SUPPLEMENTARY MATERIALS

Research Brief

Clinical acquired resistance to KRAS^{G12C} inhibition through a novel KRAS switch-II pocket mutation and polyclonal alterations converging on RAS-MAPK reactivation

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Running Title: Clinical acquired resistance to KRAS^{G12C} inhibition

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Sample	Target	AF% (pos/total events)
Tumor tissue before MRTX849	KRAS G12C	**31.4** (463/1473)
	KRAS G12V	0 (0/1157)
	KRAS G13D	0 (0/1107)
	KRAS Y96D	0 (0/1439)
	MAP2K1 K57N	0 (0/959)
	MAP2K1 E102_I103del	0 (0/830)

Sample	Target	AF% (pos/total events)
0 days post-MRTX849 discontinuation	KRAS G12C	**31.3** (600/1920)
	KRAS G12V	0 (0/2254)
	KRAS G13D	0 (0/456)
	KRAS Y96D	**0.19** (1/507)
	MAP2K1 K57N	**0.05** (1/1820)
	MAP2K1 E102_I103del	0 (0/378)

Sample	Target	AF% (pos/total events)
9 days post-MRTX849 discontinuation	KRAS G12C	**47.8** (1694/3543)
	KRAS G12V	0 (0/3690)
	KRAS G13D	**0.13** (1/757)
	KRAS Y96D	**0.14** (2/1350)
	MAP2K1 K57N	0 (0/2729)
	MAP2K1 E102_I103del	**0.12** (1/789)

Sample	Target	AF% (pos/total events)
51 days post-MRTX849 discontinuation	KRAS G12C	**21** (443/2109)
	KRAS G12V	**0.09** (1/1083)
	KRAS G13D	**0.1** (1/987)
	KRAS Y96D	0 (0/948)
	MAP2K1 K57N	**0.13** (1/744)
	MAP2K1 E102_I103del	**0.5** (2/372)

AF, allele fraction; pos, positive.

Supplementary Table S1. The variant allele fractions (AF; %) of KRAS G12C, G12V, G13D, and Y96D mutations and MAP2K1 K57N and E102_I103del mutations as detected using digital droplet PCR in pre-MRTX849 tumor tissue and post-MRTX849 plasma. Pos = positive.

	Tumor		cfDNA		
	pre-MRTX849		Days post-MRTX849 discontinuation		
			0	9	51
KRAS copy #	2	NA	2.71	3.67	2.3

#, number; NA = not assessable due to low tumor fraction.

Supplementary Table S2. Copy number of KRAS in the analyzed tumor and cell-free DNA (cfDNA) specimens pre- and post-MRTX849.

Database	Mutations (% of total)			Total Samples tested
	KRAS	KRAS G12C	KRAS Y96D	
COSMIC v92	47,339 (17.9)	5,426 (2)	0	264,108
AACR Project GENIE (cBioPortal) v.3.6.6(1,2)	29,046 (16)	4,249 (2.3)	0	186,433

Supplementary Table S3. The frequencies of all *KRAS* mutations, $KRAS^{G12C}$, and $KRAS^{Y96D}$ according to COSMIC v92 and AACR Project GENIE (cBioPortal) databases.

A

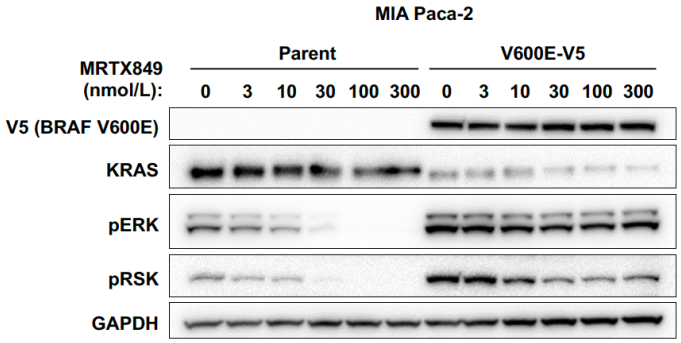
Cell line	IC_{50} (mol/L)					
	MRTX849		AMG 510		ARS-1620	
	G12C	G12C/Y96D	G12C	G12C/Y96D	G12C	G12C/Y96D
H358	2.2×10^{-8}	$>2.0 \times 10^{-6}$	6.1×10^{-9}	$>2.0 \times 10^{-6}$	2.6×10^{-7}	3.8×10^{-6}
MIA PaCa-2	2.1×10^{-8}	$>2.0 \times 10^{-6}$	6.9×10^{-9}	$>2.0 \times 10^{-6}$	1.4×10^{-7}	9.4×10^{-6}
BaF3	1.4×10^{-9}	$>2.0 \times 10^{-6}$	8.6×10^{-9}	$>2.0 \times 10^{-6}$	2.1×10^{-7}	3.0×10^{-6}

