Characterization and Overcoming of KRAS-Targeted Therapy Resistance in Resistant Cancer Cell Lines

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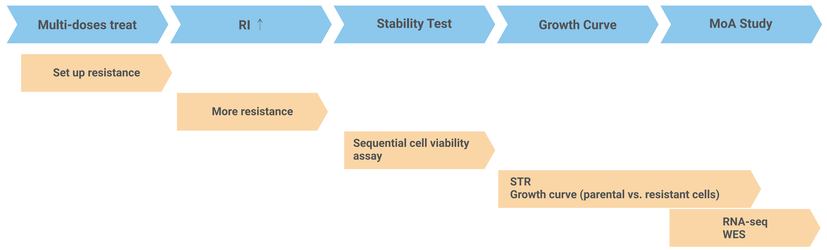
Introduction

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. Here, we established a well-validated panel of resistant cell lines against KRAS G12C/G12D 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. We further evaluated diverse combination strategies to overcome RAS-driven resistance in this platform. This model provides a robust preclinical system for investigating resistance mechanisms and supporting next-generation RAS-targeted therapy development.

Establishment Workflow of Resistant Models

Procedure of In Vitro Drug-Induced Resistance



Portfolio Overview of Resistant Cell Models

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

No.	Drug Resistant Cells	Cancer Type	Drug	Target	WES	Mutation analysis
1	MIA PaCa-2/Sotorasib R	Pancreatic	Sotorasib	KRAS G12C	Ongoing	
2	NCI-H358/Sotorasib R	Lung	Sotorasib	KRAS G12C	√	Ongoing
3	NCI-H358/Adagrasib R	Lung	Adagrasib	KRAS G12C	√	Ongoing
4	GP2d/MRTX1133 R	Colorectal	MRTX1133	KRAS G12D	√	Kras(S65R)
5	GP2d/Zoldonrasib R	Colorectal	Zoldonrasib	KRAS G12D	√	AKT(V185A), RET(T421I)
6	AsPC-1/Zoldonrasib R	Pancreatic	Zoldonrasib	KRAS G12D	Ongoing	
7	HCT 116/Daraxonrasib R	Colorectal	Daraxonrasib	pan RAS	√	Kras(Y71C), NF1(R135W)
8	LoVo/Daraxonrasib R	Colorectal	Daraxonrasib	pan RAS	√	MAP2K1(K57N)
9	GP2d/AMG 410 R	Colorectal	AMG 410	pan RAS	√	MYC(G98A), ZEB1(T4A)
10	GP2d/Daraxonrasib R	Colorectal	Daraxonrasib	pan RAS	√	BRAF(B168V), Kras(Y64C)
11	AsPC-1/Daraxonrasib R	Pancreatic	Daraxonrasib	pan RAS	Ongoing	
12	AsPC-1/AMG 410 R	Pancreatic	AMG 410	pan RAS	Ongoing	
13	NCI-H441/Daraxonrasib R	Lung	Daraxonrasib	pan RAS	Ongoing	

Table 2.Cross-resistance profile Summary.

Resistance Index (RI)		RASi Resistant Cell Lines												
Cells Drugs		NCI-H358/ Sotorasib R	MIA PaCa-2/ Sotorasib R	NCI-H358/ Adagrasib R	GP2d/ MRTX1133 R	GP2d/ Zoldonrasib R	AsPC-1/ Zoldonrasib R	LoVo/ Daraxonrasib R	HCT 116/ Daraxonrasib R	GP2d/ Daraxonrasib R	AsPC-1/ Daraxonrasib R	NCI-H441/ Daraxonrasib R	GP2d/AMG 410 R	AsPC-1/AMG 410 R
		G12C	G12C	G12C	G12D	G12D	G12D	G13D	G13D	G12D	G12D	G12V	G12D	G12D
Daraxonrasib(RMC-6236)	pan	> 10	ND#	ND#	1	3	> 5	> 20	> 20	> 100	> 100	> 20	> 100	> 100	> 5
AMG410	pan	ND#	ND#	ND#	> 20	> 5	> 100	ND#	Insensitive*	> 100	> 100	Insensitive*	> 100	> 100
Zoldonrasib(RMC-9805)	G12D	ND#	ND#	ND#	> 20	> 100	> 100	Insensitive*	Insensitive*	> 100	> 100	Insensitive*	> 100	> 100
MRTX1133	G12D	Insensitive*	ND#	Insensitive*	> 100	> 100	> 100	Insensitive*	Insensitive*	> 100	> 100	Insensitive*	> 100	> 100
Sotorasib(AMG-510)	G12C	> 100	> 10	ND#	Insensitive*	Insensitive*	Insensitive*	Insensitive*	Insensitive*	Insensitive*	Insensitive*	Insensitive*	Insensitive*	Insensitive*
Adagrasib(MRTX849)	G12C	ND#	ND#	> 20	Insensitive*	Insensitive*	Insensitive*	Insensitive*	Insensitive*	Insensitive*	Insensitive*	Insensitive*	Insensitive*	Insensitive*
Elironrasib(RMC-6291)	G12C	ND#	ND#	ND#	Insensitive*	Insensitive*	Insensitive*	Insensitive*	Insensitive*	Insensitive*	Insensitive*	Insensitive*	Insensitive*	Insensitive*
RMC-5127	G12V	ND#	ND#	ND#	Insensitive*	Insensitive*	Insensitive*	Insensitive*	Insensitive*	Insensitive*	Insensitive*	> 100	Insensitive*	Insensitive*

