoVo cell lines, with statistical significance indicated (***p < 0.001, ns = not significant). (C) KRAS mutation analysis in in WT and Daraxonrasib-resistant GP2d, HCT116, and LoVo cell lines.

Table 3. KRAS CNV and Mutation Profiles in Daraxonrasib-Resistant vs. Wild-Type Cell Lines

Cell Line	Wild-type vs. Daraxonrasib-resistant	CNV Alteration	KRAS Copy Number	Key Annotation	KRAS Mutation (Wild-Type)	de novo mutation in Daraxonrasib-Resistant Cells
HCT116	Resistant vs WT	No CNV alteration	–	No CNV observed	NM_001369786: exon2:c.G38A:p.G13D	NM_001369786:exon3:c.A212G:p.Y71C
LoVo	Resistant vs WT	Heterozygous deletion	1.4 (below normal)	–	NM_001369786: exon2:c.G38A:p.G13D	No mutation detected
GP2d	Resistant vs WT	Heterozygous deletion (KRAS region)	–	Frameshift deletion; whole-gene deletion	NM_001369786: exon2:c.G35A:p.G12D	1.NM_001369786:exon3:c.A191G:p.Y64C 2.NM_001369786:exon3:c.T190C:p.Y64H

Summary of KRAS copy number variation (CNV) and de novo secondary mutations in HCT116, LoVo, and GP2d colorectal cancer cell lines following Daraxonrasib resistance induction, compared to isogenic wild-type controls.

Summary

- A KRAS inhibitor-resistant cell line platform was successfully established covering G12C inhibitors, pan-RAS molecular glue (Daraxonrasib), and pan-RAS inhibitor (AMG410).
- Cross-resistance profiling revealed distinct resistance patterns across different KRAS mutational backgrounds.
- Genomic and transcriptomic analyses identified KRAS copy number changes and secondary KRAS mutations as key resistance mechanisms.
- This platform enables systematic characterization of resistance to KRAS/pan-RAS targeted therapies.