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MOLECULAR TARGETS AND CANCER THERAPEUTICS
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Discovery and Characterization of MRTX1133, a Selective Non-covalent Inhibitor of KRAS^{G12D}

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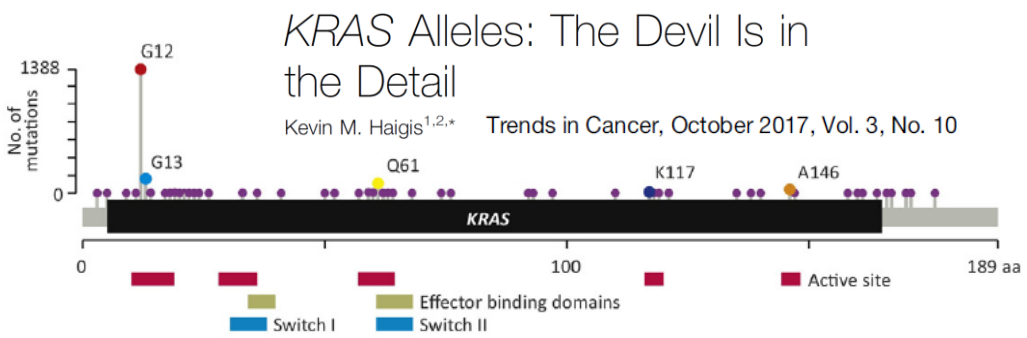
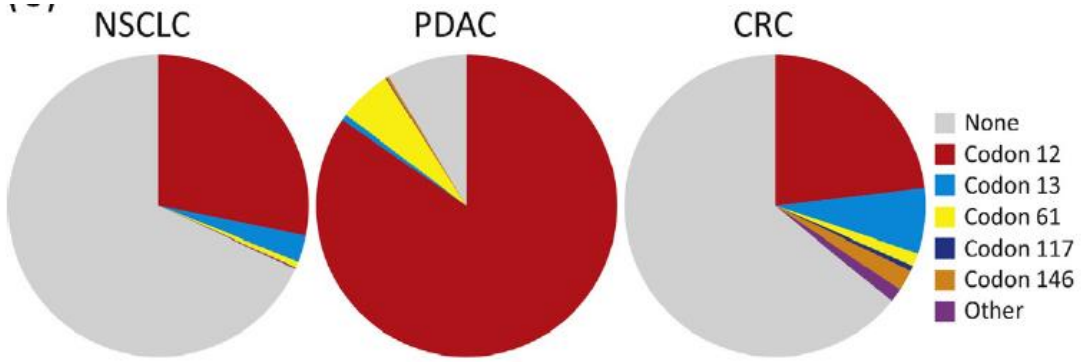
Disclosures

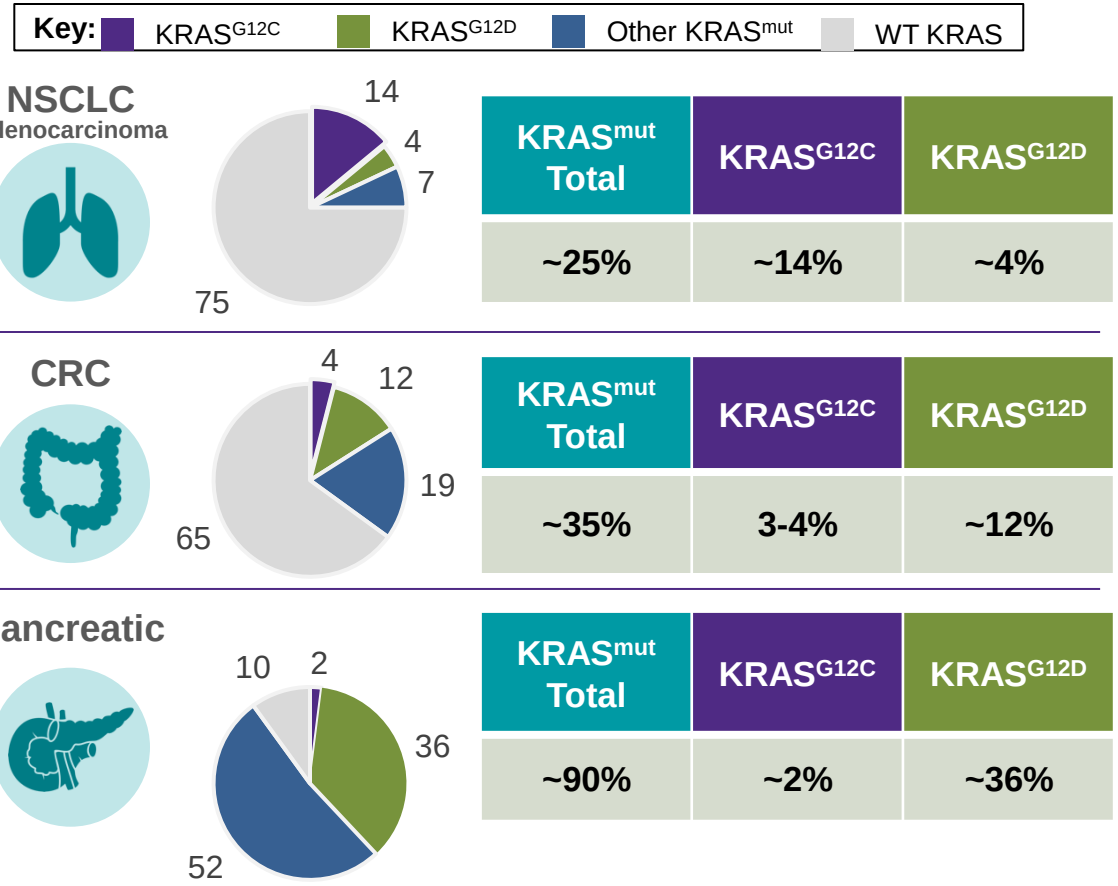
James G Christensen is an executive officer and shareholder of Mirati Therapeutics

I will discuss the investigational use of adagrasib in my presentation

Spectrum of KRAS Mutations in Human Cancers







- Different RAS family and KRAS mutations at codons 12, 13, 61 exhibit differential prevalence in across a spectrum of different cancers
- Different mutations have distinct etiology. $KRAS^{G12C}$ is a transversion mutation linked to smoking and $KRAS^{G12D}$ is a transition mutation

1. Zehir A, Benayed R, Shah RH, et al. *Nat Med*. 2017;23(6)703-713. 2. Krakstad C, Birkeland E, Seidel D, et al. *PLoS One*. 2012;7(12):e52795. 3. NIH TCGA: *The Cancer Genome Atlas*. February 11, 2021; https://www.cbioportal.org.
4. Biernacka A, Tsongalis PD, Peterson JD, et al. *Cancer Genet*. 2016;209(5):195-198. 5. Pakkala S, Ramalingam SS. *JCI Insight*. 2018;3(15):e120858.

Targeting KRAS Has Been Historically Challenging

Direct Inhibitors

- Smooth surface with limited binding sites
- High affinity for & high intracellular concentrations of GTP/GDP
- KRAS membrane targeting FTI and GGTI

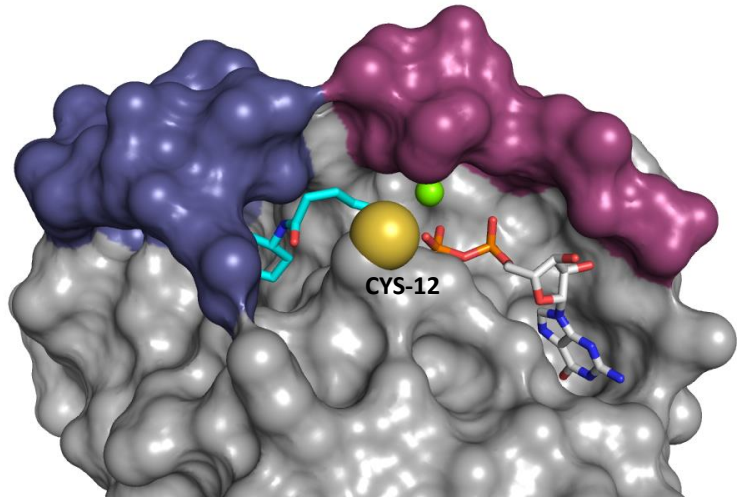
Downstream Effector Inhibitors

Raf / MEK and PI3K / AKT / mTOR

- Inhibition of WT signaling resulting in low therapeutic index
- Incomplete inhibition of signaling downstream of KRAS mut

K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions

Jonathan M. Ostrem,[1,*] Ulf Peters,[1,*] Martin L. Sos,[1] James A. Wells,[2] and Kevan M. Shokat[1]

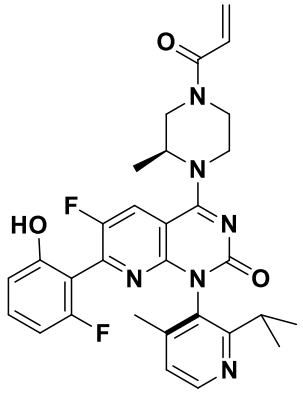


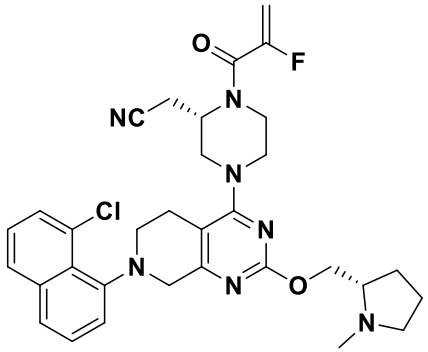
548 | NATURE | VOL 503 | 28 NOVEMBER 2013

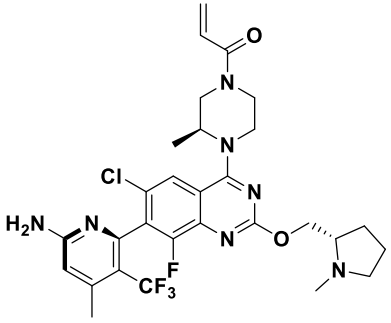
Covalent Inhibition of KRAS G12C

- Binding in the switch II pocket of GDP KRAS
- Covalent bond to cysteine 12
- Locked in the inactive conformation

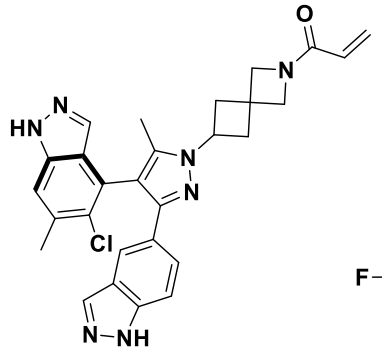
KRAS G12C Inhibitors in Clinical Trials and Representative Patent Examples







Roche
Ex 17a
(WO2020097537)



Novartis
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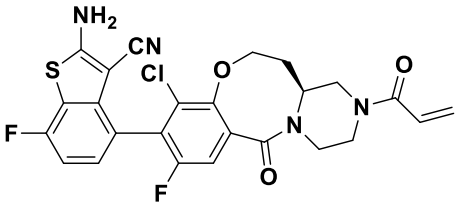
(WO2021120890)



Eli Lilly
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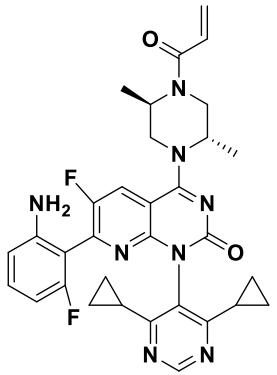
(WO2021118877)

