Structure-Based Drug Discovery of MRTX1257, a Selective, Covalent KRAS G12C Inhibitor with Oral Activity in Animal Models of Cancer

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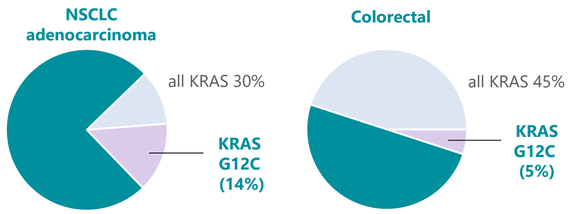
Introduction

- KRAS is the most frequently mutated driver oncogene in human cancer[1]
- The ability to target and block the function of mutated KRAS has remained elusive despite decades of research
- The KRAS G12C mutant is prevalent, and directly targeting this mutant with irreversible inhibitors has been demonstrated[2-6]
- We have previously described compound **1** as an irreversible covalent inhibitor of KRAS G12C with antitumor efficacy[7]
- The addition of an 8-substituent on the naphthyl ring of inhibitor **1** previously described inhibitors fills a hydrophobic pocket
- The tool compound MRTX1257 exhibited 31% oral bioavailability in a mouse PK experiment, robust in vivo target engagement, and antitumor efficacy in a mouse MIA PaCa-2 xenograft model

KRAS G12C Mutations

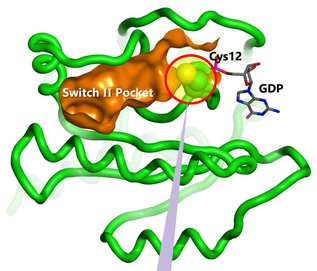


Historical Challenges in Targeting the KRAS Pathway

Upstream Inhibitors
Blocking Ras Membrane localization
Farnesyl transferase inhibitors do not block KRAS localization

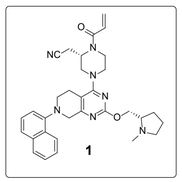
Reversible Inhibitors
Targeting KRAS^{mut} is challenging due to small, undefined catalytic site and high affinity for GTP

Downstream Effector Inhibitors
Raf / MEK and PI3K / AKT / mTOR
- Limited effectiveness in KRAS^{mut} tumors
- Incomplete inhibition of KRAS^{mut}
- Inhibition of KRAS^{wt} resulting in low therapeutic index



Covalent Inhibition of KRAS G12C
- Binding in the switch II pocket of GDP KRAS
- Covalent bond to cysteine 12
- Locked in the inactive conformation

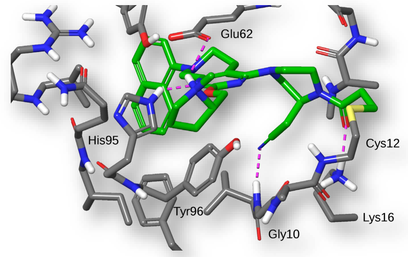
Compound 1 is an Irreversible Covalent Inhibitor of KRAS G12C



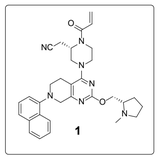
MW / ClogP / PSA	551 / 4.4 / 87
5 min/3 μM Protein Modification assay	70 %
H358 Cell IC_{50}	5 nM
Permeability	Medium/Efflux
Mouse Hepatocyte ER	90%
Mouse Microsomal ER	49%
Mouse PPB	96%

- The discovery of compound **1** has been described previously[7]
- The inhibitor **1** showed 70% modification of KRAS G12C in the 5 min/3 μM modification assay
- Compound **1** inhibited KRAS dependent ERK phosphorylation in the H358 cell assay, IC_{50} = 5 nM
- This compound has high hepatocyte clearance with medium permeability and efflux

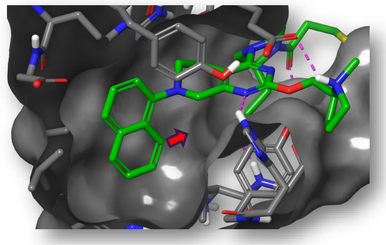
Co-Crystal Structure of 1 Reveals Key Interactions

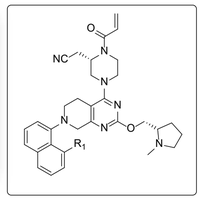


Key Interactions
- His95 - H-bond to pyrimidine nitrogen
- Tyr96 - pi-pi interaction to the pyrimidine
- Gly10 - Nitrile H-Bond to backbone N-H
- Lys16 - Acrylamide carbonyl interaction with NH_3+
- Cys12 - Covalent bond formed to acrylamide olefin
- Glu62 - C2 tail salt bridge to the acid



Surface View of Co-Crystal Structure of 1 Reveals Opportunity

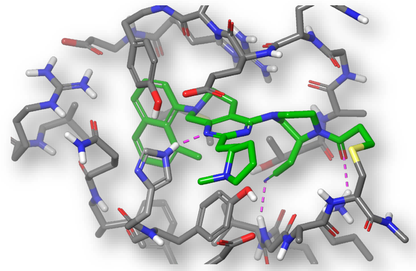




- Naphthalene 8-position extends to an open groove at the bottom of the pocket (arrow)
- Substituents in this position have the potential for increased potency

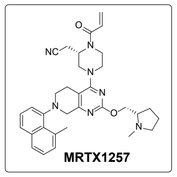
	R_1 =	POC Mod. (5min/3μM)	H358 IC50 μM		R_1 =	POC Mod. (5min/3μM)	H358 IC50 μM
2		8%	1.7	5		58%	0.005
3		37%	0.290	6		60%	0.001
4		54%	0.008	7, MRTX1257		59%	0.0009

