Insight Towards Therapeutic Susceptibility of KRAS Mutant Cancers from MRTX1257: A Prototype Selective Inhibitor of KRAS G12C

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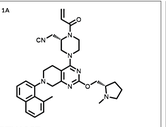
BACKGROUND

- KRAS G12C is an established driver mutation but efforts to directly target KRAS have been historically challenging.
- MRTX1257 is a mutant-selective, covalent inhibitor of KRAS G12C identified through structure-based drug design with low nanomolar cell potency and favorable oral properties (see Poster Session Sapphire ABEF, Board B30, Marx *et al.*).
- The anti-tumor activity and mechanism-of-action of MRTX1257 was evaluated across a panel of KRAS G12C-mutant and non G12C-mutant pre-clinical models in vitro and in vivo.
- Molecular mechanisms of therapeutic sensitivity and resistance were evaluated and selected resistance hypotheses were probed through combinatorial treatment strategies.

RESULTS

MRTX1257 is a Potent and Selective KRAS G12C Inhibitor

Fig. 1A



MRTX1257

- KRAS G12C H358 pERK IC_{50} 3hr = 0.9nM
- Mouse PPB = 99%
- FF adj. IC_{50} = 0.035ug/mL
- Mouse F = 31.1%
- Mouse AUC_{inf} (hr*ug/mL) = 4.91
- Mouse C_{max} (ug/mL) = 1.66

Fig. 1B

CCLE Cell Line Name	KRAS	2D 257 IC50	3D 257 IC50
NCIH2030 LUNG	G12C	37.3	0.3
HCC44 LUNG	G12C	315.3	1.6
NCI-H358 LUNG	G12C	33.4	1.6
IALM LUNG	G12C	5.6	3.0
NCI-H1792 LUNG	G12C	7214.0	3.7
SW756 CERVIX	G12C	98.5	4.1
NCI-H1373 LUNG	G12C	86.3	6.2
UMUC3 URINARY	G12C	3000.0	10.0
CALU1 LUNG	G12C	38.6	19.5
LU65 LUNG	G12C	19.3	23.2
MIAPACA2 PANCREAS	G12C	53.0	28.1
SW1573 LUNG	G12C	>10000	32.1
LU99 LUNG	G12C	1357.0	32.6
NCI-H23 LUNG	G12C	27.4	36.6
SW837 LARGE INTESTINE	G12C	24.7	40.4
NCI-H2122 LUNG	G12C	100.7	62.4
KYSE410 OESOPHAGUS	G12C	3000.0	2080.0
A549	G12S	1116.0	>3000
HCT116	G13D	1163.0	>3000
H1299	KRAS WT	3000.0	>3000

Fig. 1C

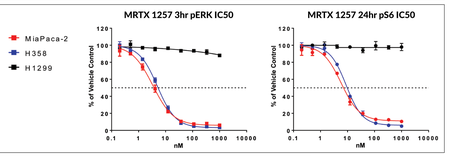


Fig. 1D

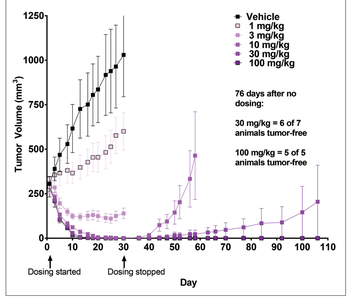


Figure 1 A-D

A. MRTX1257 structure and properties

B. A Cell Titer Glo assay was used to evaluate cell viability on cells grown in 2D (72hrs) or 3D (12 days) conditions. Cells were treated with MRTX1257 over 9 point concentration response to generate IC50 values, reported in nM. MRTX1257 was more potent in the 3D than the 2D format, as previously reported (Ref).

C. MRTX1257 was added to KRAS G12C mutant (H358, MIA PaCa-2) or KRAS WT (H1299) cells for 3hrs (pERK) or 24hrs (pS6) over 9 point concentration response and reduction of effector signaling was measured compared to vehicle control in In-Cell Western blot format. The cells with G12C mutation show potent reduction in effector signaling while KRAS WT cell line has no on-target activity.

D. Mice were treated (n=7/group) with MRTX1257 orally over dose response in MIA PaCa-2 xenograft model, which leads to tumor growth inhibition at all tested concentrations. MRTX1257 dosed at 100mg/kg daily for 28 days leads to complete, durable responses that are maintained >120 days after cessation of treatment.

MRTX1257 Demonstrates Robust Tumor Growth Inhibition with a Dose-Dependent PKPD Relationship Between KRAS Modification, Inhibition of KRAS Driven Signaling, and Anti-Tumor Activity

Fig. 2A

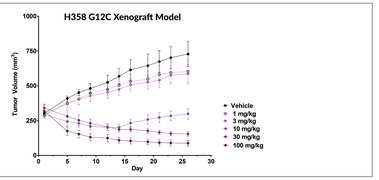


Fig. 2B

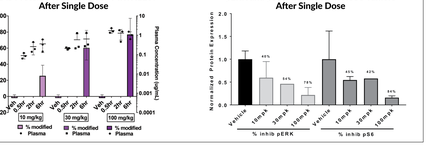


Fig. 2C

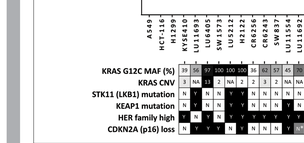


Figure 2 A-C

A. H358 xenograft-bearing, female nu/nu mice were dosed orally with MRTX1257 at indicated dose levels. Mean tumor volume + SEM, n=3.