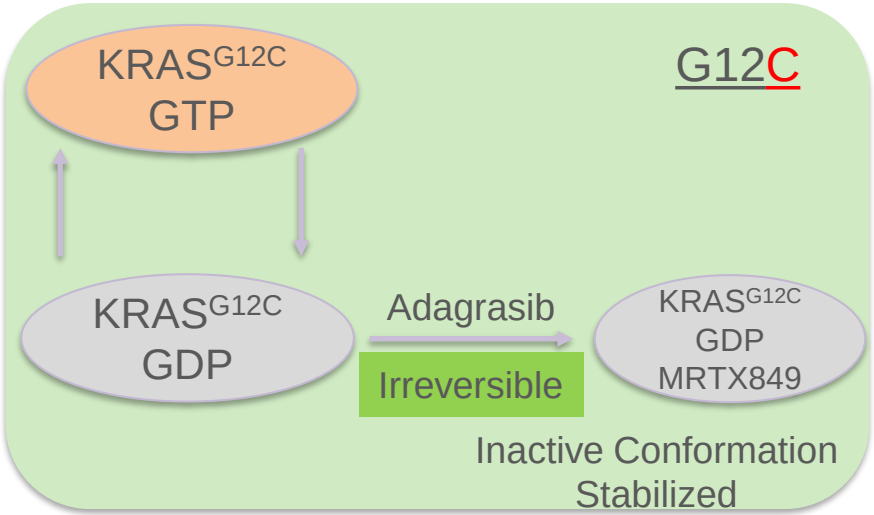


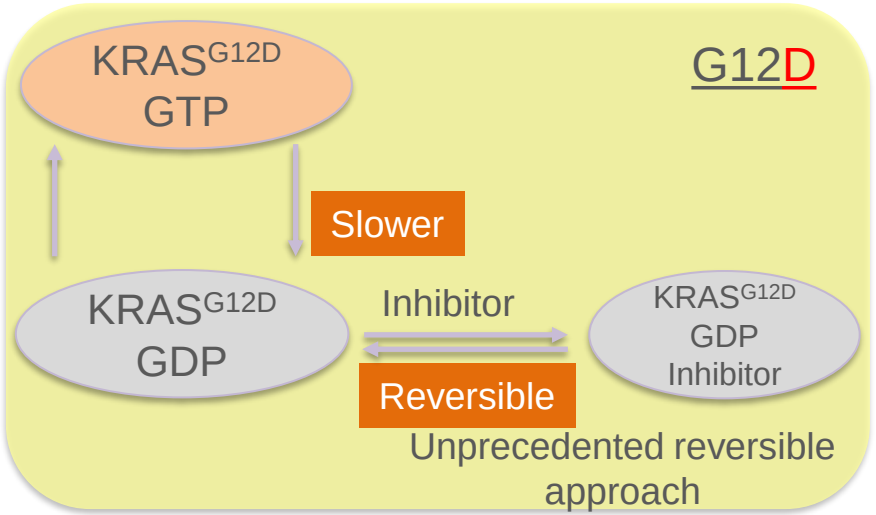
InventisBio
Cmp 2
(WO2021120045)

AMG510	MRTX849
Amgen	Mirati
Approved (2L NSCLC)	Investigational
Label reported ORR NSCLC (n=124) 36% (BICR)	Reported ORR NSCLC (n=51) 45% (Investigator) ENA Oct 24 2020

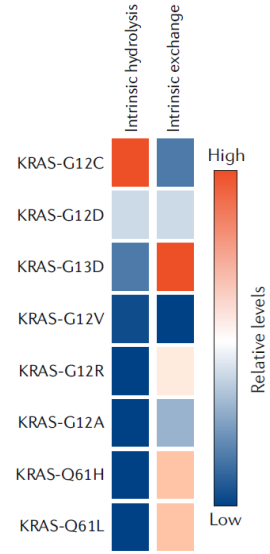
Compound	GDC-6036	JDQ443	LY3537982	D-1553
Company	Roche	Novartis	Eli Lilly	InventisBio
Status	Phase 1/2	Phase 1/2	Phase 1/2	Phase 1/2
NCT Clinical Trial No.	NCT04449874	NCT04699188	NCT04956640	NCT04585035

Challenges to Targeting KRAS^{G12D}





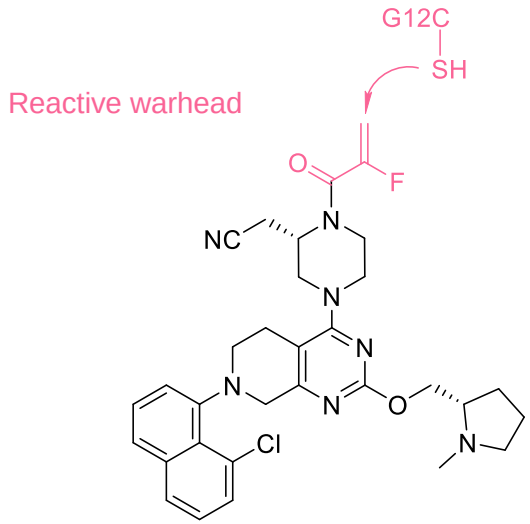
GTP Hydrolysis Rates by KRAS^{mut} allele



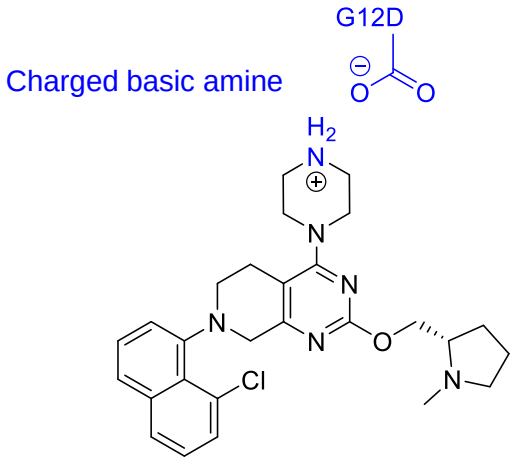
Hunter et al. *Mol Cancer Res* 13, 1325-35 2015; Moore et al. *Nature Rev Drug Disc* 19 533-52 2020.

- Impractical to target via irreversible binding. Covalent modifiers targeting Asp acid have not been well documented.
- The binding affinity of irreversible KRAS^{G12C} inhibitors is modest. Adagrasib K_I of 3.7 μM suggests that significant improvement in binding potency necessary to achieve pharmacologically meaningful G12D inhibition.
  - Potency of KRAS^{G12C} inhibitors driven, in part, by proximity to Lys16, characteristics of Cys12, and transition state reactivity of acrylamide warheads with Cys12 (Hansen et al. *Nat Struct Mol Biol* 25, 454–62 2018)
- The intrinsic GTPase activity of KRAS^{G12D} is about 2.5-fold lower than for KRAS^{G12C}. Potential drugs targeting GDP-bound KRAS^{G12D} must address this additional challenge

Proof of Concept: Modification of Adagrasib to Target Asp12 Residue with Reversible Inhibitor



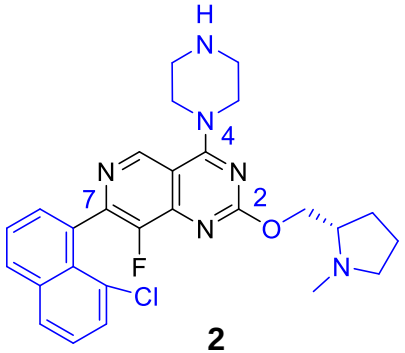
Adagrasib
KRAS^{G12C} inhibitor
G12C K_I 3.7 µM; k_{inact}/K_I 35 $mM^{-1}s^{-1}$



1
KRAS^{G12D} SPR K_D 19 µM
KRAS^{WT} SPR K_D ~82 µM

Modest activity against KRAS^{G12D}/GDP confirms plausibility of reversible approach; however, the likelihood of a need for a 4 order of magnitude improvement in binding affinity is apparent

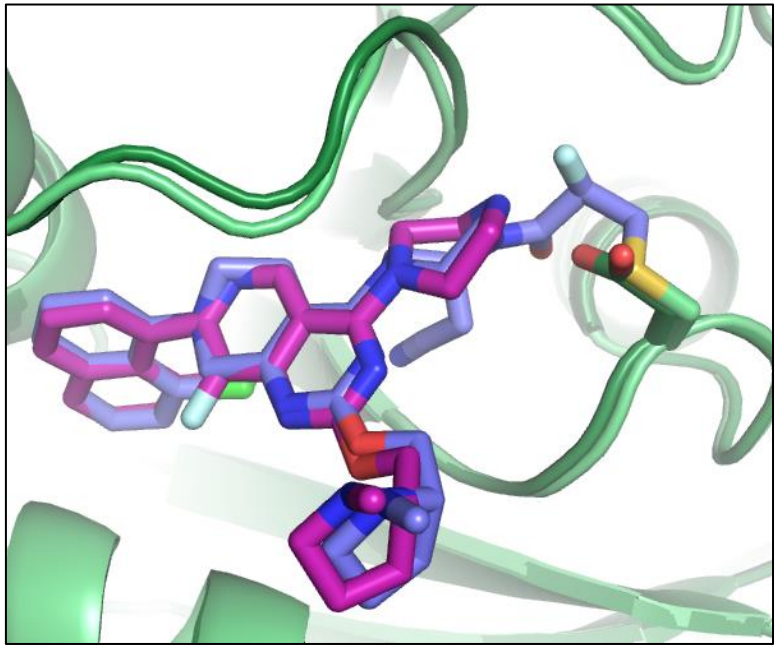
Pyrido[4,3-d]pyrimidine Scaffold Identified for Further Optimization



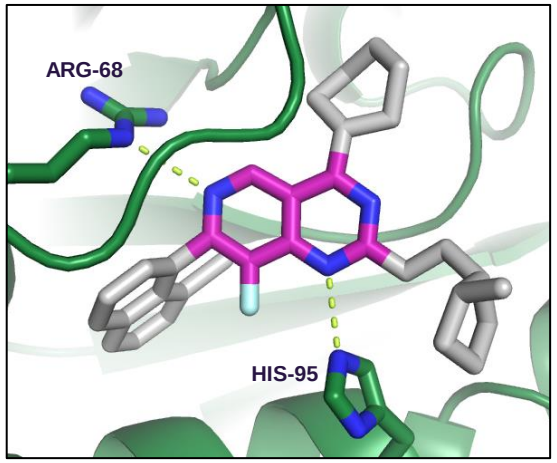
2

$KRAS^{G12D}$ HTRF IC_{50} 6 µM
$KRAS^{G12D}$ SPR K_D 3.5
$KRAS^{WT}$ SPR K_D 36 µM

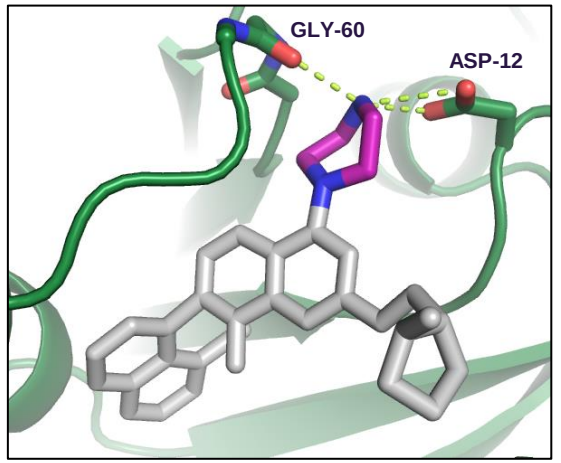
*Overlay of **2**/G12D and Adagrasib/G12C*



