Chemical Genomics Identify Novel Druggable Nodes and Resistance Pathways in the Presence of Concomitant SOS1 and KRAS Inhibition

Abstract LB193

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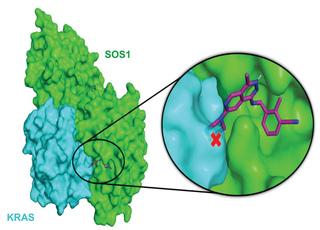
BACKGROUND

- **MRTX849 (adagrasib)** is an irreversible covalent KRAS^{G12C} inhibitor presently under evaluation in clinical trials in cancers with confirmed KRAS^{G12C} mutations, including NSCLC, CRC and PDAC.[1,2]
- **MRTX0902** is a potent and selective SOS1 inhibitor that disrupts the KRAS::SOS1 protein-protein interaction, thereby preventing SOS1-mediated nucleotide exchange on KRAS, and ultimately resulting in the accumulation of inactive GDP-bound KRAS^{G12C}.
- **We have found that MRTX0902 enhances the anti-tumor activity of MRTX849 in KRAS^{G12C} mutant models both *in vitro* and *in vivo***. Although the MRTX0902 + MRTX849 combination leads to deep pharmacological inhibition of RAS/MAPK signaling, **intrinsic or adaptive resistance mechanisms are anticipated**.
- We conducted drug-anchored CRISPR screens in two KRAS^{G12C} models and uncovered both resistance-associated and co-dependency genes that inform putative targets for triple combination therapies. Resistance with the dual combination treatment was associated with tumor suppressor genes PTEN, KEAP1, TSC1/2 and NF1. Newly discovered genetic co-dependencies included the SOS1 homolog SOS2, PRMT5, PIK3CA, and mTOR.
- To validate the newly discovered co-dependency hits, we conducted *in vitro* triple combination viability screens and observed drug synergy between MRT0902, MRTX849 and the mTOR inhibitor vistusertib.
- These data suggest that co-targeting KRAS^{G12C} and SOS1 with additional anti-cancer therapies may be a useful strategy to overcome potential mechanisms of resistance associated with robust RAS/MAPK pathway inhibition.

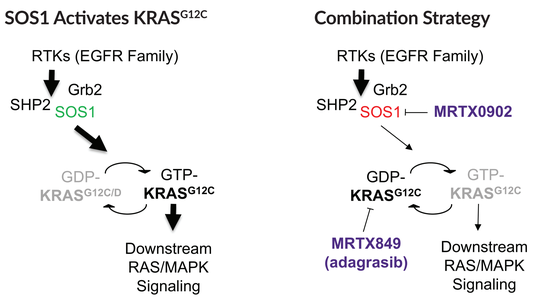
Fig. 1: MRTX0902 Disrupts the KRAS::SOS1 Dimer



MRTX0902 partially overlaps the KRAS binding interface of SOS1, thus occluding KRAS binding

Please see New Drugs on the Horizon presentation "MRTX0902: A SOS1 inhibitor for therapeutic intervention of KRAS-driven cancers" (Abstract #7687) for additional information.

Fig. 2: SOS1 Inhibition Shifts KRAS^{G12C} Into an Inactive State and Augments MRTX849 (adagrasib) Activity



RESULTS

MRTX0902 with MRTX849 Demonstrates a More Durable Antitumor Effect

Fig. 3

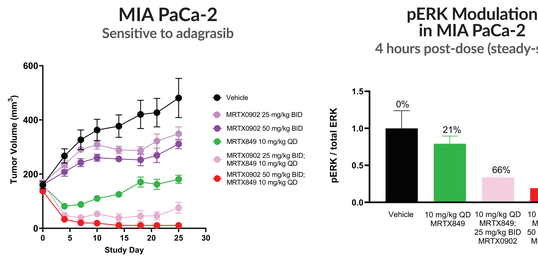


Fig. 4

Fig. 5

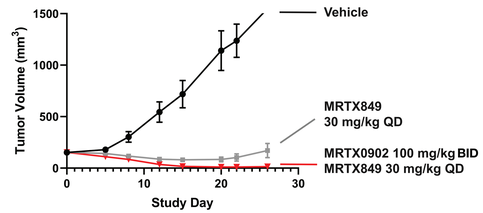
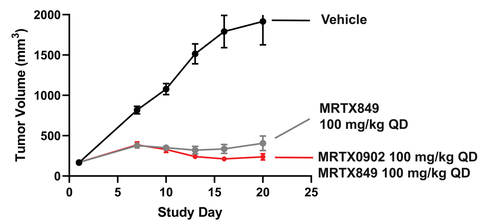


Fig. 6



Mice bearing cell line derived xenografts (MIA PaCa-2, LU99, or H2122) were treated with Vehicle BID/QD PO, 25/50 mg/kg BID or 100 mg/kg BID/QD PO MRTX0902, MRTX849 at 10, 30, or 100 mg/kg QD PO, or a combination of MRTX0902 and MRTX849 at the same doses for the duration of the study. Data shown as average tumor volume +/- SEM, n=5/group. Tumors (n=3) were harvested at 4 hours post final treatment and subsequently lysed and analyzed by Western Blot for pERK and total ERK proteins.