B. MRTX1257 was administered orally as a single dose to mice bearing H358 tumors. Plasma was collected at 0.5, 2, and 6hrs post dose and tumors were collected at 6hrs post dose. The fraction of KRAS G12C covalently modified by MRTX1257 relative to total evaluable KRAS G12C protein was measured using mass spectrometry.

C. From the same H358 tumors as in Fig B, immunoblots were run to assess downstream effectors pERK and pS6, bands were quantified by densitometry analysis and normalized to total ERK and S6 respectively. (% of vehicle control). The bar graphs represent mean tumor volume + SEM, n=3.

MRTX1257 Inhibits pERK and pS6 in H358 Tumors; Deflection is Diminished After Multiple Dosing Days in H358 Tumors.

Fig. 3A

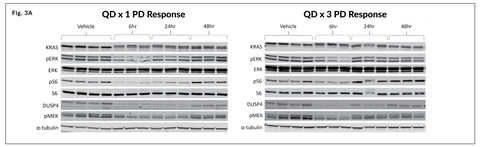
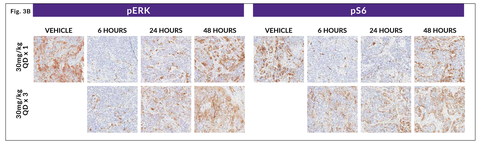


Fig. 3B



MRTX1257 Treatment Inhibits Proliferation and Induces Apoptosis in H358 Tumors

Fig. 3C

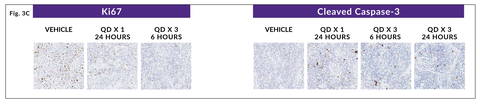


Figure 3 A-C

A. Mice with H358 tumors were treated with MRTX1257 @ 30mg/kg for single dose or QD x 3 days. Tumors were harvested at 6, 24, and 48hrs post last dose (n=3/ treatment group) and lysates were generated to assess biomarkers by immunoblot.

B/C. The same tumors from Panel A were cut and processed for immunohistochemistry analysis, using the antibodies indicated.

MRTX1257 Demonstrates Broad Anti-Tumor Efficacy in KRAS G12C Mutant Cell Lines and Patient Derived Xenografts with Evidence of Adaptation to Treatment Observed in a Subset of Models

Fig. 4A

Fig. 4B

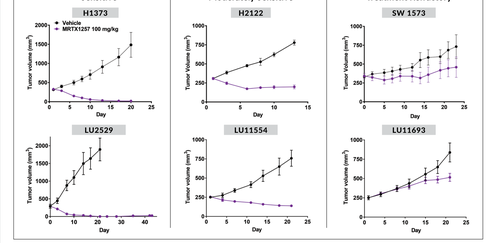


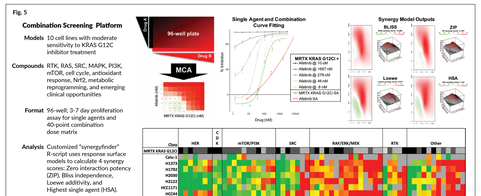
Figure 4 A-B

A. Female nu/nu mice were implanted with tumor cells subcutaneously and animals were treated with MRTX1257 @ 50 or 100mg/kg PO, daily for at least 20 days in most models. % change from baseline is calculated as tumor growth inhibition on a given day as compared to vehicle control.

B. Xenograft models implanted and treated as described in A. Data reported as tumor mean + SEM, n=5.

In Vitro Combination Screen to Interrogate Bypass Pathways and Identify Combinatorial Treatment Strategies

Fig. 5



KRAS G12Ci Treatment Alters RAS, MYC, Cell Cycle and Apoptosis Pathway Gene Expression In Vivo

Fig. 6A

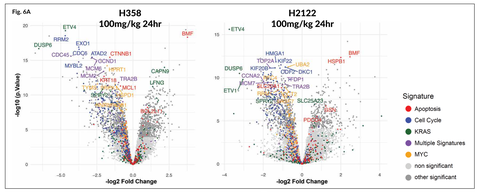


Fig. 6B

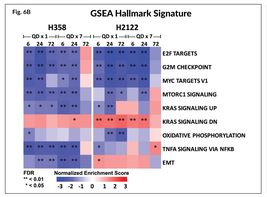


Fig. 6C

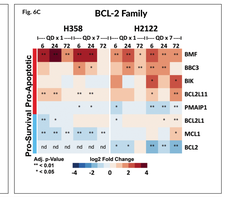
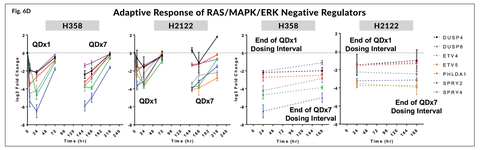


