Discovery and Characterization of Potential Drivers of Resistance to the KRAS^{G12D} Inhibitor MRTX1133 in Cancer Cell Line Models

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BACKGROUND

KRAS^{G12D} is the most common KRAS mutation and is present in ~34% of pancreatic cancer, ~10% of colorectal cancer, ~4% of lung adenocarcinoma, ~11% of bile duct carcinoma, ~5% of endometrial cancer. Effective therapies for these cancers are needed.

MRTX1133 is a potent and selective non-covalent KRAS^{G12D} inhibitor that binds the Switch II binding pocket with high-affinity and binds to active and inactive forms of KRAS^{G12D}, in an ongoing phase 1 clinical trial.

Previous pharmacodynamic and pharmacogenomic profiling in sensitive and partially resistant models identified mechanisms implicated in limiting the anti-tumor response including signaling pathways that induce feedback reactivation and/or bypass KRAS dependence using KRAS^{G12C} inhibitors[1]. Mechanisms of acquired resistance to KRAS^{G12D} inhibition have not yet been fully elucidated. Thus, KRAS^{G12D} cell lines (GP2d and SNU-1033, colorectal cancer; HPAC and AsPC-1, pancreatic cancer) were utilized to study acquired resistance to MRTX1133 following continuous treatment at increasing concentrations over time. These cell lines were confirmed to be stably resistant as determined by evaluation of response to MRTX1133 in vitro and in vivo and were extensively profiled to determine underlying mechanisms of resistance utilizing molecular profiling strategies as well as drug combination screens to interrogate pathways that could circumvent resistance.

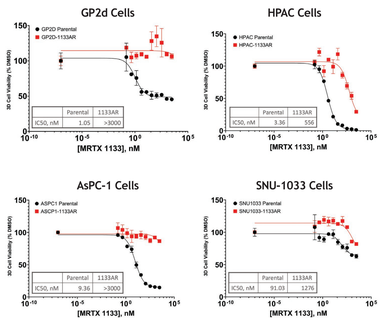
Development of in vitro and in vivo models of acquired resistance to MRTX1133

Figure 1. Generation of resistance to MRTX1133 in 4 KRAS G12D cell lines

Cell Lines with KRAS G12D mutation were treated with MRTX1133 at escalating doses starting at 10nM until cells reach 90% confluency.

Once cells reached confluency dose was increased again until cells were able to grow in the presence of 5uM MRTX1133 in growth media.

Dose levels were 10nM → 30nM → 100nM → 300nM → 1uM → 2uM → 5uM. It took between 5 to 8 months for all cells to reach 5uM MRTX1133 acquired resistance.

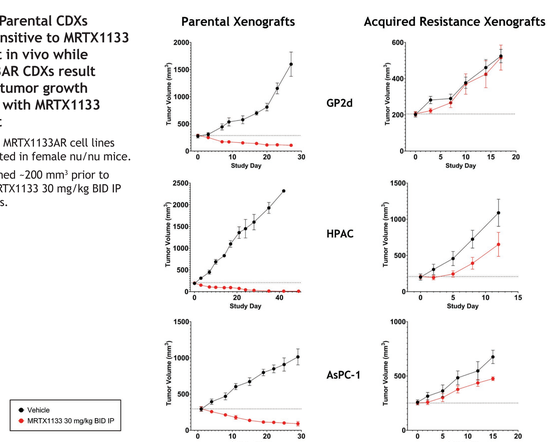


- 3D cell viability IC_{50} shift seen after MRTX1133 dose response in parental vs acquired resistance cells

Figure 2. Parental CDXs remain sensitive to MRTX1133 treatment in vivo while MRTX1133AR CDXs result in lack of tumor growth inhibition with MRTX1133 treatment

Parental and MRTX1133AR cell lines were implanted in female nu/nu mice.

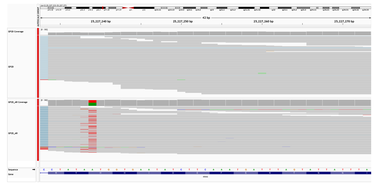
Tumors reached ~200 mm³ prior to beginning MRTX1133 30 mg/kg BID IP for 2-3 weeks.



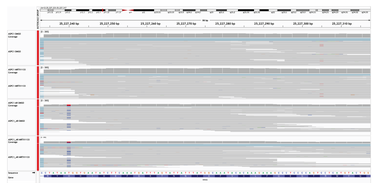
Emergence of KRAS H95 and Y96 switch II pocket mutations in two of four acquired resistance models

Figure 3. GP2D and AsPC-1 mechanism of resistance: Secondary KRAS switch II mutation

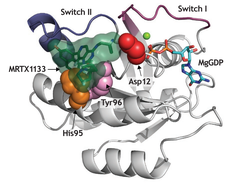
A. GP2d Parental and AR cell lines by RNAseq



B. AsPC-1 Parental and AR cell lines by RNAseq



C. Model of MRTX1133 binding in SWII pocket highlights interactions with neighboring residues



A and B: RNAseq was performed on GP2d and AsPC-1 parental and acquired resistant (AR) cell lines and heterozygous mutations were discovered in the switch II pocket.