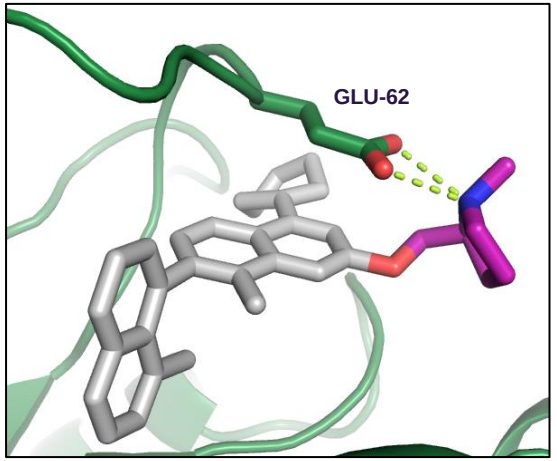
Pyrido[4,3-d]pyrimidine core



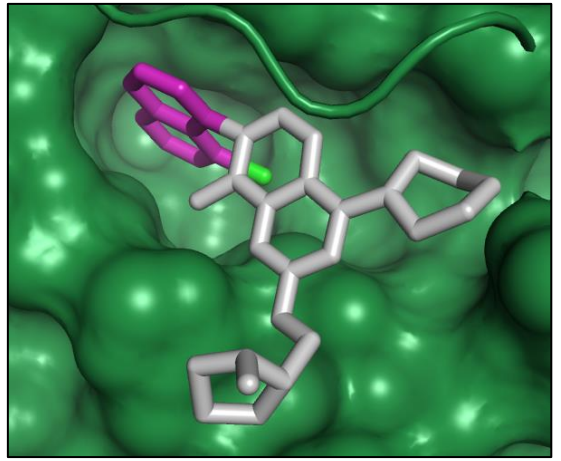
Cationic C4 piperazine



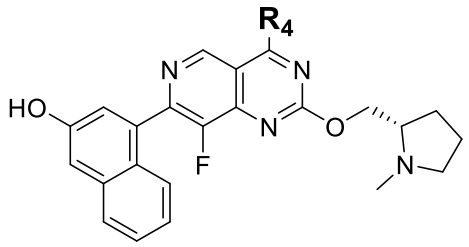
Cationic C2 pyrrolidine

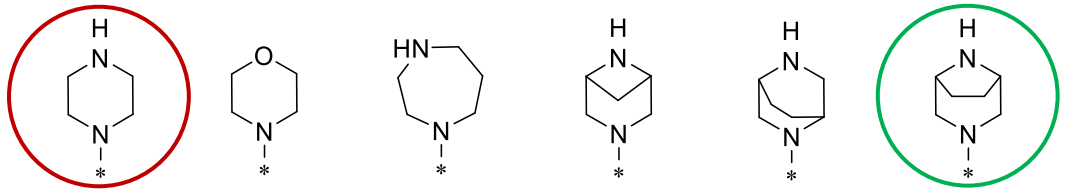


Hydrophobic C7 naphthyl



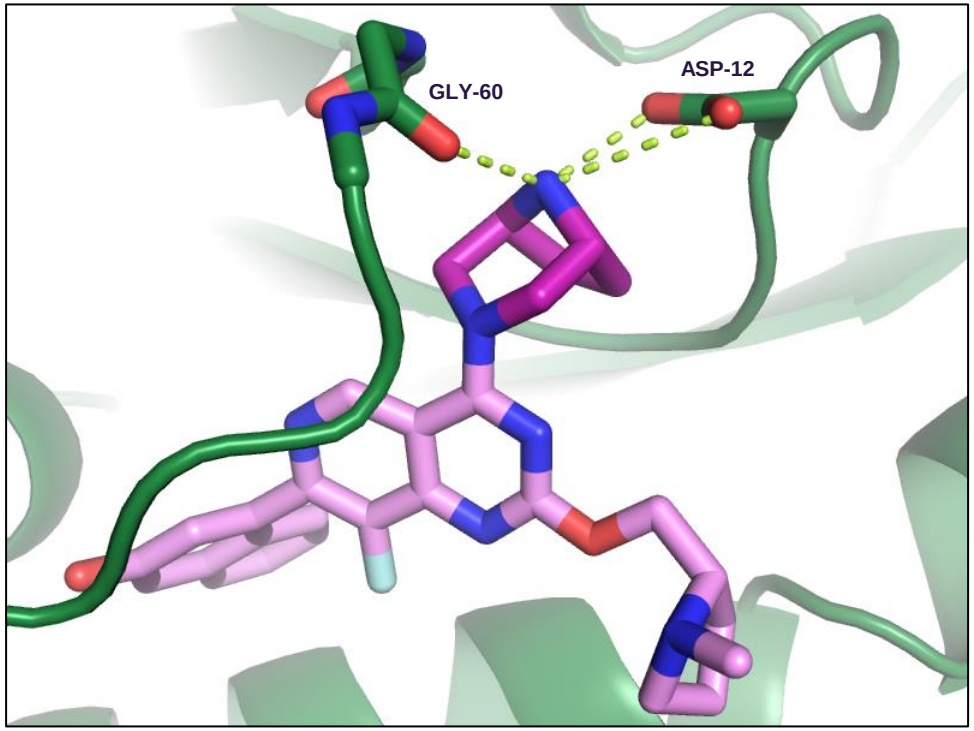
Optimization of Interactions with the Mutated Asp12





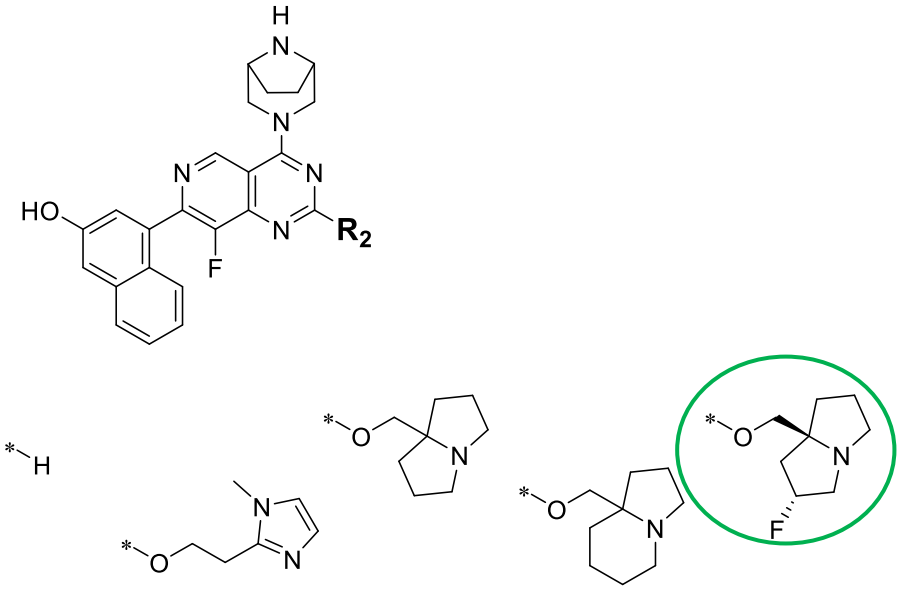
Compound	3	4	5	6	7	8
HTRF $KRAS^{G12D}$ IC_{50} (μM)	0.98	12	2.6	0.31	0.068	0.005
SPR $KRAS^{G12D}$ K_D (μM)	0.19	1.5	0.42	0.073	0.009	0.0008
SPR $KRAS^{WT}$ K_D (μM)	1.2	0.56	-	-	-	0.18

Compound **8** with $KRAS^{G12D}$



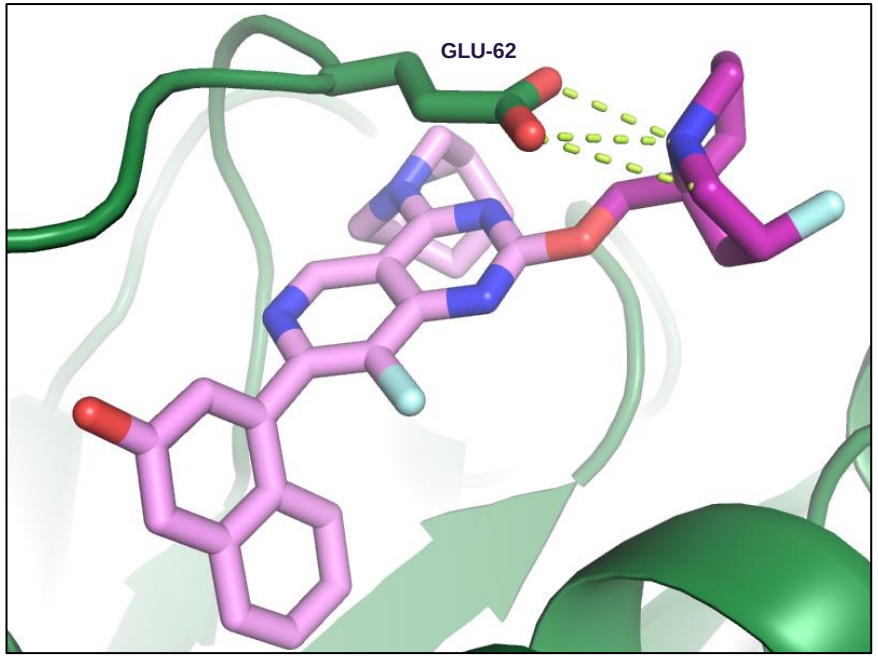
- Preferred bicyclic protonated amine interacts with both Gly60 and Asp12 and provides activity and selectivity
- Cellular pERK IC_{50} = 0.53 μM for compound **8** (G12D mutant cell line AGS)

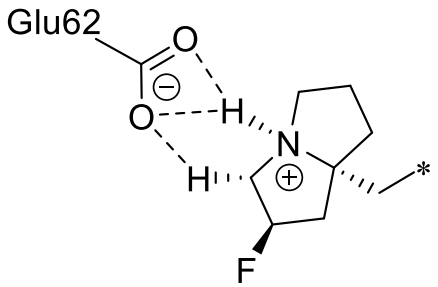
Optimization of Polar Interaction with Glu62



Comp.	9	10	11	12	13	14
HTRF $KRAS^{G12D}$ IC_{50} (µM)	0.005	2.96	0.030	<0.002	0.012	<0.002
pERK IC_{50} (AGS) K_D (µM)	0.53	> 10	3.5	0.27	0.65	0.024

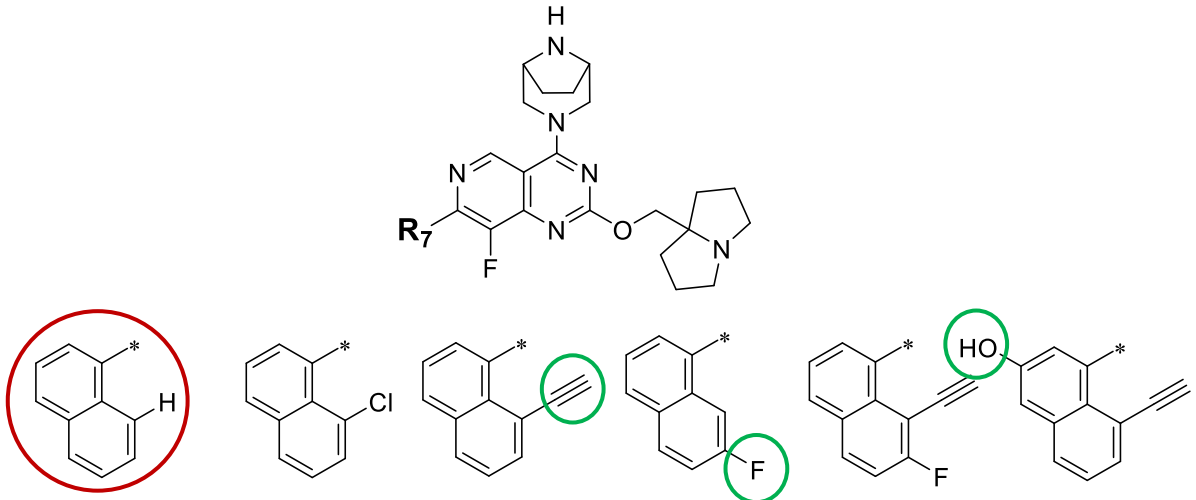
Compound **14** with $KRAS^{G12D}$





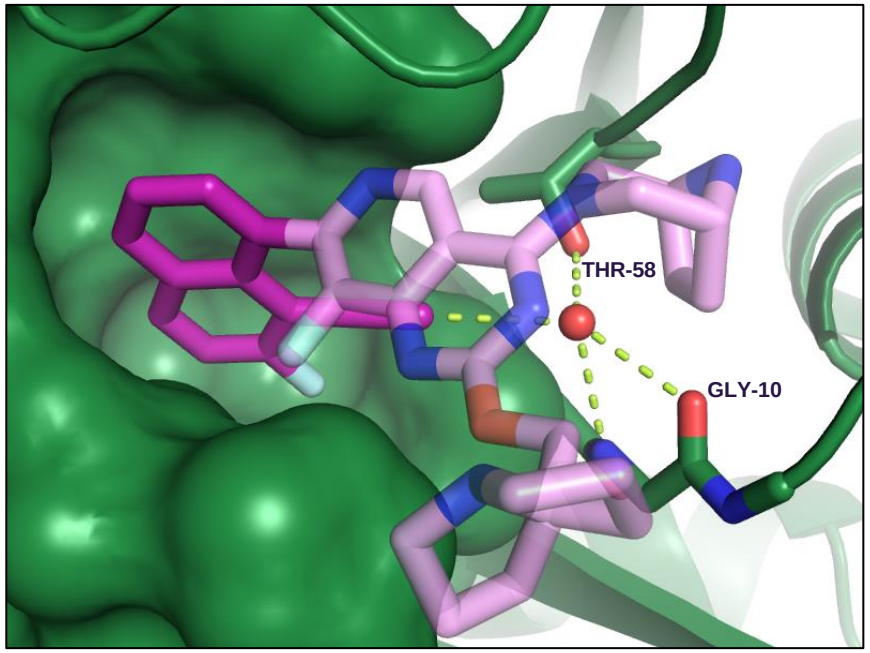
- A strong salt bridge interaction and a plausible non-classical C-H-O hydrogen bond between the pyrrolizidine and Glu62 revealed by co-crystal structure.
- The addition of the fluoro to the pyrrolizidine functions as a strong electron withdrawing substituent enhancing both interactions for an optimal engagement with Glu62.