Combination of MRTX0902 + MRTX849 results in:

- -92% regression, tumor-free animals, and correlative PD (pERK modulation) in the MIA PaCa-2 CDX model
- Substantial efficacy in additional KRAS^{G12C} CDX models LU99 and H2122

Drug-Anchored CRISPR Screen Reveals Resistance Biomarkers and Combination Targets for Dual MRTX0902 and MRTX849 Treatment

Fig. 7A

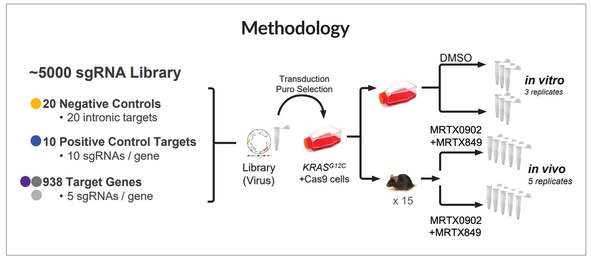


Fig. 7B MIA PaCa-2 *In Vitro* / MIA PaCa-2 *In Vivo*

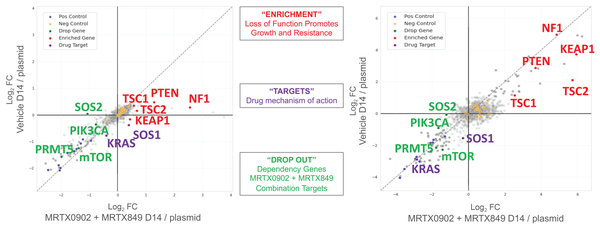
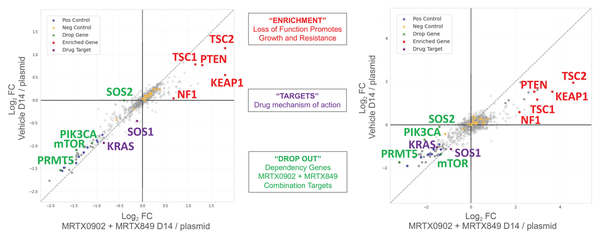


Fig. 7C LU99 *In Vitro* / LU99 *In Vivo*



Overall methodology for the MRTX0902/MRTX849 drug-anchored CRISPR screen using a 5000 sgRNA library (5-10 sgRNAs/gene) is shown in **Figure 7A**.

Contrast plots display differentially expressed genes (sgRNA **enrichment**, **target**, or **dropout**) following 14 days of treatment with either vehicle or the MRTX0902 + MRTX849 combination in the MIA PaCa-2 or LU99 cell line (**Figures 7B and 7C**, respectively).

Log_2 fold change (FC) in Vehicle-treated samples/Plasmid vs. log_2 fold change (FC) in MRTX0902/MRTX849-treated samples/Plasmid are displayed for *in vitro* (**left**) and *in vivo* (**right**) studies.

Triple Combination with MRTX0902, MRTX849, and mTOR Inhibitor Vistusertib Leads to Synergistic Effects *In Vitro*

Fig. 8 Combination Targets and Potential Resistance Biomarkers

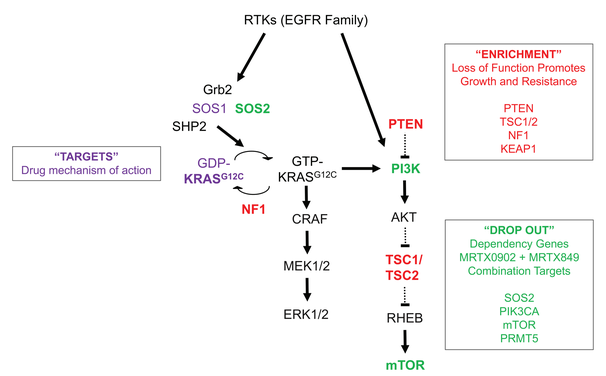


Table 1

Cell Line	3 Day Viability (CTG) Assay GI_{50} (nM)			Triple Combination Synergy Scores			
	MRTX0902	MRTX849	Vistusertib	ZIP Score	Bliss Score	HSA Score	Combined
LU99	>3000	588	142	14.0	12.4	12.6	**39.0**

Inhibitory effects on LU99 (KRAS^{G12C}) 2D cell proliferation following 72-hour drug treatment was assessed via the Cell-Titer Glo assay. The GI_{50}, or concentration of drug that reduced total cell growth by 50%, is tabulated for MRTX0902, MRTX849, and vistusertib. A custom R-script was used to generate a composite synergy score (Mirati Combination Analysis or MCA) for combination treatment of all three drugs tested at a 5-point or 6-point dose-response curve.

CONCLUSIONS

- MRTX0902 is a potent and selective SOS1 inhibitor with high oral bioavailability across multiple preclinical species.
- MRTX0902 treatment in combination with MRTX849 (adagrasib) can more fully inhibit mutant KRAS/MAPK signaling, which leads to enhanced *in vitro* efficacy and deeper and more durable regressions *in vivo*.
- Drug-anchored CRISPR screens reveal both resistance and co-dependency mechanisms associated with the MRTX0902 + MRTX849 combination. Additional triple combination strategies with inhibitors of the PIK3CA/mTOR signaling pathway and PRMT5 are currently being characterized.
- Our studies uncover the potential utility of additional drug partners for the MRTX0902 and adagrasib combination and aide in the understanding of SOS and RAS biology in targeted cancer therapy.

ACKNOWLEDGEMENTS

- The Drug Discovery and Research teams at Mirati Therapeutics, Inc.

1. Hallin J, et al. *Cancer Discovery* 10(1): **54-71** (2020) doi: 10.1158/2159-8290.
2. Ou SI, et al. *J Clin Oncol.* (2022) doi: 10.1200/JCO.21.02752