Insensitive*: The parental cell line shows no response to the tested drugs.
ND#: Not Done

MOA Exploaration of Rasi Resistant Cell Lines

Table 3. Summary of Reported Genes and Signaling Pathways Associated with RAS Resistance.

DATA	Category	Genes/Pathway
WES	Secondary mutations in RAS itself	KRAS (secondary mutations such as Y96D/R68S), NRAS, HRAS, copy number variation analysis
	Upstream RTK gene mutations	EGFR/MET/HER2/FGFR1, FGFR-RET fusions(amplifications / mutations / fusions)
	Downstream gene mutations	BRAF/MEK1/2/PIK3CA/AKT
	Negative regulation inactivation	NF1/PTEN
	Metabolic / immune / epigenetic regulation	KEAP1/SMARCA4/STK11
	Wnt pathway	APC/CTNNB1
	Cell cycle / tumor suppressor pathway abnormalities	TP53/CDKN2A/SMAD4/MYC/APC
RNA-seq	MAPK activation	DUSP ↓ / ETV ↑
	Other RAS isoforms	HRAS, NRAS
	RTK bypass signaling	MET/AXL/IGF1R
	PI3K/AKT pathway compensation	PIK3CA / AKT1 / MTOR / PTEN ↓
	EMT	VIM/ZEB1 ↑ / CDH1 ↓
	Cell cycle / apoptosis	CCND1/MYC/BCL2
	Immune microenvironment / immune evasion	PD-L1 / CXCL1/2 / IL-6/8 / CD163
	Stemness / CSC characteristics	SOX2 / OCT4 / Wnt/β-catenin / Notch

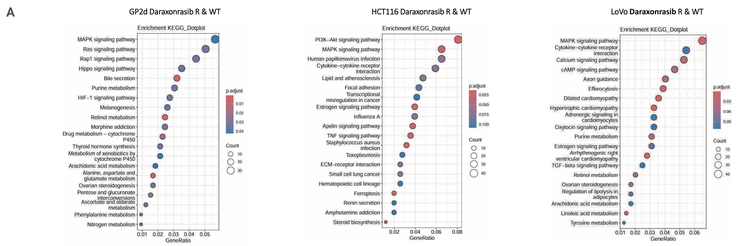
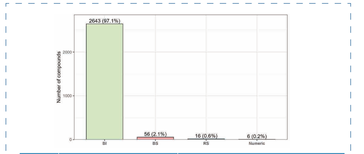


Figure 1. Genomic and transcriptomic characterization of Daraxonrasib resistance in KRAS G12C/G12D/G13D cancer cell lines.(A) KEGG pathway enrichment analysis of differentially expressed genes in Daraxonrasib-resistant (R) cells relative to their isogenic wild-type (WT) counterparts. The dot plot displays the top enriched pathways, including MAPK signaling. (B) Gene expression Ration in Rasi-resistant Colorectal, Pancreatic, and NSCLC cell lines to their WT. (C) Schematic of mutation analysis in Rasi-resistant variants of GP2d, HCT116, and LoVo cell lines, showing mutation for Single Nucleotide Variant (SNV) and Deletion Mutation(DEL).