Optimization of Naphthyl Substituents



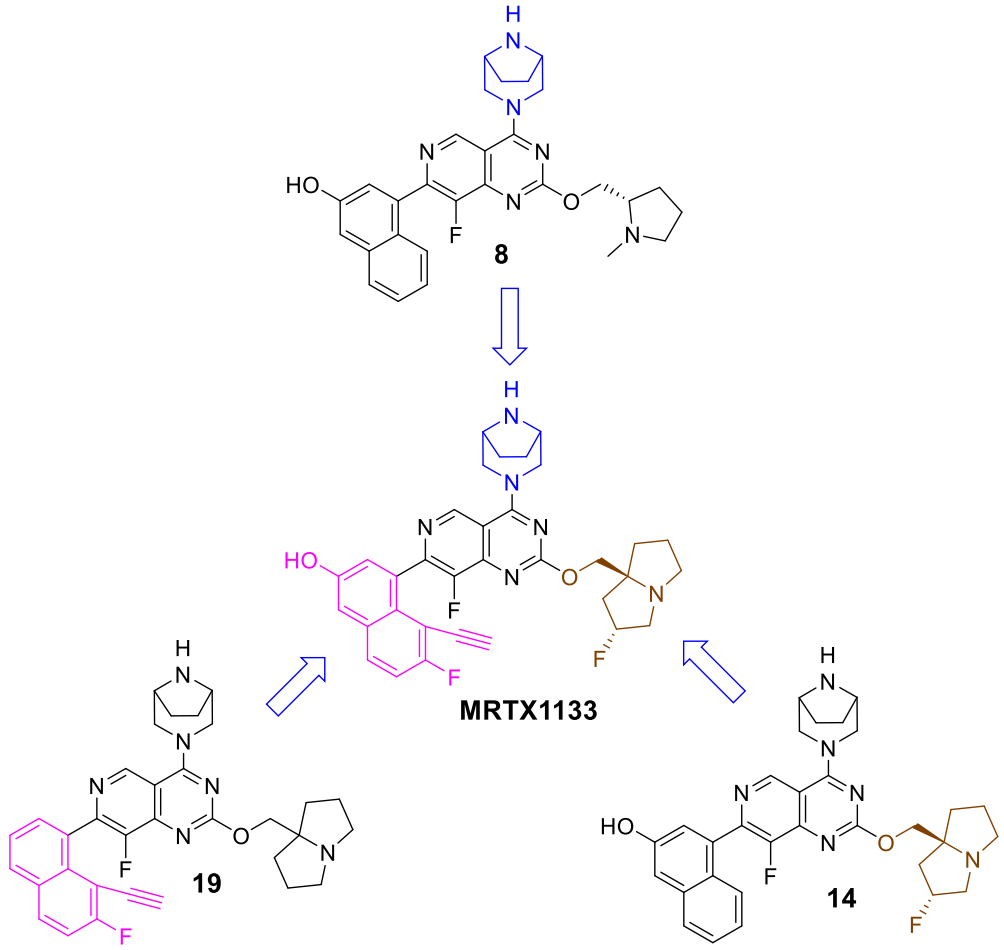
Comp.	15	16	17	18	19	20
HTRF KRAS^{G12D} IC_{50} (μM)	0.36	0.015	0.003	0.027	<0.002	<0.002
pERK IC_{50} (AGS) K_D (μM)	> 10	0.19	0.071	0.37	0.024	0.014

Compound **19** with KRAS^{G12D}

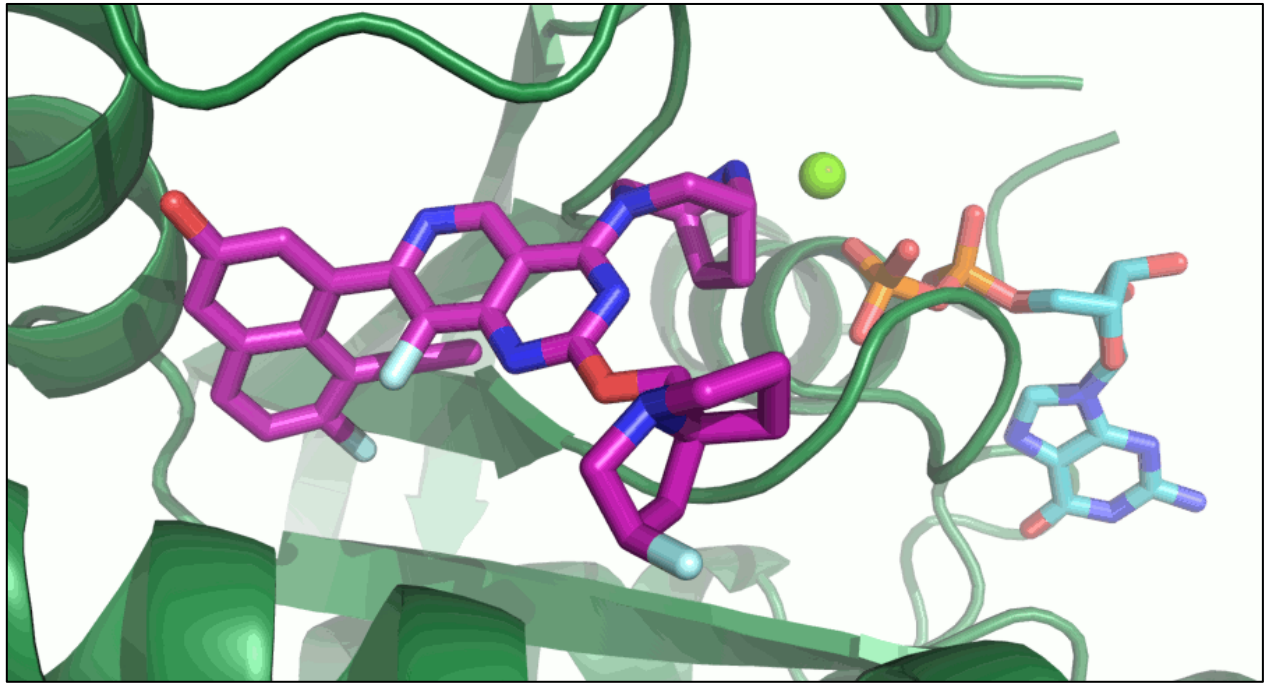


- 8-alkyne forms a non-classical C-H O hydrogen bond with the conserved G10 water
- The C7-fluorine fills a small cavity in the protein
- The 3-hydroxyl naphthol exhibits a hydrogen bonding interaction with Asp69
- The combined improvements from each of the 3 positions contribute to overall improvement in affinity

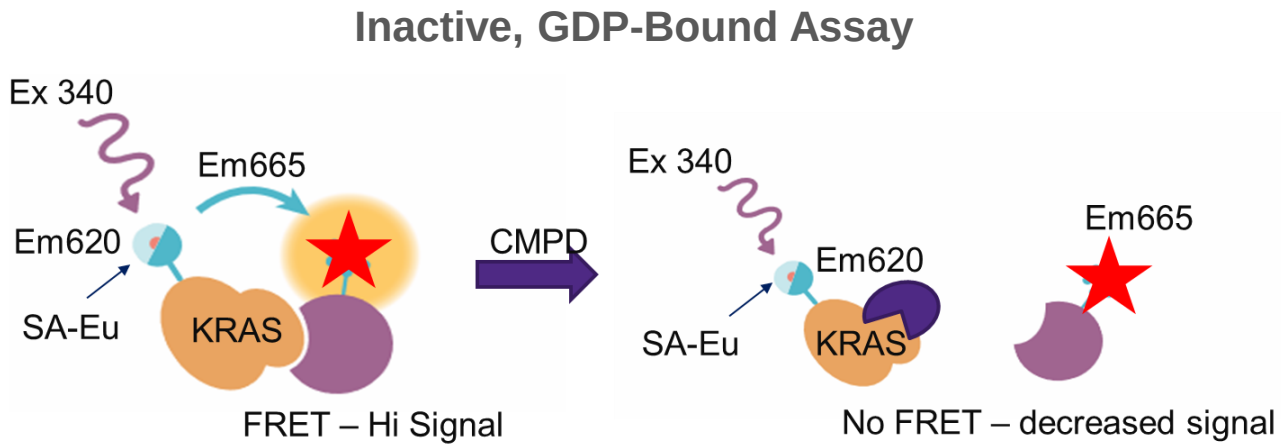
MRTX1133 Inhibits KRAS^{G12D} with sub-nM Binding Affinity



Assay	Activity
KRAS^{G12D} K_D (nM)	~0.0002*
AlphaLISA IC_{50} (nM)	5
pERK AGS IC_{50} (nM)	2
2D viability AGS (KRAS^{G12D}) IC_{50} (nM)	6
2D viability MKN1 (KRAS^{WT}) IC_{50} (nM)	>3000

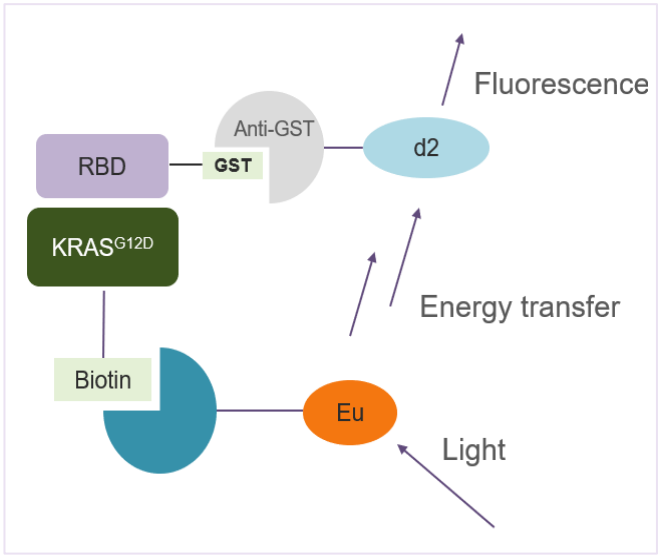


MRTX1133 Binds the Inactive and Active States of KRAS G12D and Demonstrates >500-fold Selectivity for KRAS^{G12D} vs WT KRAS by SPR



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

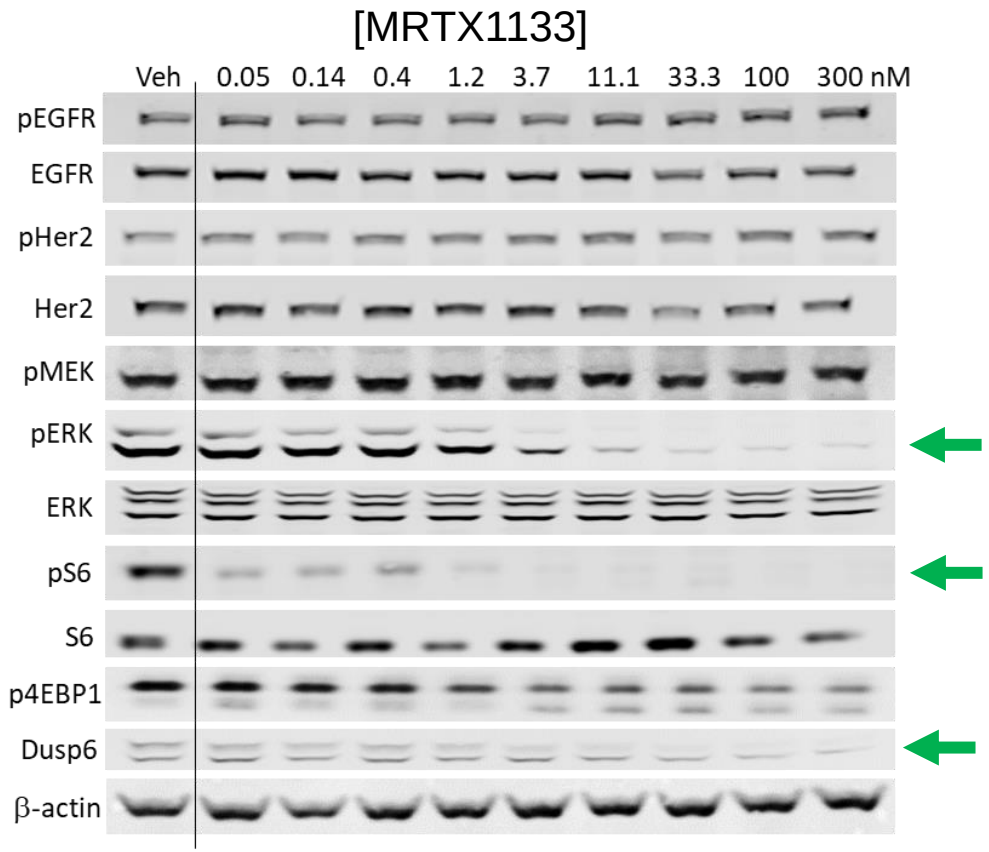
***MRTX1133 bottoms out the inactive assay**



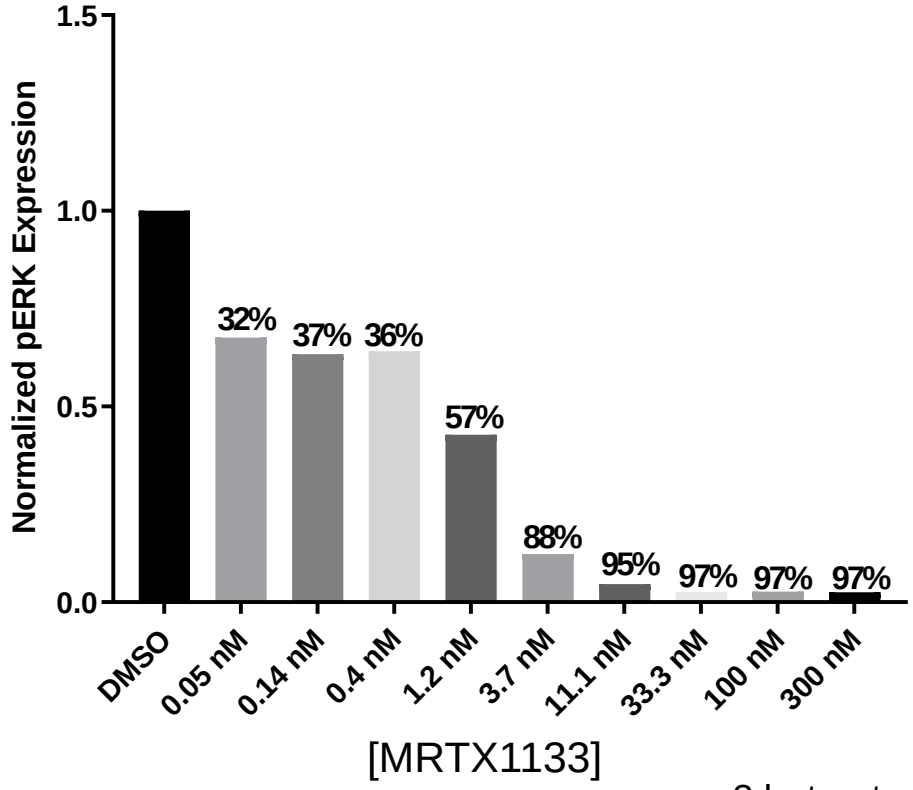
- MRTX1133 Binds the inactive, GDP-bound KRAS^{G12D} with high affinity (<2nM)
- Ability to inhibit binding of active KRAS^{G12D} to RBD binding may contribute to the pharmacological MOA