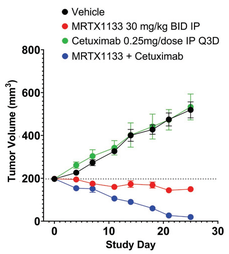
RNAseq samples were aligned to the human reference genome (GRCh38) using STAR 2.7.1.

Samples were visualized in IGV (v2.17.3). Lower track (blue) shows the reference genome annotation. Read length = 150bp.

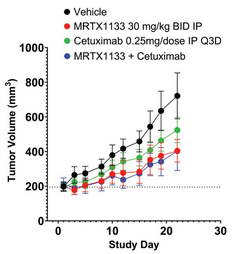
- **GP2d AR:** Two KRAS variants with base substitutions A->T found at positions chr12:25,227,238 and chr12:25,227,239 which results in missense mutations Y96N and H95Q. 2x increase in KRAS expression in MRTX1133AR line compared to parental.
- **AsPC-1 AR:** Heterozygous KRAS missense mutation H95R identified. No differences in KRAS expression between parental and MRTX1133AR line. MAPK signaling regulators are increased in MRTX1133AR samples compared to parental samples.
- In addition to KRAS mutations, hallmark pathways for Hedgehog and WNT signaling are downregulated across all models in MRTX1133AR cell lines

Figure 4. Secondary KRAS mutations are likely a main driver of resistance and typical combination strategies are not sufficient to re-sensitize tumors to MRTX1133 treatment

A. AsPC-1 Parental Efficacy Study



B. AsPC-1 MRTX1133AR Efficacy Study



C. Differences in tumor time to size

Cell Line		Time to size
GP2d	Parental	14 days
	AR	14 days
HPAC	Parental	10 days
	AR	> 40 days
AsPC-1	Parental	17 days
	AR	32 days
SNU-1033	Parental	30 days
	AR	no tumor

A. Efficacy study with parental AsPC-1 CDX shows improved antitumor activity with MRTX1133 and cetuximab combination

B. Efficacy study with AsPC-1 MRTX1133AR CDX shows loss of antitumor activity with MRTX1133 single agent and the combination of MRTX1133 with cetuximab does not improve the outcome

C. In 3 of 4 CDX models, the MRTX1133AR line takes longer to grow to 200 mm3 than the parental lines

Increased signaling through pAKT and pSTAT3 is evident in HPAC MRTX1133AR, a resistant model without secondary KRAS mutations

Figure 5. HPAC parental vs MRTX1133AR cell line in vitro characterization shows increased reliance on AKT and STAT3 signaling

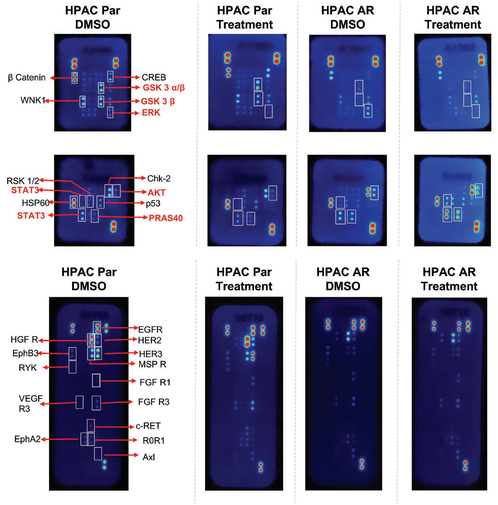


Table 1. Quantification of phosphorylation changes of HPAC Parental and MRTX1133AR cells after MRTX1133 treatment

		Log2 FC 1133 vs DMSO Parental	Log2 FC 1133 vs DMSO AR	Log2 FC AR vs parental DMSO	Log2 FC AR vs parental MRTX1133		
AKT1/2/3	T308	-0.03	0.83	1.60	2.45	AKT1/2/3	T308
B-Catenin	na	0.31	-0.26	-1.84	-2.41	B-Catenin	na
Chk2	T68	0.51	0.81	2.07	2.37	Chk2	T68
CREB	S133	1.50	0.44	-1.48	-2.55	CREB	S133
ERK1/2	T202/Y204	-1.56	-2.98	1.28	-0.14	ERK1/2	T202/Y204
GSK3a/b	S21/S9	0.05	-0.76	-3.05	-3.85	GSK3a/b	S21/S9
GSK3b	S9	-0.59	-1.04	-3.59	-4.04	GSK3b	S9
HSP60	na	0.48	0.03	0.09	-0.36	HSP60	na
p53	S46	0.59	0.99	-0.49	-0.08	p53	S46
PRAS40	T246	0.43	1.69	2.15	3.42	PRAS40	T246
RSK1/2	S221/S227	1.22	0.33	1.84	0.94	RSK1/2	S221/S227
STAT3	S727	-0.61	2.01	0.73	3.36	STAT3	S727
STAT3	Y705	0.75	1.62	2.14	3.01	STAT3	Y705
WNK1	T60	0.00	-0.40	-1.19	-1.58	WNK1	T60
Axl		2.26	0.63	2.52	0.89	Axl	
c-Ret		0.38	-0.83	-4.40	-5.60	c-Ret	
EGFR		-0.96	-0.26	-1.68	-0.98	EGFR	
EphA2		-1.42	1.34	-4.07	-1.31	EphA2	
EphB3		-0.47	-0.51	-0.93	-0.96	EphB3	
FGFR1		1.34	0.42	0.32	-0.60	FGFR1	
FGFR3		1.21	0.76	-0.77	-1.22	FGFR3	
HER2		0.26	0.46	-1.95	-1.75	HER2	
HER3		-0.33	0.45	-6.46	-5.68	HER3	
HGFR		-0.47	0.50	-4.36	-3.40	HGFR	
MSPR		-1.27	0.49	-6.25	-4.48	MSPR	
ROR1		-0.39	0.40	-1.04	-0.25	ROR1	
RYK		1.09	-0.61	-0.21	-1.91	RYK	
VEGFR3		4.05	0.80	2.60	-0.66	VEGFR3	

