Pharmacogenomic Insight into Targetable Vulnerabilities and Modifiers of Response to MRTX1133 in KRAS^{G12D} Mutant Models

Abstract #1131

Vickie Bowcut[1], Andrew Calinisan[1], David M. Briere[1], Lauren Hargis[1], Lars D. Engstrom[1], Jade Laguer[1], Darin Vanderpool[1], Xiaolun Wang[1], J. David Lawson[1], Nicole Thomas[1], Robin Gunn[1], Christopher R. Smith[1], Julio Fernandez-Banet[2], Leo He[2], Adam Pavlicek[2], Matthew A. Marx[1], Peter Olson[1], James G. Christensen[1], Jill Hallin[1]*

*corresponding author

[1]Mirati Therapeutics, Inc., San Diego, CA, 92121, USA.
[2]Monoceros Biosystems LLC, San Diego, CA, 92172, USA.

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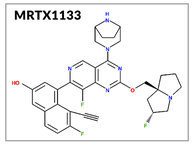
BACKGROUND

- 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}.
- MRTX1133 has been evaluated across a panel of cell and patient-derived xenograft models and demonstrates strong single agent activity in the majority of models tested. However, a subset of less responsive models were evaluated in genetic screens to elucidate specific targetable vulnerabilities.
- Using CRISPR/Cas9 screens, several targets upstream or downstream of KRAS were depleted, supporting exploration of strong combination opportunities. Conversely, enrichment of several hallmark tumor suppressor genes illuminates potential mechanisms of resistance to KRAS^{G12D} inhibition.

RESULTS

MRTX1133 is a Potent and Selective KRAS^{G12D} Inhibitor Broadly Active Across a Panel of KRAS^{G12D}-Mutant Cell Lines

Fig. 1



A.

KRAS Protein	MRTX1133		
	Inactive IC_{50} (nM)	Active IC_{50} (nM)	SPR K_D (pM)
G12D	<2*	9	~0.2
WT	2.4	112	140

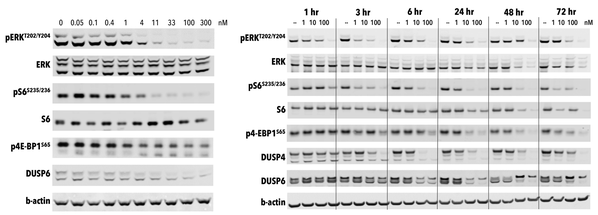
B.

Cell Line	KRAS Mutation	Histology	ICW IC_{50} (nM) pERK 3 hr	Viability Assay IC_{50} (nM) 2D 3-Day	3D 8-Day
GP2D	G12D	Colon	0.6	1.5	1.7
Colo678	G12D	Colon	0.7	5.4	1.7
SNU410	G12D	Pancreas	1.6	23.2	9.5
A427	G12D	Lung	1.8	6.8	35.3
HPAFII	G12D	Pancreas	1.9	13.9	3.6
AGS	G12D	Gastric	2.0	6.1	1.4
Panc0813	G12D	Pancreas	2.1	32.4	4.9
SNU1197	G12D	Colon	2.6	27.8	6.0
Panc1005	G12D	Pancreas	2.8	20.3	5.0
KP4	G12D	Pancreas	3.0	299	7.6
LS513	G12D	Colon	4.6	14.1	9.9
Panc0504	G12D	Pancreas	5.9	7.2	3.8
SNU407	G12D	Colon	6.2	30.9	3.4
ASPC1	G12D	Pancreas	6.2	55.4	4.8
Panc02.03	G12D	Pancreas	6.3	26.0	6.9
HPAC	G12D	Pancreas	8.0	20.3	3.8
Suit2	G12D	Pancreas	8.0	17.0	5.0
SW1990	G12D	Pancreas	9.2	61.2	4.7
SNU1033	G12D	Colon	9.2	1199	42.3
Panc04.03	G12D	Pancreas	10.1	41.6	7.4
SU8686	G12D	Pancreas	10.5	54.0	10.4
SNUC2B	G12D	Colon	10.6	34.8	14.0
LS180	G12D	Colon	11.6	55.0	31.5
HEC1B	G12D	Endometrial	13.7	181	5.6
HCC1588	G12D	Lung	250	3000	1000
MKN1	WT (KRAS dep)	Gastric	151	188	331
NCIH358	G12C	Lung	288	3000	211
HCT116	G13D	Colon	524	1093	573
PSN1	G12R	Pancreas	3000	3000	1000
A549	G12S	Lung	3000	103	256
NCIH727	G12V	Lung	3000	3000	1000
NCIH1299	WT (NRAS Q61K)	Lung	3000	3000	1000

A. MRTX1133 IC_{50} and K_D values determined by inactive biochemical binding assay, Raf-Ras binding domain assay, and Surface Plasmon Resonance (SPR) assay.

