CO69

Discovery and Preclinical Development of MRTX849: A Mutation-Selective KRAS G12C Inhibitor

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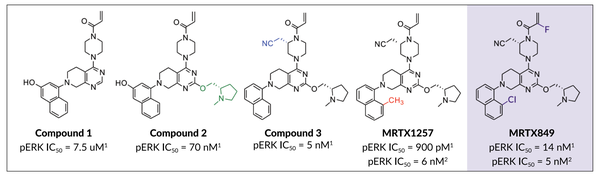
BACKGROUND

- Despite decades of research, efforts to directly target KRAS have been challenging.
- The MRTX chemical series is comprised of mutant-selective, covalent inhibitors that target the GDP-bound form of KRAS G12C, locking it in the inactive state.
- The oral bioavailability and ADME properties of a previously described tool compound, MRTX1257, was limited by GST-mediated glutathione (GSH) scavenging, leading to unfavorable predicted human PK properties.
- Optimization of the 8-substituent of the naphthyl ring and modulation of the acrylamide reactivity led to MRTX849, with improved GST-mediated GSH stability and favorable human PK projections.
- The clinical candidate molecule MRTX849, a KRAS G12C mutant-selective, covalent inhibitor of KRAS G12C was identified through structure-based drug design with low nanomolar cell potency and favorable oral properties.

Previous Poster Disclosure:

Hallin J. et al. *Insight Towards Therapeutic Susceptibility of KRAS Mutant Cancers from MRTX1257: A prototype selective inhibitor of KRAS G12C.* Poster presented at: 2019 AACR Mar 29-Apr 3; Atlanta, GA.

Drug Discovery Progression Toward MRTX849



Compound 1
pERK IC_{50} = 7.5 uM[1]

Compound 2
pERK IC_{50} = 70 nM[1]

Compound 3
pERK IC_{50} = 5 nM[1]

MRTX1257
pERK IC_{50} = 900 pM[1]
pERK IC_{50} = 6 nM[2]

MRTX849
pERK IC_{50} = 14 nM[1]
pERK IC_{50} = 5 nM[2]

[1] pERK IC_{50}, 3hrs in H358 cells, [2] pERK IC_{50}, 24hrs in MIA PaCa-2 cells

- The addition of the **C2 substituent** significantly improved solubility and cellular potency compared with the lead compound 1 and demonstrated more rapid modification of the protein.
- The introduction of the **cyanomethyl substituent** on the piperazine further improved potency, allowing for the elimination of the 3-hydroxyl group on the naphthyl ring, improving ADME properties.
- **The 8-position of the naphthyl group was substituted** to fill a hydrophobic pocket, increasing potency an additional 5-fold.
- **Warhead modification** and final optimization for reactivity and bioavailability provided MRTX849.

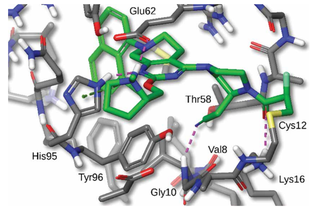
	MRTX1257	MRTX849
Cellular Potency[1]	6 nM	5 nM
Half-life in whole blood[2], m/r/d/h	8, 5, 4, 16	> 50 in all species
Bioavailability, % m/r/d	31/8/4 (30mg/kg mouse and rat, 10mg/kg dog)	63/30/26 (30mg/kg)
$V_{d,ss}$ L/Kg, m/r/d	2/6/6	2/21/17

[1] ICW average value, pERK IC_{50}, MIA PaCa-2 cells, 24 hour incubation
[2] GST-mediated GSH conjugation, hours

- The reactivity of the acrylamide species of MRTX1257 leads to short half-lives in whole blood, due in part to GST-mediated GSH uptake.
- Systematic adjustment of the acrylamide reactivity and further optimization of the naphthyl 8-substituent led to MRTX849, which shows greater stability in whole blood.
- The cross-species bioavailability of MRTX849 was improved relative to MRTX1257, leading to favorable human PK and dose predictions.
- PBPK modeling utilizing PK Sim and Gastro Plus projects 50% oral bioavailability and >20 hour half-life in humans. Present PK data in MRTX849-001 clinical trial supports these projections.