Fig. 6D



MRTX KRAS G12Ci + Afatinib Exhibits Strong Combination Activity in Models with High HER Family Expression

Fig. 7

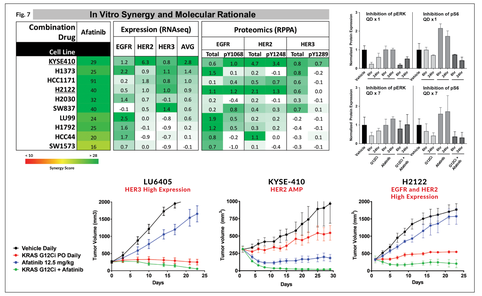


Figure 7

Summary of in vitro combination screen results for MRTX KRAS G12C inhibitor + pan-HER inhibitor afatinib (Synergy Score: Green - strong synergy; Yellow to orange - moderate; Red- none). HER family mRNA (RNAseq), protein, and phospho protein expression (RPPA) from CCLE were analyzed and sorted by average mRNA expression. HER Family RNA expression (RNAseq) across a panel of KRAS G12C-mutant lung PDX models was evaluated (HUBase; Crown BioSciences). The LU6405 model exhibits high HER3 expression.

Mice with H2122 tumors were treated with G12Ci @ 100mg/kg, Afatinib @ 12.5mg/ kg, or in combination for single dose or QD x 7 days. Tumors were harvested at 6 and 24hrs post last dose (n=3/treatment group) and lysates were generated to assess biomarkers by immunoblot. Bands were quantified by densitometry analysis and normalized to total ERK and S6 respectively (% of vehicle control).

Palbociclib Significantly Enhances Antitumor Activity of MRTX KRAS G12Ci – Particularly in Tumors Exhibiting CDKN2A Deletion

Fig. 8

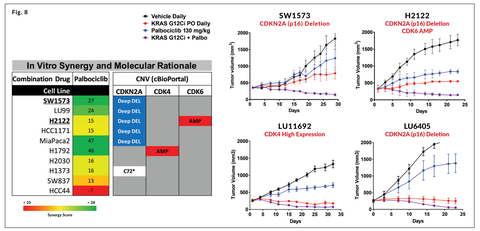


Fig. 8

Summary of in vitro combination screen results for MRTX KRAS G12C inhibitor + CDK4/6 inhibitor palbociclib (Synergy Score: Green – strong synergy; Yellow to orange – moderate; Red- none). CDKN2A (p16), CDK4 and CDK6 copy number alteration CCLE data were analyzed using cBioPortal.

KRAS G12C PDX models were evaluated for copy number alterations and mRNA expression (RNAseq) using HuBase (Crown Biosciences). The LU6405 model exhibits a deep deletion in CDKN2A (p16) and the LU11692 model exhibits high CDK4 expression.

MRTX KRAS G12Ci + Vistusertib Combination Active in STK11^{MUT} Models

Fig. 9

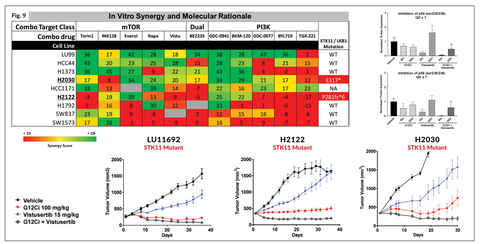


Fig. 9

Summary of in vitro combination screen results for MRTX KRAS G12C inhibitor + mTOR or PI3K inhibitors (Synergy Score: Green – strong synergy; Yellow to orange – moderate; Red- none). STK11 (LKB1) mutation status was determined using CCLE and HuBase (Crown Biosciences).

Mice with H2030 tumors were treated with G12Ci @ 100mg/kg, Vistusertib @ 15mg/kg, or in combination for single dose or QD x 7 days. Tumors were harvested at 6 and 24hrs post last dose (n=3/treatment group) and lysates were generated to assess biomarkers by immunoblot. Bands were quantified by densitometry analysis and normalized to total S6 (% of vehicle control).

CONCLUSIONS

- MRTX1257 potently and selectively inhibits KRAS-dependent signal transduction and viability of KRAS G12C-mutant cell lines grown under 2D and 3D conditions.
- MRTX1257 administered daily PO at well tolerated dose levels induced 30% or greater tumor regression in 18 of 23 cell lines and patient-derived xenograft models with durable complete regression observed in a subset of models (e.g., MIA Paca-2).
- A subset of in vivo models exhibited sub-maximal responses with MRTX1257 treatment, possibly due to relief of negative feedback or additional co-mutations.
- Feedback signaling through HER family RTKs, uncoupling of KRAS from cell cycle entry, and mTOR-mediated bypass signaling were identified as potential mechanisms of decreased response to single agent KRAS G12Ci treatment and are each addressable through clinically actionable combination strategies.

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WE WOULD LIKE TO THANK:

- Monoceros Biosystems, LLC for their work on RNA sequencing tools and genomics data analysis
- Crown Biosciences for their support with in vivo PDX models
- Flagship Biosciences for their contributions to immunohistochemistry images and analysis