B. Activity of MRTX1133 in cells by pERK In-Cell Western assay and 2D/3D viability assay.

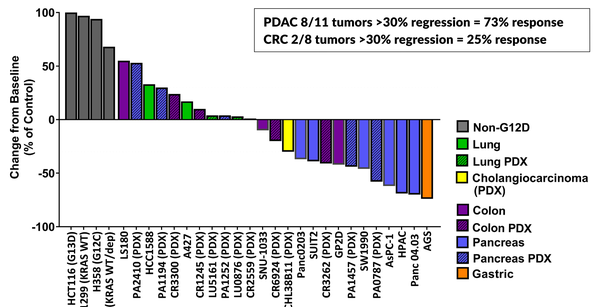
Fig. 2: MRTX1133 Modulates KRAS Pathway Targets In Vitro



A. HPAC cells were treated with MRTX1133 over a dose response for 3 hours and analyzed by western blot.

B. HPAC cells were treated with MRTX1133 at 1, 10, and 100nM from 1 to 72 hours and analyzed by western blot.

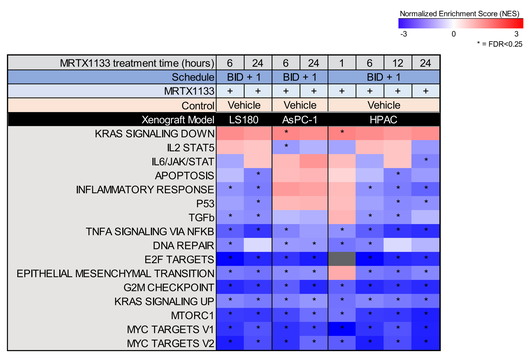
Fig. 3: Anti-tumor Activity of MRTX1133 in KRAS^{G12D}-mutant and non-KRAS^{G12D}-mutant Human Tumor Xenograft Models



MRTX1133 was administered 30 mg/kg BID IP. The % change from baseline control was calculated at day 14 for most models.

MRTX1133 Treatment Regulates KRAS-dependent Oncogenic Signaling and Feedback Inhibitory Pathways

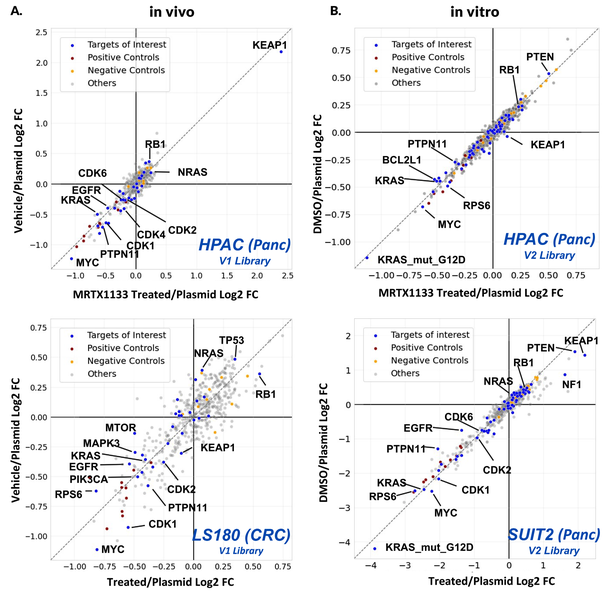
Fig. 4



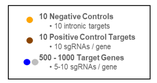
GSEA Hallmark signature analysis of RNAseq differential expression following in vivo MRTX1133 treatment. LS180, AsPC-1 and HPAC xenograft models were treated with Vehicle or three doses of MRTX1133 (30 mg/kg IP) and tumors were harvested for RNA extraction at indicated timepoints post last dose.

MRTX1133-anchored CRISPR Screens Reveal Combination Targets and Potential Resistance Mechanisms

Fig. 5



- EGFR, PI3Kα, and SHP2 dropout and represent potential combination targets
- Tumor suppressor genes PTEN, KEAP1, NF1, RB1, and TP53 are enriched and may confer partial resistance to MRTX1133



Libraries V1 and V2 each have ~5000 sgRNAs (5-10 sgRNAs/gene).

A. Genes that exhibited sgRNA dropout or enrichment in two-week, Vehicle-treated HPAC cells *in vivo* (top panel) or LS180 cell *in vivo* (bottom panel) relative to MRTX1133-treated. Log2 fold change (FC) in Day 14 MRTX1133-treated samples/Plasmid vs. log2 fold change (FC) in Day 14 Vehicle-treated samples/Plasmid.

B. Genes that exhibited sgRNA dropout or enrichment in two-week, DMSO-treated HPAC cells *in vitro* (top panel) or SUIT2 cells (bottom panel) relative to MRTX1133-treated. Log2 fold change (FC) in Day 14 MRTX1133-treated samples/Plasmid vs. log2 fold change (FC) in Day 14 DMSO-treated samples/Plasmid.