MRTX1133 Treatment Demonstrates Dose-dependent pERK, pS6 & DUSP6 Modulation in *KRAS*^{G12D}–mutant HPAC Cells

Concentration-dependent KRAS Pathway Target Inhibition



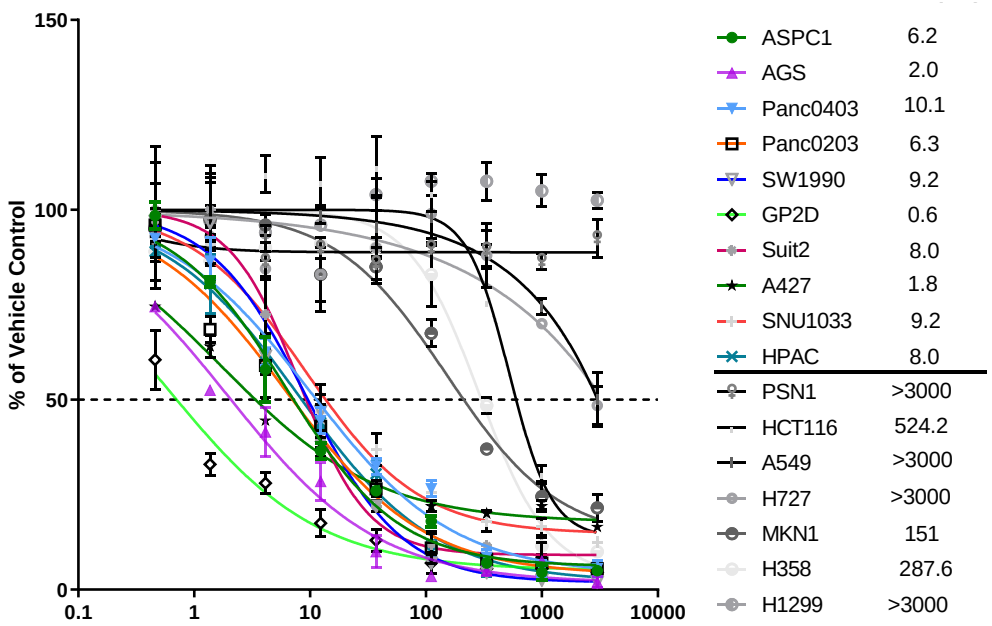
Normalized pERK Dose Response and % Inhibition



3 hr treatment

MRTX1133 is Broadly Active Across a Panel of *KRAS*^{G12D}-mutant Cell Lines and is Selective for KRAS^{G12D}

- MRTX1133 potently inhibits KRAS signaling in *KRAS*^{G12D}-mutant cell lines
- MRTX1133 potently inhibits viability of *KRAS*^{G12D}-mutant cell lines
- Significantly less active in non *KRAS*^{G12D}-mutant cell lines in pERK and 3D viability assays



Cell Line	KRAS Mutation	pERK IC_{50} (nM)	3D Viability IC_{50} (nM)
GP2D_Colon	G12D	0.64	1.7
Colo678_Colon	G12D	0.69	1.7
SNU-410_Pancreas	G12D	1.6	9.5
HPAF-II_Pancreas	G12D	1.9	3.6
AGS_Stomach	G12D	2	1.4
Panc 08.13_Pancreas	G12D	2.1	4.9
SNU-1197_Colon	G12D	2.7	6
KP-4_Pancreas	G12D	3	7.6
AsPC-1_Pancreas	G12D	6.2	4.8
SUIT-2_Pancreas	G12D	8	5
HPAC_Pancreas	G12D	8	3.7
SNU-1033_Colon	G12D	9.2	42.3
Panc 04.03_Pancreas	G12D	10.1	7.4
LS180_Colon	G12D	11.6	31.5
MKN1_Stomach	WT AMP (dependent)	151	599.7
H358_Lung	G12C	288	213.3
HCT116_Large Intestine	G13D	524	977.8
A549_Lung	G12S	>3,000	752.4
H1299_Lung	NRAS (Q61K)	> 3,000	1000

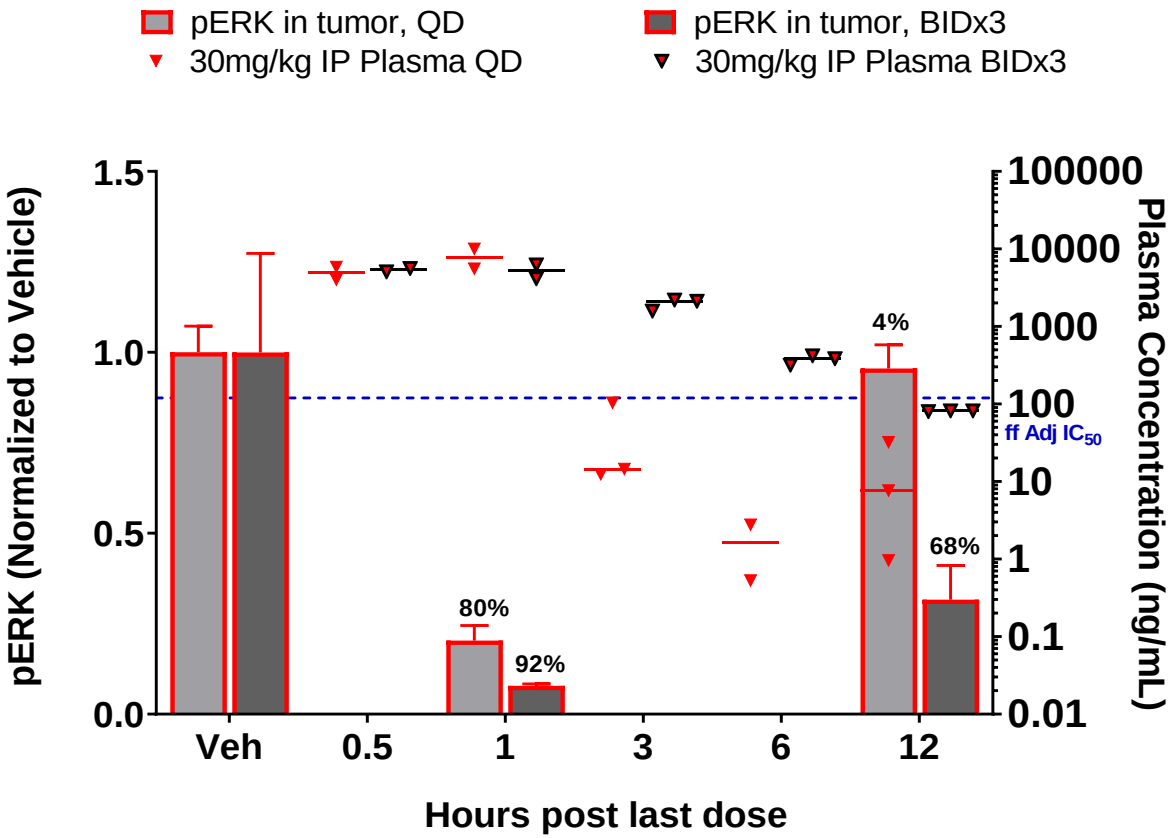
MRTX1133 Target Profile and Key Attributes

Assay	Criteria	MRTX1133
$KRAS^{G12D}$ cell activity	< 10 nM	✓
Selectivity over $KRAS^{WT}$	> 100-fold	✓
Aq. Solubility (pH 5.8)	> 5 mg/mL	✓
Half-life (mouse)	>10 hr	✓
Predicted human half-life	> 24 hr	✓
hERG activity	> 10 µM	✓
CYP inhibition, TDI, Induction	Low risk	✓
Eurofins safety screening	Low risk	✓
Screening Ames	Nonmutagenic	✓

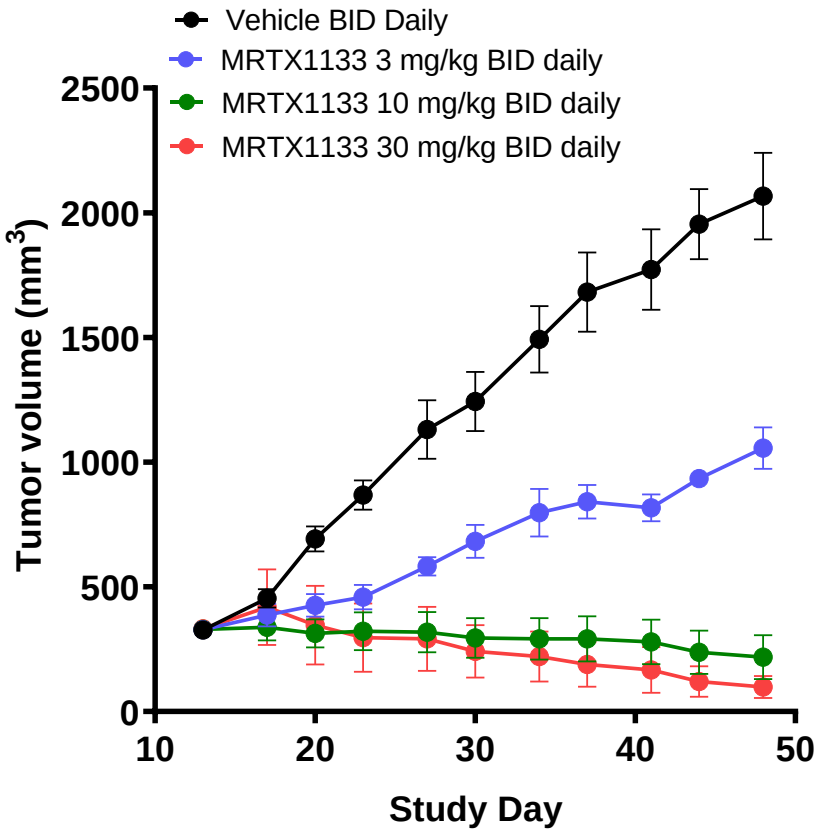
- MRTX1133 is a potent, selective inhibitor of KRASG12D with a favorable predicted human half life and low risk for drug-drug interactions
- The optimization of MRTX1133 binding affinity via amine-based H-bonding interactions result in limited permeability and oral bioavailability
- Formulations to enable IV delivery and maximize plasma exposure are being pursued

IP Administration of MRTX1133 to Xenograft Tumor-bearing Mice Inhibits KRAS Signaling and Exhibits Strong Antitumor Activity

