LB-271

Insight Towards Therapeutic Susceptibility of KRAS Mutant Cancers From MRTX1257, a Novel KRAS G12C Mutant-Selective Small Molecule Inhibitor

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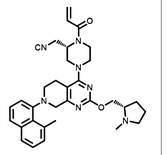
BACKGROUND

- 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 PK properties.
- 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 and demonstrated selective KRAS-dependent antitumor activity 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

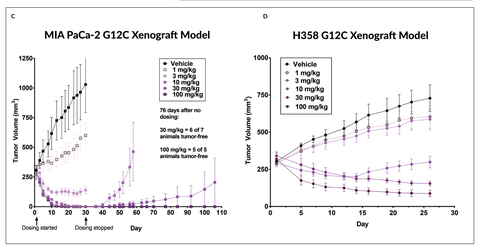
Fig. 1: MRTX1257 is a Potent and Selective KRAS G12C Inhibitor and Research Tool Molecule that Demonstrates Robust Anti-Tumor Activity



MRTX1257

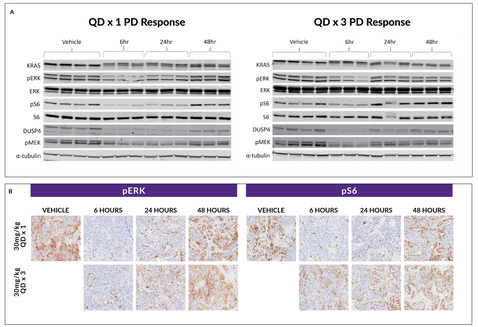
- KRAS G12C H358 pERK IC_{50} 3hr = 0.9nM
- Mouse PPB = 99%
- FF adj. IC_{50} = 0.035ug/mL
- Mouse F = 31.1%
- Mouse AUC_{inf} (hr*ug/mL) = 4.91
- Mouse C_{max} (ug/mL) = 1.66

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	19.1	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



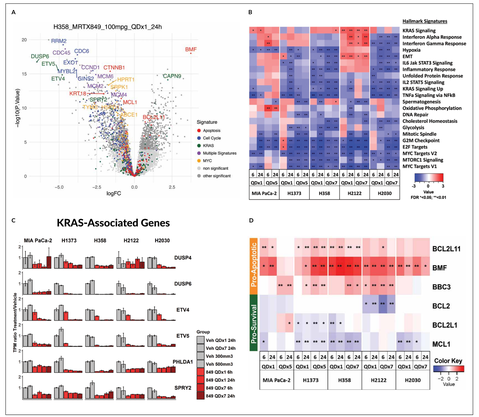
B. A Cell Titer Glo assay was used to evaluate cell viability on cells grown in 2D (72hrs) or 3D (12 days) conditions. **C.** MIA PaCa-2 xenograft-bearing female nu/nu mice were dosed orally with MRTX1257. MRTX1257 dosed at 100mg/kg daily for 28 days leads to complete, durable responses that are maintained >120 days after cessation of treatment. Mean tumor volume + SEM, n=7 **D.** H358 xenograft-bearing, female nu/nu mice were dosed orally with MRTX1257. Mean tumor volume + SEM, n=5.

Fig. 2: MRTX1257 Inhibits pERK and pS6 in H358 Tumors



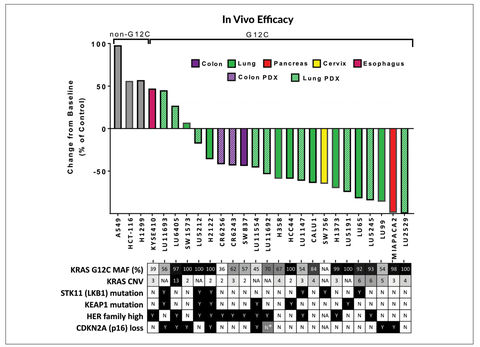
A. H358 xenograft-bearing mice were treated with MRTX1257 @ 30mg/kg for a single dose or QD x 3 days. Tumors were harvested at 6, 24, and 48hrs post last dose (n=3) and biomarkers were assessed by immunoblot. **B.** The same tumors from panel A were analyzed by immunohistochemistry.

Fig. 3: KRAS G12Ci Treatment Alters RAS, MYC, Cell Cycle and Apoptosis Pathway Gene Expression, and Shows Evidence for Relief of Feedback Inhibition with Repeat Dosing



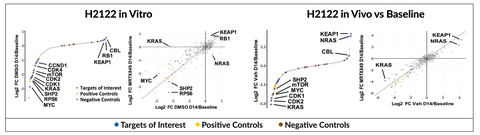
A-D. Female nu/nu mice bearing H358, H2122, H2030, H1373 and MIA PaCa-2 xenografts were treated with KRAS G12Ci @ 100mg/kg as a single dose or QD x 5-7 days. Tumors were harvested at 6 and 24hrs post last dose and RNA-sequencing was performed. Differential expression analysis was performed with limma-trend (version 3.36.2; Ritchie et al. 2015) in R (version 3.5.1). Gene Set Enrichment Analysis tool (GSEA; gsea2-2.2.4) and Molecular Signature Database (MSigDB; msigdb_v6.0) were used for gene enrichment analysis (Subramanian et al. 2005). * = FDR < 0.05; ** = FDR < 0.01.

Fig. 4: MRTX1257 Demonstrates Broad Efficacy in KRAS G12C Mutant Cell Line and Patient Derived Xenografts



Female nu/nu mice were implanted with tumor cells subcutaneously to generate either cell line derived or patient derived xenograft models. 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, n=5.