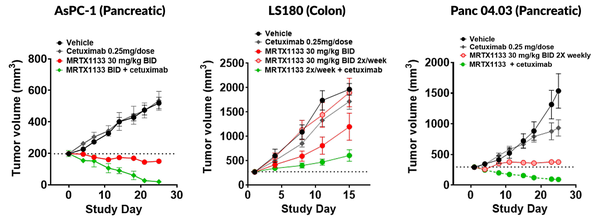
Fig. 6: Overview of In Vitro MRTX1133 and Combination Partner Screens

G12D Cell Lines	Co-Occurring Mutations						MCA Scores					
	PI3KCA	APC	CDKN2A	CTNNB1	TP53	SMAD4	EGFR cetuximab	ErbB Family afatinib	SHP2 RMC-4550	SOS BI-I-13	PI3K BYL-719	mTOR vistusertib
LS180							65	76	63	42	18	16
Suit2							45	45	31	6	6	15
HP AC							33	33	11	25	44	12
AsPC-1							34	28	9	1	16	14
GP2D							48	26	27	-10	49	35

Co-occurring alterations in each cell line are indicated by **red** (activating), **green** (inactivating), or **blue** (deletion). Heatmap summary of synergy (Mirati Combination Analysis or MCA Score) generated by combination treatments of MRTX1133 dose response with each partner compound in a dose response, run in a 72-hour, 2D CellTiter-Glo assay to assess proliferation with either single agent and in combination in the cell lines listed. A custom R-script was used to generate a composite synergy (MCA) score. **Green** indicates synergy and **yellow** indicates additivity, as described in He L et al.

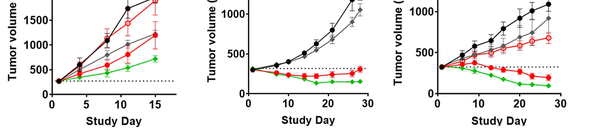
EGFR Inhibition (cetuximab) or PI3Kα Inhibition (alpelisib) Enhance KRAS-dependent Anti-tumor Activity of MRTX1133

Fig. 7: Anti-tumor Activity of MRTX1133 in Combination with Cetuximab



MRTX1133 at 30 mg/kg dosed BID daily or BID daily for 2 consecutive days followed by 5 days off, cetuximab at 0.25 mg per dose Q3D, or the combination of cetuximab with either schedule of MRTX1133 was administered intraperitoneally to mice bearing the AsPC-1, LS180, or Panc 04.03 cell line xenograft (n=5 per group). Tumor volumes at study end for the MRTX1133 BID daily for 2 days a week + cetuximab combination treatment group were determined to be statistically significant vs MRTX1133 BID daily for 2 days a week or cetuximab single agent treatment group using two-tailed Student's *t*-test (p-value <0.05) for all models tested.

Fig. 8: Anti-tumor Activity of MRTX1133 in Combination with Alpelisib (BYL-719)



MRTX1133 at 30 mg/kg dosed BID daily or BID daily for 2 consecutive days followed by 5 days off, BYL-719 at 15 mg/kg QD, or the combination of BYL-719 with either schedule of MRTX1133 was administered intraperitoneally to mice bearing the LS180, GP2D, or AsPC-1 cell line xenograft (n=5 per group). Tumor volumes at study end for the MRTX1133 BID daily for 2 days a week + BYL-719 combination treatment group were determined to be statistically significant vs MRTX1133 BID daily for 2 days a week or BYL-719 single agent treatment group using two-tailed Student's *t*-test (p-value <0.05) for all models tested.

CONCLUSIONS

- MRTX1133 is a potent and selective KRAS^{G12D} inhibitor that demonstrates marked anti tumor activity across a panel of xenograft models, including regression in the majority of pancreatic ductal adenocarcinoma (PDAC) models tested.
- A subset of models were less responsive to MRTX1133 monotherapy. CRISPR screening and RNAseq analyses identified potential mechanisms of partial resistance including EGFR, PIK3CA, PTPN11, mTOR and CDK2/4/6. Hallmark tumor suppressor genes were enriched including KEAP1, RB1 and PTEN.
- Cetuximab and BYL-719 represent combination strategies that may help augment the anti tumor activity of MRTX1133.
- These data illustrate the therapeutic susceptibility and broad dependence of KRAS^{G12D} mutant cancers, including pancreatic and colorectal cancers, on *KRAS* for tumor cell growth and survival, and provide insight into development of therapeutic strategies for patients with this mutation.

Manuscript submitted:
Hallin, et al., ***A Non-Covalent KRAS^{G12D} Allele Specific Inhibitor Demonstrates Potent Inhibition of KRAS-dependent Signalling and Regression of KRAS^{G12D} Mutant Tumors.***

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