MRTX849 Binds GDP-KRAS G12C and Locks the Protein in the Inactive State

Fig. 1: MRTX849 Key Small Molecule Interactions with KRAS G12C Protein



KEY PROTEIN-LIGAND INTERACTIONS

Cys12: Covalent bond formed to acrylamide olefin

Lys16: Acrylamide carbonyl interaction with NH3+

Gly10: Nitrile H-bond to backbone N-H

Glu62: C2 tail salt bridge to the acid

His95: H-bond to pyrimidine nitrogen

Tyr96: pi-pi interaction to the pyrimidine

MRTX849 Is A Potent, Selective and Orally Bioavailable Clinical Compound

	MRTX849
MW/cLogP/tPSA	604 / 5.8 / 87
K_{inact}/K_i	35 +/- 0.3mM^{-1}s^{-1}
5 min / 3uM Protein Modification[1]	66%
Hepatocyte ER % (m/h)	61 / 50
Plasma Protein Binding % (m/h)	99.0 / 98.3
F % (m/h)	63 / 50*

[1] LCMS-based KRAS G12C protein modification assay utilizing MRTX849 pre-loaded with GDP
* human projected F%

Fig. 2: Cysteine Selectivity in H358 Cells with MRTX849 (1uM)

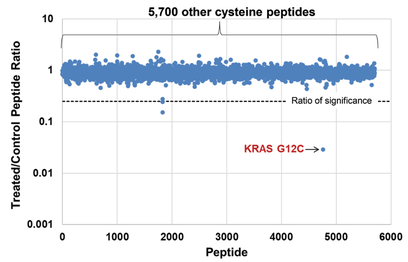
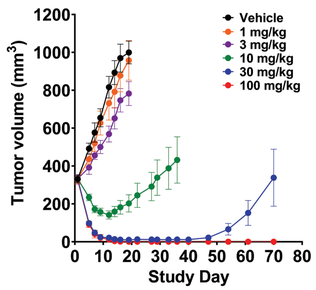


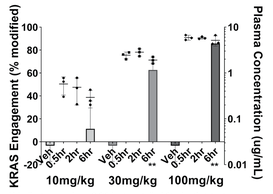
Fig. 3: Antitumor Efficacy in MIA PaCa-2 Xenograft Model



- 100mg/kg MRTX849 PO daily for 16 days demonstrates complete response with no tumor recovery after dosing cessation.

MRTX849 Treatment Achieves KRAS Modification, Biomarker Modulation, and Antitumor Efficacy

Fig. 4: KRAS G12C Protein Modification 6hrs Post Single Dose of MRTX849



** indicates p-value <0.01 by two-tailed Student's t-test

Fig. 5: Normalized pERK and pS6 Inhibition 6hrs Post Single Dose of MRTX849

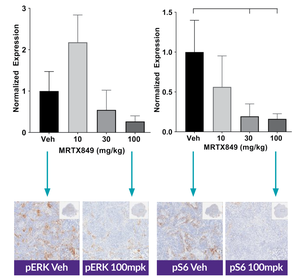
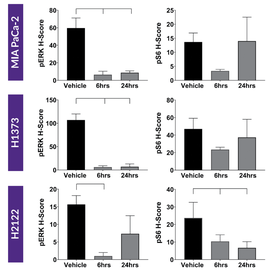


Fig. 6 MRTX849 at 100mg/kg leads to significant pERK and pS6 inhibition by IHC in multiple xenograft models



Brackets indicate p-value <0.05 compared to left-most sample, by two-tailed Student's t-test*

- IHC shows near complete inhibition of KRAS-mediated pathway inhibition and provides a more accurate representation of signal modulation than bulk tumor lysate.
- Near complete durable inhibition of KRAS-dependent signaling for full dose interval correlates with maximum anti-tumor activity.
- Near complete durable inhibition of pERK is associated with deep and durable responses, while transient and/or incomplete pERK inhibition is associated with submaximal antitumor activity