HPAC, IP PKPD Study

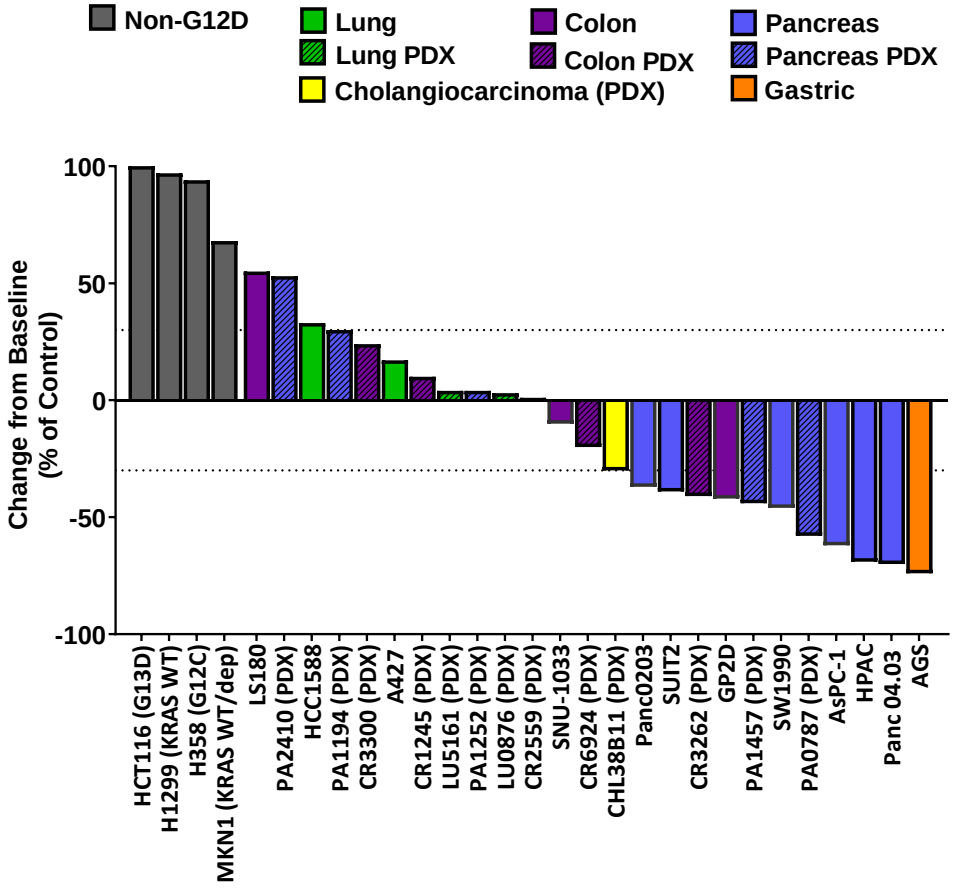


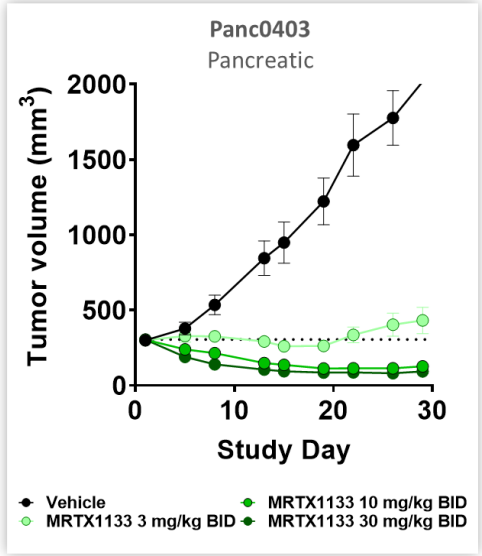
HPAC IP Efficacy Study

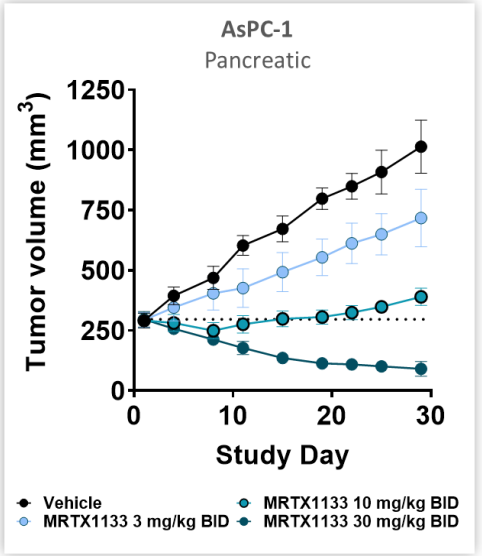


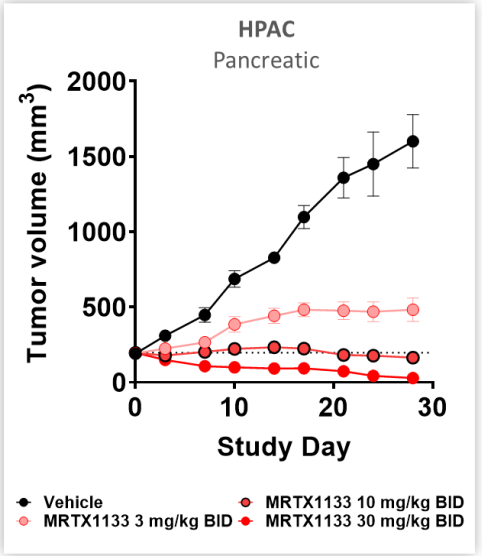
- Near maximal pERK inhibition after a single dose of MRTX1133
- BIDx3 administration demonstrates robust pERK inhibition for entire dose interval and correlates with maximal antitumor efficacy

MRTX1133 Demonstrates Cytoreductive Antitumor Efficacy Across a Panel of Cell- and Patient-derived Xenograft Tumor Models

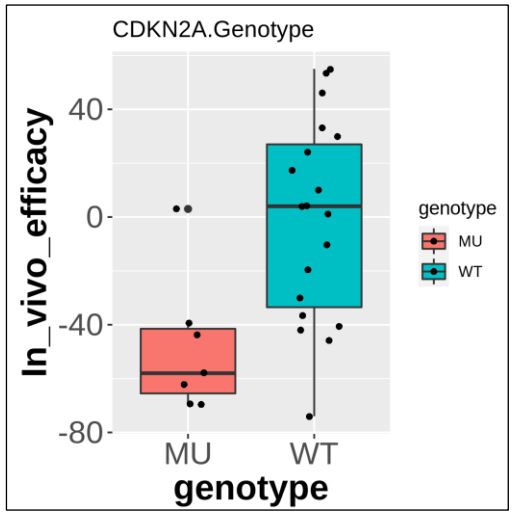








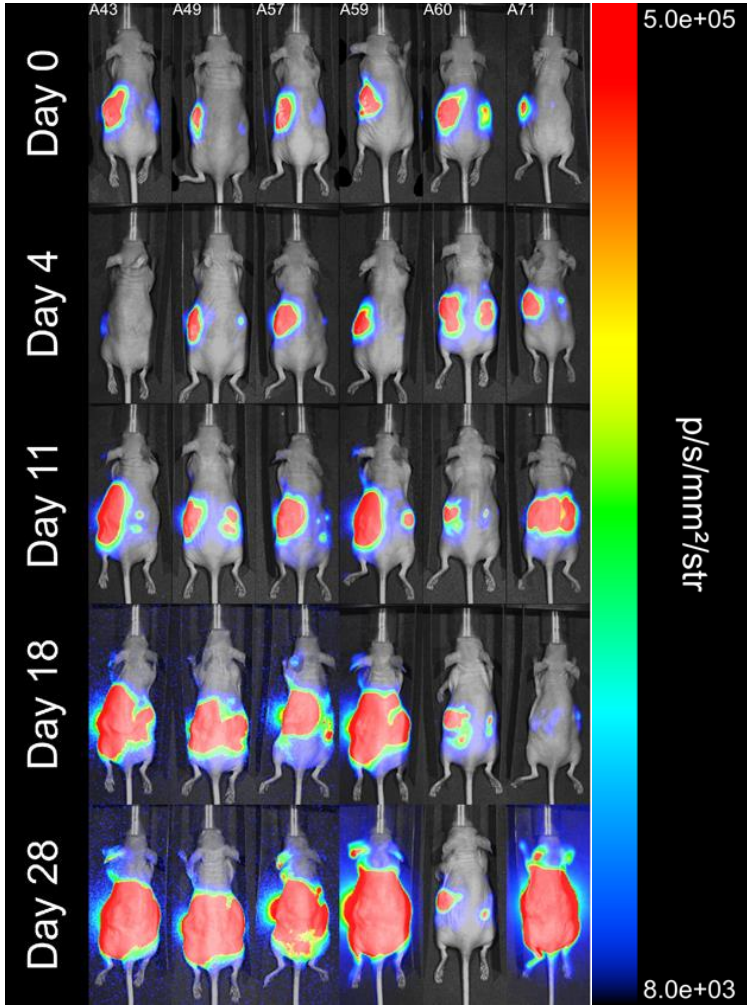
Regression in 15/25 (60%) models & response in 8/11 (73%) PDAC models and in 2/8 (25%) CRC models



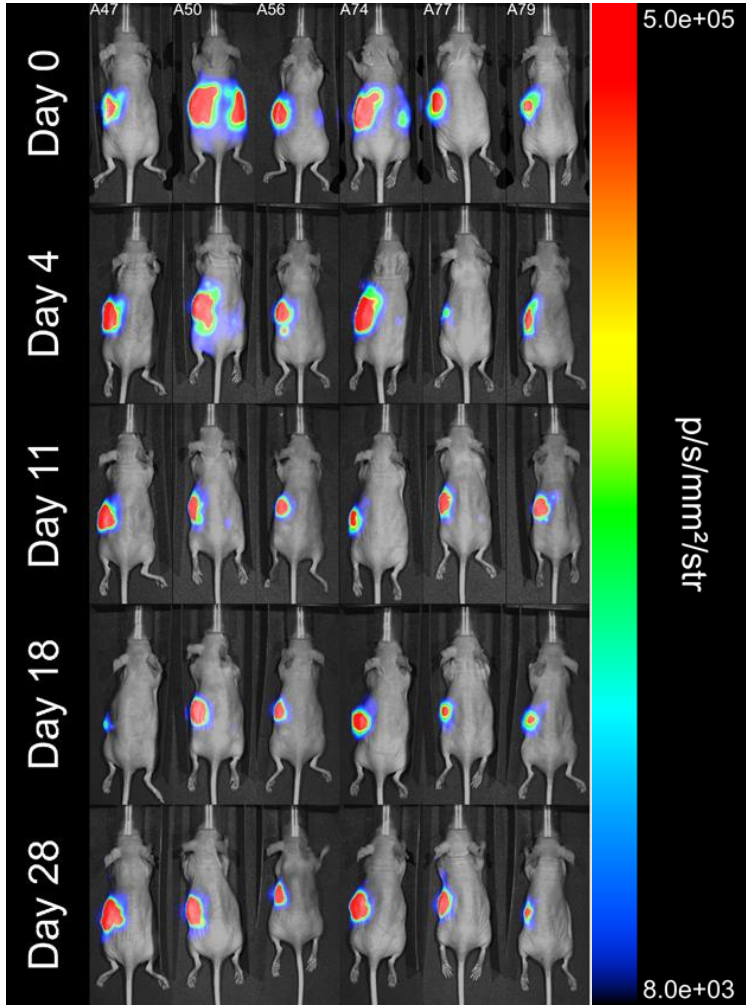
- Loss of PTEN expression correlates with resistance & CDKN2A LOF with response
- BYL719 (PI3Kalpha) effective and palbociclib (CDK4/6i) ineffective in combination with MRTX1133

MRTX1133 Inhibits pERK and Induces Regression in a Luc-labeled Orthotopic Model of Pancreatic Cancer