Fig. 5: Vulnerabilities and Modulators of Response to G12Ci Identified by CRISPR/Cas9 Screen



A custom sgRNA library targeting ~400 genes (8-10 sgRNA/gene) was generated (Cellecta; Mountain View, CA) and used to transduce Cas9 expressing H2122 cells. Following puromycin selection, baseline samples were harvested and cells were plated for treatment with DMSO or an IC75-IC90 concentration of G12Ci for 2 weeks. In parallel, cells were implanted into nu/nu mice. When tumors reached ~300 mm^3, mice were treated with Vehicle or KRAS G12Ci @ 100 mg/kg, QDx14 (n=5). DNA extraction, PCR of sgRNAs and next generation sequencing was performed and average log2 fold changes relative to baseline were calculated for each gene.

Fig. 6: In Vitro Screen to Identify Synergistic and Clinically Translatable Combinations

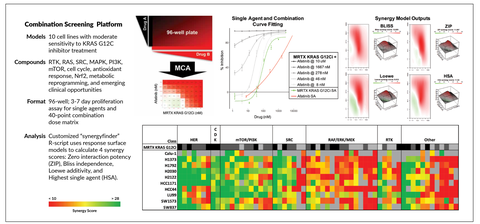
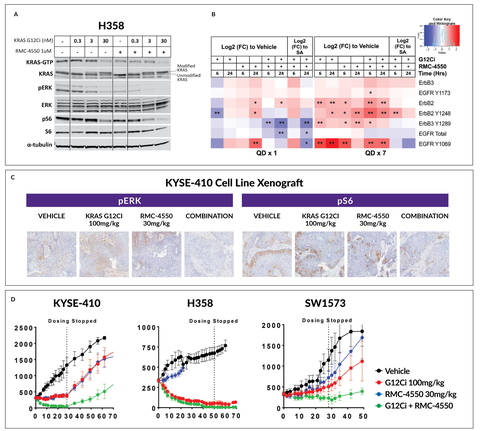


Fig. 7: Combination of KRAS G12Ci + SHP2 Inhibitors Lead to Increased Modulation of Pathway Biomarkers and Exhibits Deeper Anti-Tumor Responses



A. H358 cells were treated with G12Ci @ 0.3, 3, and 30nM +/-RMC-4550 @ 1uM for 24hrs. Lysates were generated to assess biomarkers by immunoblot. **B.** KYSE-410 xenograft bearing mice were treated with G12Ci @ 100mg/kg +/-RMC-4550 @ 30mg/kg for a single dose or daily for 7 days. Tumors harvested at 6 and 24hrs post last dose and processed for Reverse-Phase Proteomic Analysis. **C.** Fragments from the same tumors from Panel B were also processed for immunohistochemistry analysis

Fig. 8: Palbociclib Significantly Enhances Anti-Tumor Activity of MRTX KRAS G12Ci – Particularly in Tumors Exhibiting CDKN2A Deletion

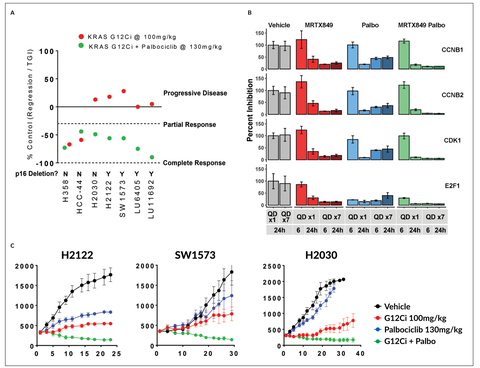
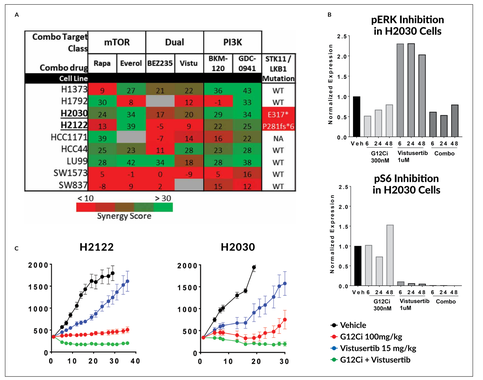


Fig. 9: MRTX KRAS G12Ci + Vistusertib Combination Active in STK11/KRAS ^{G12C} MUT Models



CONCLUSIONS

- MRTX1257 is a research tool molecule that potently and selectively inhibits KRAS-dependent signal transduction in vitro and in vivo.
- MRTX1257 administered daily PO at well tolerated dose levels induced 30% or greater tumor regression in 18 of 23 cell line and patient-derived xenograft models with durable complete regression observed in some models (e.g., MIA Paca-2).
- Comprehensive pharmacodynamic and pharmacogenomic profiling identified potential mechanisms responsible for the incomplete responses seen in a subset of models.
- Feedback signaling through HER family RTKs, uncoupling of KRAS from cell cycle entry, and mTOR-mediated bypass signaling were identified as top mechanisms for decreased response to single agent KRAS G12Ci treatment.
- Combinations addressing these resistance mechanisms augmented the activity of KRAS G12C inhibition in multiple models.
- With MRTX849 demonstrated comparable selective activity in KRAS ^{G12C} mut disease models and is presently under evaluation in clinical trials.

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- *"Structure-Based Drug Discovery of MRTX1257, a Selective, Covalent KRAS G12C Inhibitor with Oral Activity in Animal Models of Cancer"*, Marx et al.
- *"Insight Towards Therapeutic Susceptability of KRAS Mutant Cancers from MRTX1257: A Prototype Selective Inhibitor of KRAS G12C"*, Hallin et al.

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