HPAC cells were treated for 3hrs with MRTX1133 at IC_{50} 3.3nM for parental or 5uM for MRTX1133AR cell lines. Protein lysates were incubated on dot micro arrays overnight, per protocol R&D systems Proteome Profiler Human Phospho-Kinase Array and Human Phospho-RTK Array. Arrays were imaged on Licor Odyssey XF Imaging system.

- Baseline phosphorylated ERK1/2 levels are elevated in MRTX1133AR cells vs parental cells, but MRTX1133 treatment decreases pERK levels in both parental and MRTX1133AR cells
- MRTX1133 treatment increases pAKT and pSTAT3 levels in MRTX1133AR cells but not parental cells
- Data suggests alternative signaling pathways beyond MAPK that become activated upon acquired resistance to KRAS^{G12D} inhibition and may allow for continued cell growth

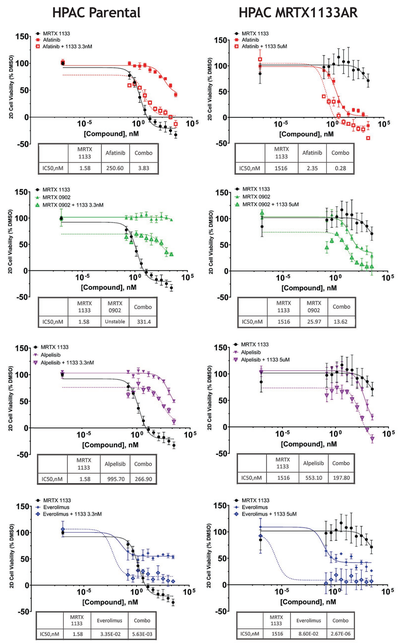
In vitro evaluation of MRTX1133 + combination partners can reduce cell viability in HPAC MRTX1133AR cell line

Figure 6. HPAC MRTX1133AR cell line treated with MRTX1133 + EGFR, SOS1, PI3K, or mTOR inhibitors result in reduced viability compared to either single agent

Cells were treated with MRTX1133, afatinib, MRTX0902, alpelisib, or everolimus as single agents over a dose response starting at 3uM, with a 1:3 dilution series.

Additionally, a combination of MRTX1133 at 3.3nM for parental or 5uM for MRTX1133AR cells and a dose response curve for the combination partner was used for the combo treatment. Cell viability was assessed after 72hrs (3days) of treatment using Promega CellTiter-Glo assay.

- HPAC MRTX1133AR cells are more sensitive to single agent inhibition of EGFR, SOS1 and mTOR than parental cells. This may be due to a shift away from KRAS dependence in the MRTX1133AR cells and instead reliance on alternative oncogenic drivers as a mechanism to preserve viability, though further exploration in this area is required.
- Increased HPAC MRTX1133AR cell line sensitivity to everolimus and alpelisib is consistent with molecular analysis indicating increased AKT dependence, as compared to parental HPAC.
- Treatment of MRTX1133 with combination partners potentiates the dose response in the HPAC MRTX1133AR cells.



CONCLUSIONS

- Using long term dose escalation of KRAS^{G12D} inhibitor MRTX1133 in 4 cell lines (2 colorectal cancer, 2 pancreatic cancer) resulted in all cell lines gaining acquired resistance over time, which was validated in vitro and in vivo as compared to parental cell lines
- Using RNAseq to compare gene expression outcomes from parental and MRTX133AR cell lines, secondary KRAS mutations H95 and Y96 in the SWII pocket of MRTX1133 were identified in 2 of the 4 cell line pairs evaluated and indicate that this mechanism may be a common path to resistance for KRAS G12D inhibition
- In a cell line pair without secondary KRAS mutations driving resistance, inhibition of pathways downstream of KRAS such as PI3Ka and mTOR, and upstream pathways such as EGFR and SOS1, suggest mechanisms by which activation of these pathways may contribute to a resistance phenotype. This data may contribute to understanding which combinations may best augment tumor cell killing in the context of KRAS G12D resistance.
- The possible resistance mechanisms of KRAS G12D inhibition are diverse and complex and require further exploration to identify rational combination partners that can overcome the various mechanisms of resistance

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