Case Study 1: HTS Compound Library Screening in RASi Resistant cell lines

Hit Screening with single dose Sensitive or Resistant → Full doses testing of Candidate cpds RI testing → 6×6 dose Matrix combination Synergy score

STEP 1. Hits Screening



	Item	% Inhibition
△	Difference between 2 cells	Parental cells ≥ 50%, Resistant cells < 50%
RS	Resistantcellssensitive	Parental cells < 50%, Resistant cells ≥50%
BS	Both cells sensitive	Parental cells ≥ 50%, Resistant cells ≥ 50%
BI	Both cells insensitive	Parental cells < 50%, Resistant cells < 50%

RS compounds Compounds classified as RS (excluding Daraonrasib)	
Panobinostat	RS
Lonafarnib	RS
Bardoxolone methyl	RS
Azvudine	RS
Givinostat	RS
Econazole	RS
Temposide	RS
Selinexor	RS
Disulfiram	RS
Talazoparib	RS
Talazoparib tosylate	RS
Ingenol Mebutate	RS
Amsacrine	RS
Axitinib	RS
Decitabine	RS
Midostaurin	RS
Compound	Type

Figure 2. High-throughput screening of 2,721 FDA-approved compounds (1000 nM, 7 days) in GP2d and Daraxonrasib-resistant GP2d cells using CTG.

STEP 2. Dose response curve validation

Table 4. Dose-Response Data and Resistance Index (RI) of Hit Compounds Identified from the Library Screen in step 1, Comparing Daraxonrasib-Resistant and Parental Cell Lines.

Compound_ID	RI (GP2d/Daraxonrasib R)	Target
Lonafarnib	0.01	Autophagy; Farnesyl Transferase; Ras
Olverembatinib	6.25	Protein Tyrosine Kinase/RTK
Oxyphenisatin acetate	5.38	Autophagy
Talazoparib	0.08	PARP
Ingenol Mebutate	0.01	PKC
Decitabine	0.18	Apoptosis; DNA Methyltransferase; Nucleoside Antimetabolite/Analog

Note:
RI > 5 indicates the resistant cells are resistant to the compounds comparing with parental cells.
RI < 0.2 indicates the resistant cells are sensitive to the compounds comparing with parental cells.

STEP 3. Combination Evaluation

Table 5. Combination Synergy Analysis of Daraxonrasib with Hit Compounds (Lonafarnib, Talazoparib) in Daraxonrasib-Resistant GP2d Cells

Drug A	Drug B	Drug B Target	Cell Line	Parameter	Synergy Score
Daraxonrasib	Lonafarnib	FTase/FPTase	GP2d/Daraxonrasib R	HSA	14.3
Daraxonrasib	Lonafarnib	FTase/FPTase	GP2d/Daraxonrasib R	Loewe	12.8
Daraxonrasib	Talazoparib	PARP1,PARP2	GP2d/Daraxonrasib R	HSA	14.5
Daraxonrasib	Talazoparib	PARP1,PARP2	GP2d/Daraxonrasib R	Loewe	12.6

Note: The synergistic parameter > 10 indicates that this drug group shows synergy effect.

Case study 2: Combination Small Library screening

Drug A (single-dose: EC20 + EC70) Drug B: Customized Library → Combination Candidates IC_{50} shift ≥ 10 folds → 10×10 dose Matrix combination Synergy score>=10

Table 6. Combination Synergy Analysis of Daraxonrasib with Hit Compounds in Daraxonrasib-Resistant GP2d Cells

Drug A	Drug B	Drug B Target	Cell Line	ZIP Score
Daraxonrasib	FHD-286	SMARCA4/SMARCA2	GP2d	4.12
Daraxonrasib	FHD-286	SMARCA4/SMARCA2	GP2d/Daraxonrasib R	16.49
Daraxonrasib	Pelabresib	BET(BRD2/3/4)	GP2d	5.66
Daraxonrasib	Pelabresib	BET(BRD2/3/4)	GP2d/Daraxonrasib R	13.95
Daraxonrasib	Saruparib	PARP1	GP2d	4.29
Daraxonrasib	Saruparib	PARP1	GP2d/Daraxonrasib R	15.57

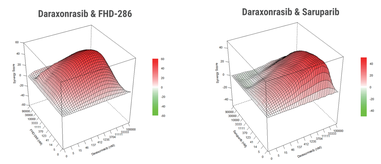


Figure 3. Synergy landscapes of Daraxonrasib in combination with FHD-286 or Saruparib in Daraxonrasib-Resistant GP2d Cell, analyzed using SynergyFinder.

Summary

- A KRAS inhibitor-resistant cell line platform was successfully established covering G12C, G12D 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 the mechanism of resistance.
- Different compound combination strategies were assessed for their ability to overcome RAS resistance in our established resistant cell platform.