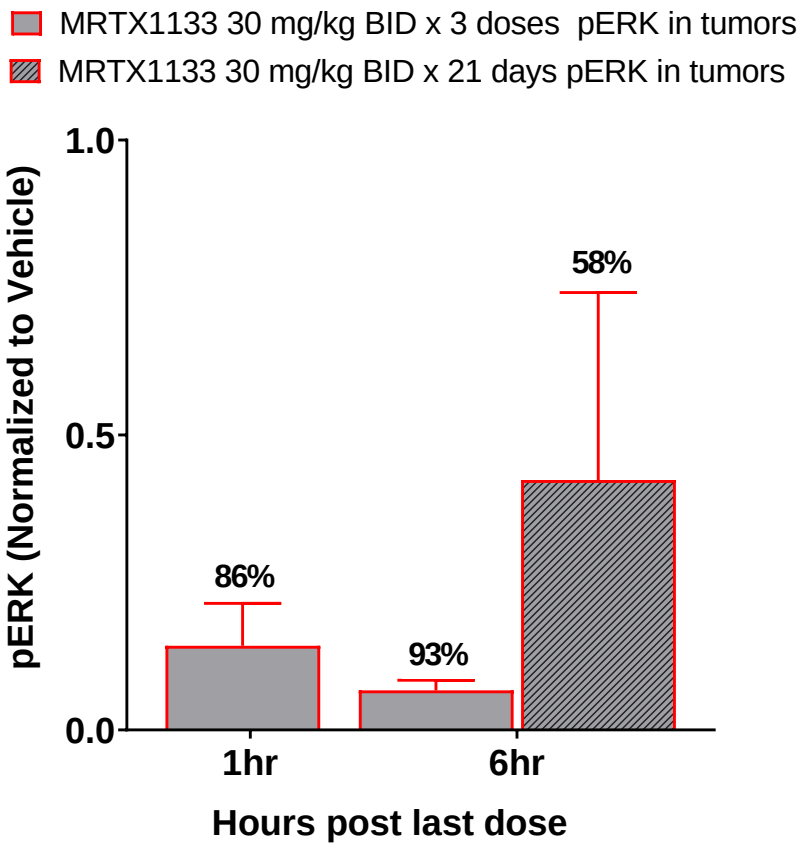
Vehicle



MRTX1133 at 30mg/kg IP BID

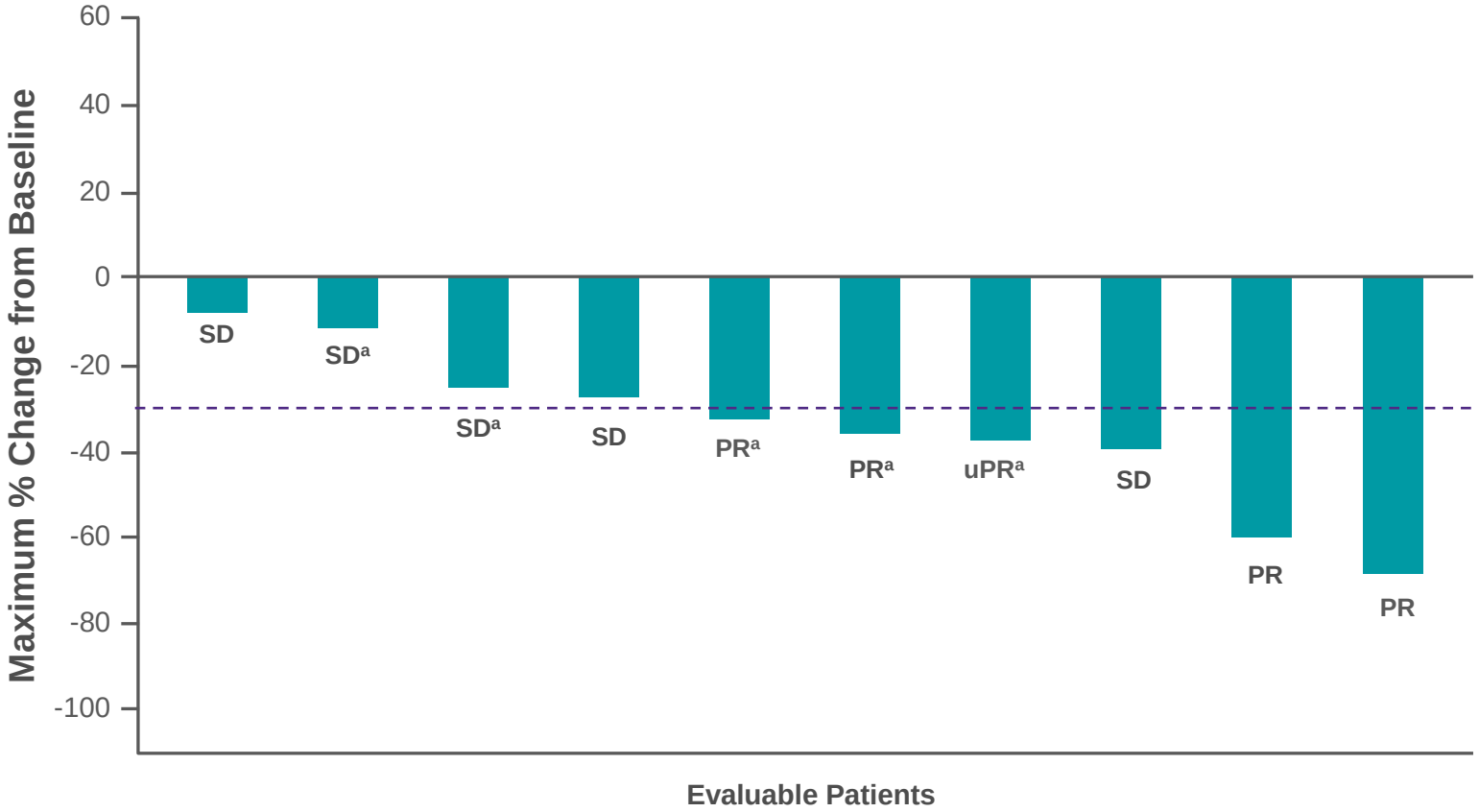


PD in AsPC-1



Proof of Concept for Mutant KRAS inhibition in PDAC with Adagrasib ($KRAS^{G12C}$)

Best Tumor Change From Baseline in Patients With Pancreatic Cancer (n=10)



Selected Patient Demographics

	Pancreatic Cancer (n=12)
Median age, y (range)	67 (40-80)
Female, n (%)	4 (33%)
ECOG PS, n (%)	
0	0 (0%)
1	12 (100%)
Prior lines of systemic therapy, median (range)	3 (1-4)
Prior lines of systemic therapy, %	
1	8%
2	42%
3	33%
≥4	17%

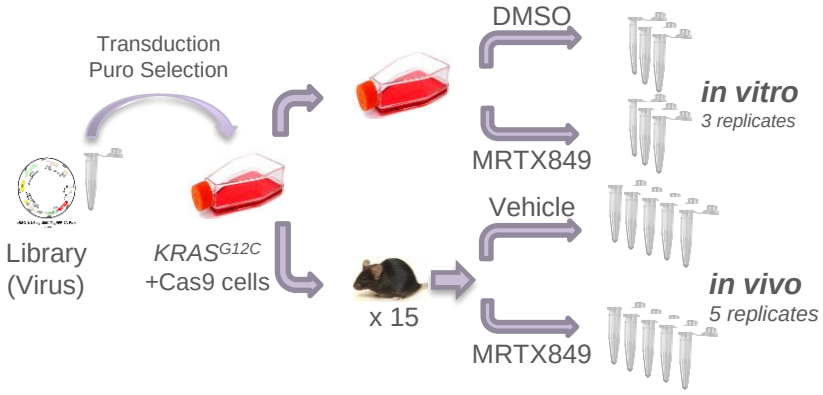
- Preliminary response rate per investigator assessment was 50% (5/10), including 1 unconfirmed PR
- Clinical benefit (DCR) was observed in 100% (10/10) of patients
- All patients received gemcitabine-based regimens and 10/12 received prior fluoropyrimidine-based regimens

At the time of the 10 September 2021 data cutoff, 2 of the 12 enrolled patients had discontinued treatment prior to 1st scan due to unrelated adverse events and were not evaluable for clinical activity
[a]Treatment ongoing.

Drug-anchored CRISPR Screens Reveal Combination Targets & Potential Resistance Mechanisms

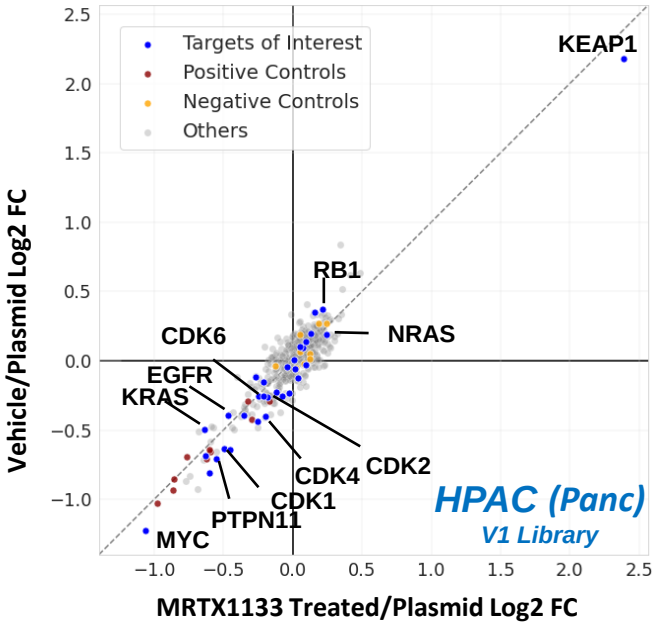
~5000 sgRNA Library

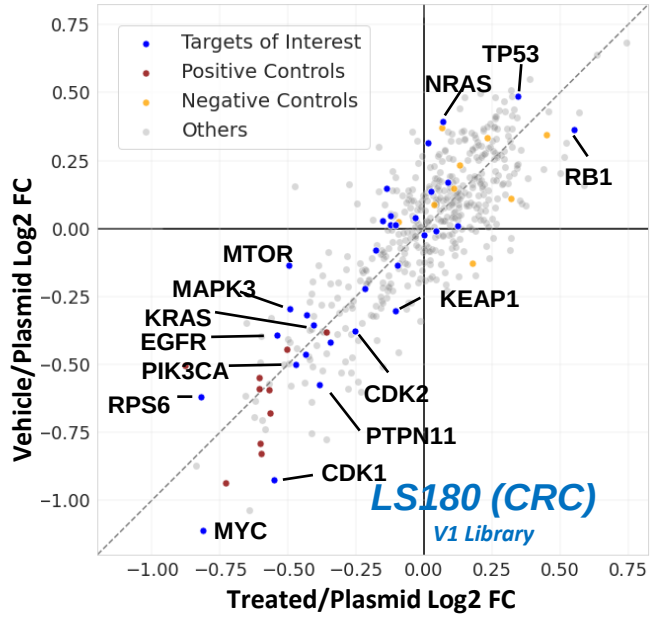
- **20 Negative Controls**
  - 20 intronic targets
- **10 Positive Control Targets**
  - 10 sgRNAs / gene
- **938 Target Genes**
  - 5 sgRNAs / gene



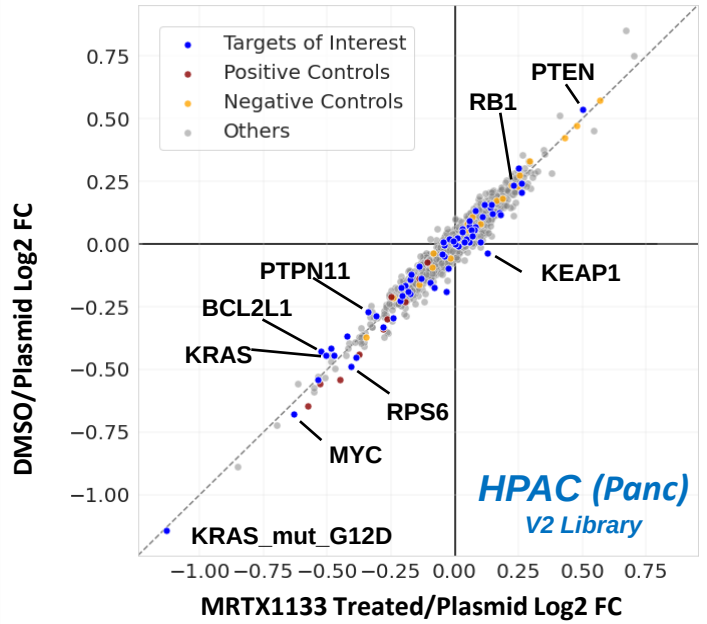
- Upstream genes EGFR, SHP2 dropout and represent potential combination targets
- Several TSGs enriched and may confer partial resistance to MRTX1133
- KEAP1 enriched in 2 PDAC models