Co-Crystal Structure of MRTX1257 Bound in KRAS G12C



- MRTX1257 binds in the switch II pocket of KRAS G12C in the GDP bound form
- All of the key polar and hydrophobic contacts previously mentioned are maintained
- The 8-methyl group fills the lipophilic pocket between the naphthyl and cyanomethyl substituent

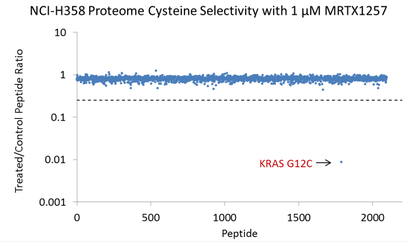
MRTX1257 is an Irreversible Covalent Inhibitor of KRAS G12C



MW / ClogP / PSA	565 / 4.9 / 88
5 min/3 μM Protein Modification assay	59 %
H358 Cell IC_{50}	900 pM
Permeability	Medium/Efflux
Mouse Hepatocyte ER	82%
Mouse Microsomal ER	36%
Mouse PPB	99%

- MRTX1257 showed 59% modification of KRAS G12C in the 5 min/3 μM modification assay
- MRTX1257 inhibited KRAS dependent ERK phosphorylation in the H358 cell assay, IC_{50} = 900 pM
- This compound has high hepatocyte clearance with medium permeability and efflux

MRTX1257 Selectively Modifies KRAS G12C in H358 Cells



- The selectivity of MRTX1257 toward the targeted Cys12 of KRAS G12C was evaluated in NCI-H358 cells
- Free cysteine residues were surveyed by labeling with desthiobiotin-iodoacetamide, digesting the proteins, and analyzing the streptavidin-captured peptides by LCMS
- Less than 1% of the KRAS G12C peptide remained at 1 μM, indicating near complete target engagement
- No other cysteine-containing peptides decreased >4-fold (dotted line), demonstrating a high degree of selectivity for KRAS G12C

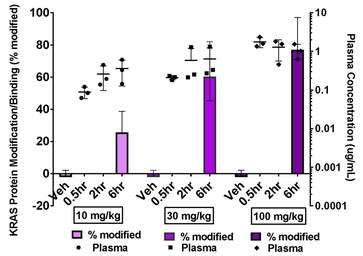
MRTX1257 Demonstrated 31% Bioavailability in Mice

ff adj. H358 cell IC_{50} = 0.035 μg/mL (0.7 μM; PPB 98.9%)

MRTX1257 Mouse PK				
IV Dose	AUC_{inf} (hr*μg/mL)	$t_{1/2}$ (hr)	Clearance (mL/min/kg)	V_{ss} (L/kg)
3 mg/kg	1.58	1.3	32	1.9
PO Dose	AUC_{inf} (hr*μg/mL)	C_{max} (μg/mL)	T_{max} (hr)	F (%)
30 mg/kg	4.91	1.66	0.5	31

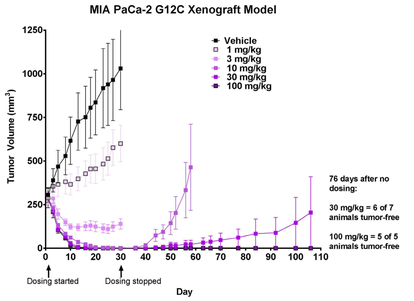
- MRTX1257 is a moderate clearance compound in vivo
- A 30 mg/kg oral dose resulted in 4.91 hr*μg/mL AUC and 1.66 μg/mL maximal total concentration
- The 30 mg/kg dose covers the free fraction adjusted cell IC_{50} for >8 hours

MRTX1257 Demonstrated Robust Target Engagement in vivo



- MRTX1257 showed dose response of target engagement that correlates well with plasma exposure in H358 tumor model
- The fraction of KRAS G12C covalently modified by MRTX1257 relative to total evaluable KRAS G12C protein was measured using a mass spectrometry-based assay
- MRTX1257 has 77% target engagement at the 100 mg/kg dose

MRTX1257 Exhibits Rapid and Sustained Efficacy Against MIA PaCa-2 Xenografts



- MRTX1257 showed rapid tumor growth inhibition at all dose groups
- MRTX1257 showed sustained regression at 3,10, 30, and 100 mg/kg dose groups
- MRTX1257 dosed at 100 mg/kg daily leads to complete responses that are maintained >70 days after cessation of treatment

Conclusions

- We have identified the tetrahydro-pyridopyrimidine MRTX1257 as an irreversible covalent inhibitor of KRAS G12C
- The addition of an 8-position substituent on the naphthyl ring filled a hydrophobic pocket in the protein, resulting in increased potency
- MRTX1257 inhibited KRAS dependent ERK phosphorylation in the H358 cell assay with an IC_{50} = 900 pM
- MRTX1257 demonstrated 31% bioavailability in the mouse, with free fraction exposures well above the cellular potency
- In a PK/PD experiment, 77% target engagement was seen in MIA PaCa-2 tumors
- An efficacy experiment with MRTX1257, dosed orally in mice bearing MIA PaCa-2 tumors, showed rapid growth inhibition and durable efficacy even after dosing was stopped
- MRTX1257 is a benchmark compound; the clinical candidate MRTX849 will be discussed in future publications

References and Acknowledgements

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