Clinical Target Concentration Projections Derived from Nonclinical PKPD Modeling

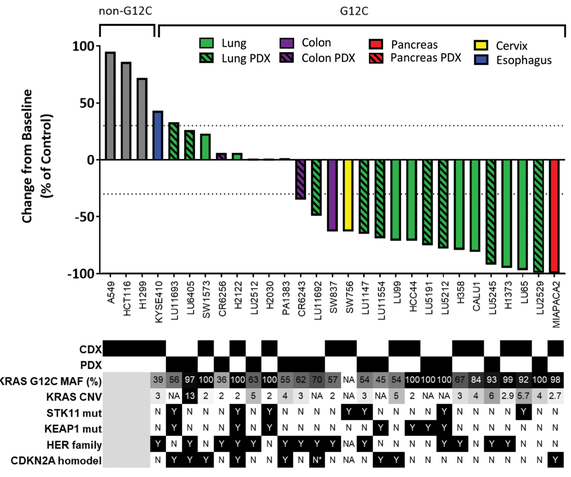
Relationship Between Dose, $AUC_{0\text{-}24}$ and Antitumor Efficacy in Nonclinical Human Xenograft Models

Model	Dose (mg/kg)	$AUC_{0\text{-}24}$ (ug*h/mL)	FF adj $AUC_{0\text{-}24}$ (ug*h/mL)	% Regression (Day)	Projected Efficacious Total/FF adj AUC (human, ug*h/mL)
MIA PaCa-2	10	7	0.0697	-52% (13)	-
MIA PaCa-2	30	24	0.243	-96% (13)	14.3
HCC-44	100	63	0.63	-61% (13)	37.1

- Schedule dependence and infusion studies were utilized to define plasma exposure parameters that best fit antitumor activity regardless of dose schedule. AUC and C_{avg} were the closest fit for PK parameters driving antitumor activity.
- The $AUC_{0\text{-}24}$ values determined at the 30 mg/kg and 100 mg/kg dose levels which demonstrated maximum antitumor activity in the MIA PaCa-2 (sensitive) and HCC44 (partially sensitive) models, respectively, corrected for mouse plasma protein binding were utilized for human efficacious exposure projections. The free-fraction adjusted $AUC_{0\text{-}24}$ corrected for human plasma protein binding (98.3%) were calculated as **14.3 ug*h/mL** and **37.1 ug*h/mL** in these 2 models.
- PBPK Link modeling approaches utilizing PK-Sim™ or GastroPlus 9.5™ software were applied, and data inputs including the total human projected target AUC values, along with nonclinical ADME data inputs, and in vivo clearance determined in mouse, rat and dog studies were applied to project a human efficacious dose and map human exposure relative to target efficacious concentrations.
- The data inputs fit well and human PK data was predictable based on PBPK modeling. Mean human AUCs achievable at steady-state at 600 mg BID well-exceeded even the most aggressive target plasma concentrations.

MRTX849 Demonstrates Broad Spectrum Tumor Regression in KRAS G12C Nonclinical Models

Fig. 7: 17 of 26 KRAS G12C Xenografts Show >30% Regression with MRTX849 at 100mg/kg QD



CONCLUSIONS

- Optimization of the warhead reactivity and naphthyl substituent of tool compound MRTX1257 led to the optimized compound MRTX849 with favorable human PK and dose predictions.
- Dose response administration of MRTX849 in preclinical models led to KRAS modification, pERK and pS6 modulation, and antitumor activity which defined the PKPD relationship and target AUC needed for human dose predictions.
- MRTX849 administered daily PO at well-tolerated dose levels induced 30% or greater tumor regression in ~65% of cell line and patient-derived xenografts with durable complete regressions observed in a subset of models.
- Nonclinical PKPD modeling predict that target AUC is achievable at dose levels presently safely achieved in clinical trials. Projected human half-life exceeding 20 hours is predicted to be advantageous in limiting KRAS-dependent signaling feedback reactivation.

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