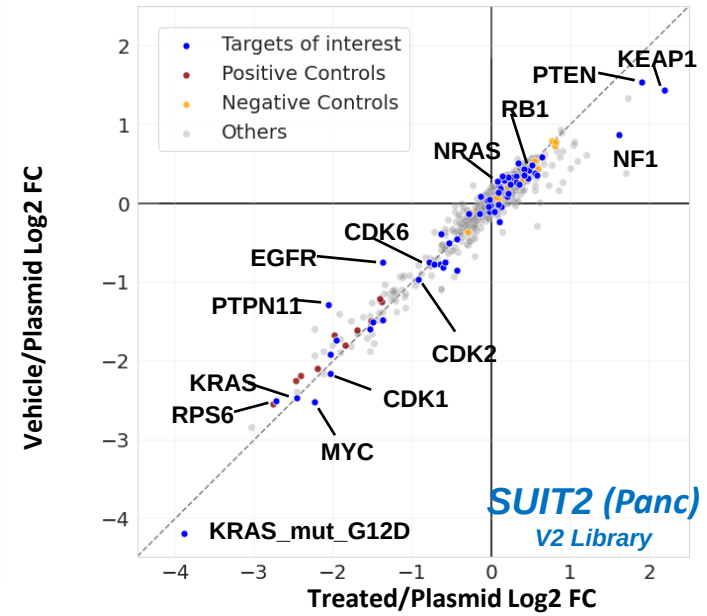
in vivo





in vitro

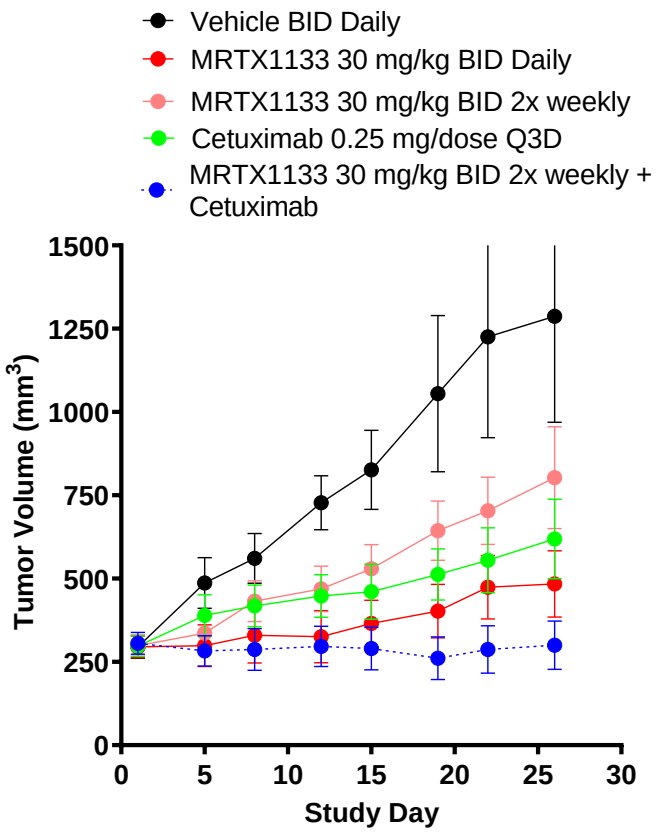




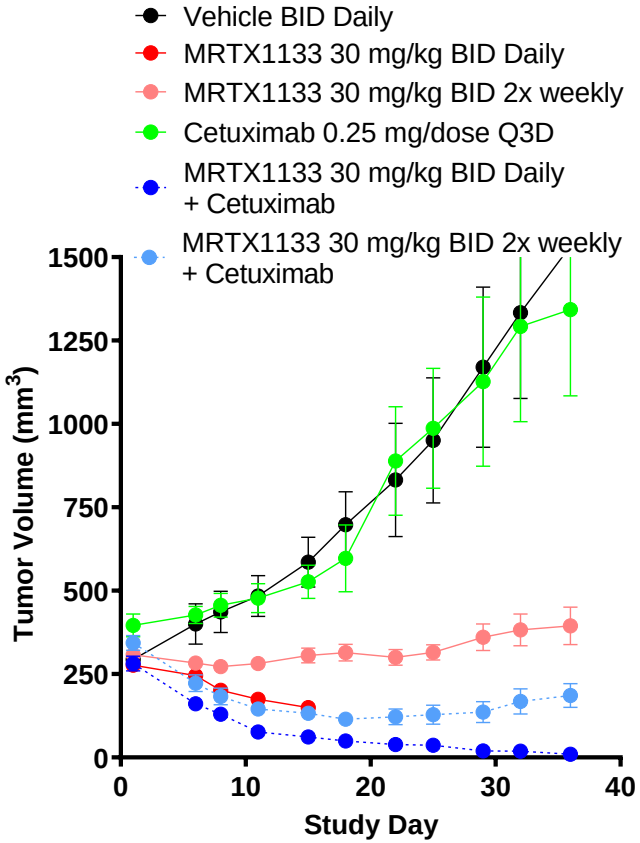
Sustained Regression with Cetuximab Combination

Cetuximab Combinations Active in PDAC and Colon Xenograft Models

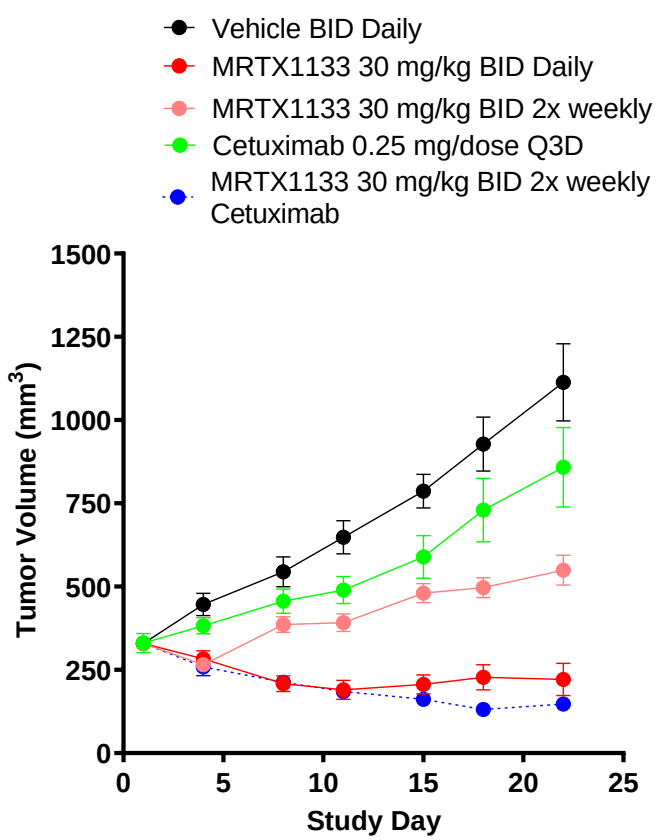
SNU-1033 (colon) + Cetuximab



AsPC-1 (panc) + Cetuximab



Panc0203 (panc) + Cetuximab



Legend

synergistic | additive | no benefit

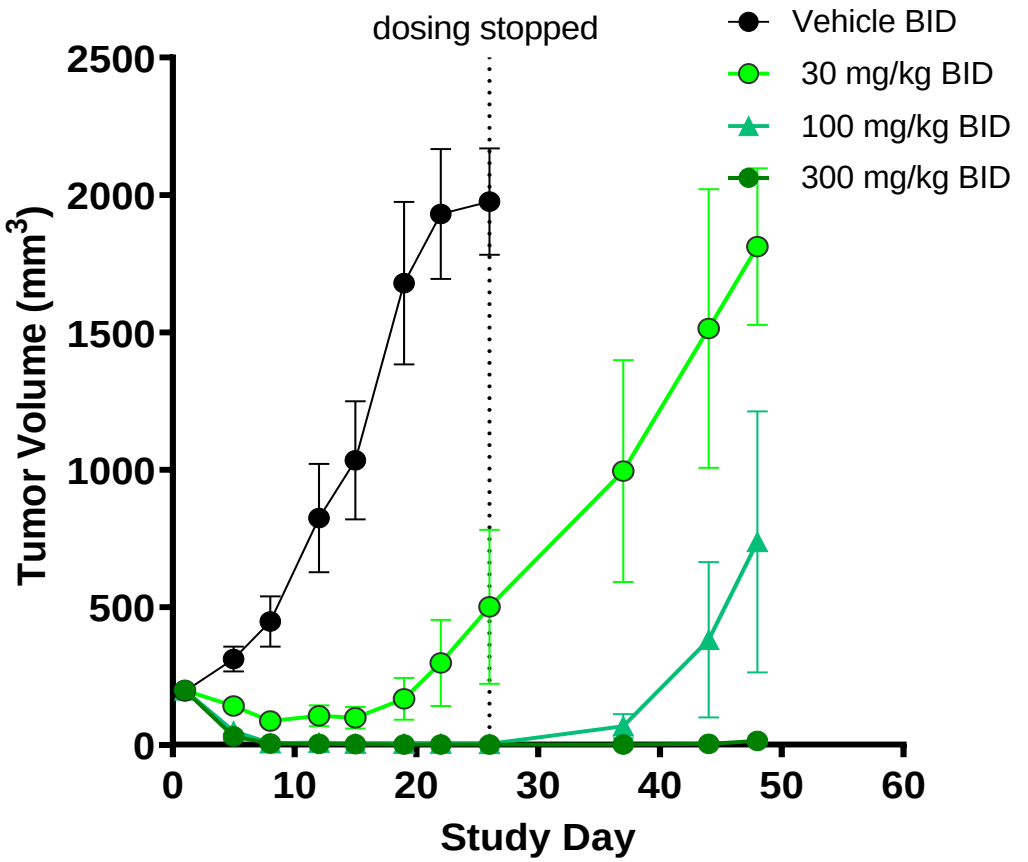
In Vitro MCA Scores	A427	AGS	ASPC1	CT26 M8V	GP2D	HCC1588	Hec1B	HPAC	HPAFII	KP4	LS180	LS513	Panc0203	Panc0403	Panc0504	Panc0813	Panc1	Panc1005	SNU1033	SNU1197	SNU407	SNU410	SNU61	SNUC2B	SU8684	SUIT2	SW1990
Cetuximab		53	43	8	60	-7	1	38	24		69	60	49	44	59	32	-19	29	36	53	14	2	52	53	31	45	7

Prototype MRTX Pan KRAS Inhibitor Inhibits KRAS-Dependent Signaling and Demonstrated Complete response in a $KRAS^{G12V}$-mutant Model

KRAS mutant pathway inhibition selectivity spectrum

Cell Line	KRAS Mutation	MRTXA pERK IC_{50} (nM)	MRTXB pERK IC_{50} (nM)
H358_Lung	G12C	13.7	4.1
MiaPaca2_Pancreas	G12C	9.9	4.4
NIH3T3 G12D	G12D	45.7	9.4
ASPC1_Pancreas	G12D	44.2	9.8
PSN1_Pancreas	G12R	1193.9	533.8
HUPT3_Pancreas	G12R	2317.7	3,807.0
A549_Lung	G12S	23.0	20.5
H727_Lung	G12V	31.2	28.6
RKN_Soft Tissue	G12V	35.1	47.8
HCT116_Large Intestine	G13D	34.4	22.6
H460_Lung	Q61H	8.7	8.3
MKN1_Stomach	WT AMP (dependent)	1.6	3.5

Antitumor Activity of MRTXA in RKN Model (LMS, $KRAS^{G12V}$) In Vivo (PO Administration)



- IC_{50} values for NRAS and HRAS mut cell lines each >3,000 nM
- Modification of molecule structural and physiochemical properties modulate mutational selectivity profile and oral PK properties
- Although molecule targets WT KRAS, well tolerated in mice suggesting compensatory role for NRAS/HRAS in normal tissue

Summary and Conclusions

- $KRAS^{G12D}$ is an oncogenic driver and compelling drug target across a unique spectrum of cancers including pancreatic ductal adenocarcinoma
- MRTX1133 demonstrates proof of principle for development of selective and noncovalent inhibitors of mutant variants of KRAS in either the active or inactive conformation, particularly $KRAS^{G12D}$
- MRTX1133 is being explored for introduction into clinical trials utilizing novel parenteral formulations and administration strategies
  - Stealth liposomal formulations have demonstrated feasibility in preclinical studies including potential for durable plasma exposure and reduced clearance
- Targeting other oncogenic forms of KRAS are also feasible and candidate seeking discovery is ongoing

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New York, NY

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Fairfax, VA

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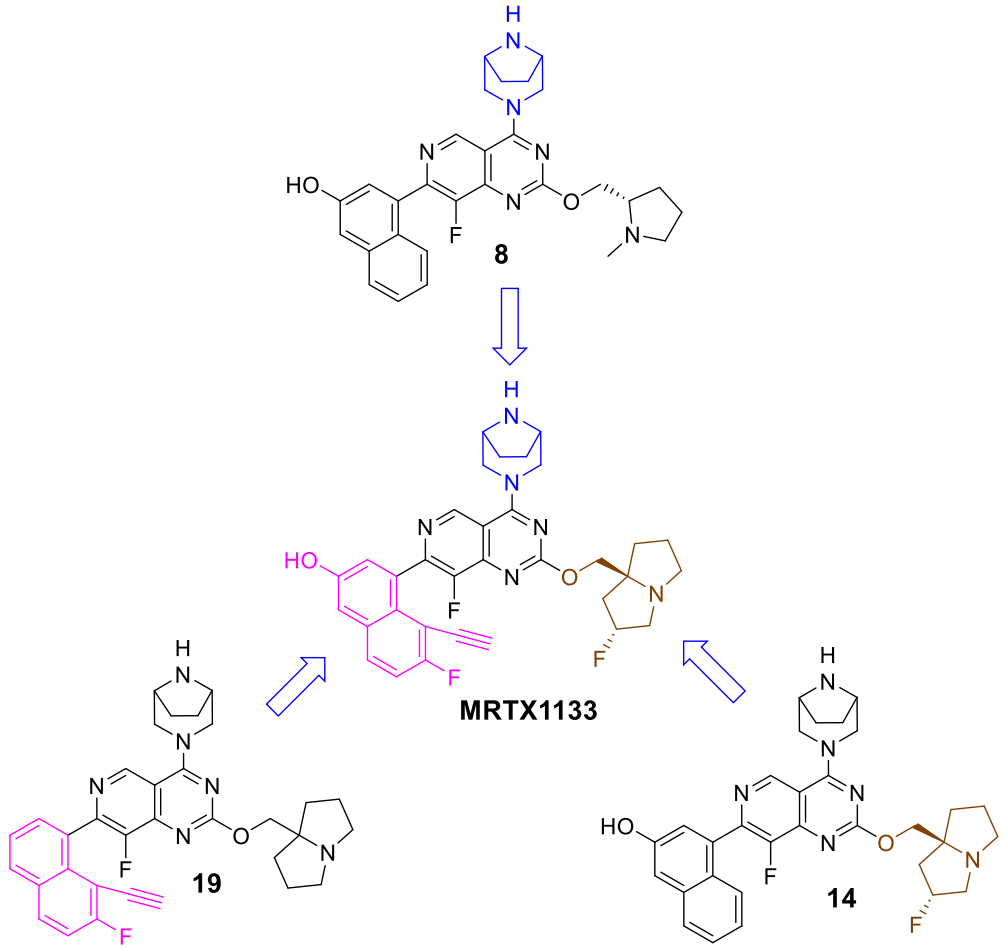
MRTX11133 Inhibits KRAS^{G12D} with sub-nM Binding Affinity



Assay	Activity
KRAS^{G12D} K_D (nM)	~0.0002*
AlphaLISA IC_{50} (nM)	5
pERK AGS IC_{50} (nM)	2
2D viability AGS (KRAS^{G12D}) IC_{50} (nM)	6
2D viability MKN1 (KRAS^{WT}) IC_{50} (nM)	>3000