B

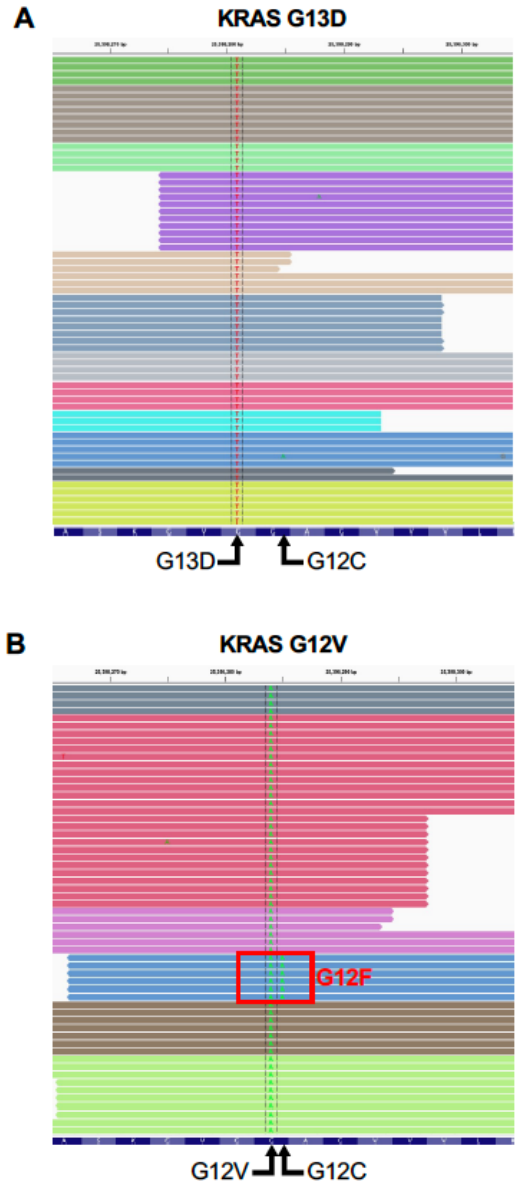
Cell line	IC_{50} (mol/L)	
	RM-018	
	G12C	G12C/Y96D
H358	3.5×10^{-9}	7.3×10^{-9}
MIA PaCa-2	1.4×10^{-9}	3.4×10^{-9}
Ba/F3	1.4×10^{-9}	2.8×10^{-9}

Supplementary Table S4. The IC_{50} values of KRAS inhibitors in cell lines expressing $KRAS^{G12C}$ or $KRAS^{G12C/Y96D}$. A, The IC_{50} values of MRTX849, AMG 510, ARS-1620 (A) and RM-018 (B) were shown as the average of at least two biologic experiments; each biologic experiment was run in triplicate.

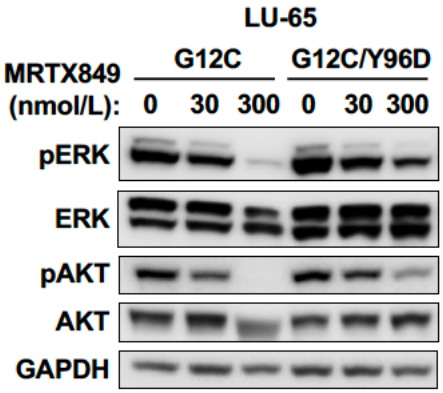
SUPPLEMENTARY FIGURES



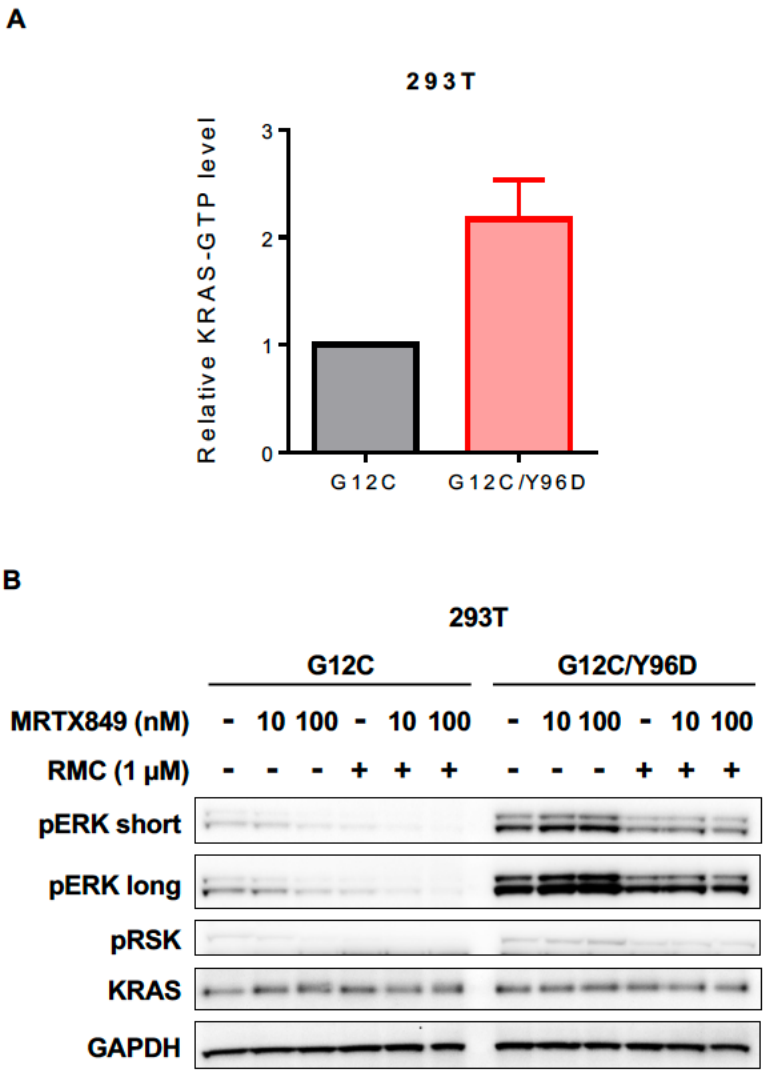
Supplementary Figure S1. BRAF^{V600E} maintains MAPK signaling downstream of KRAS^{G12C} in the presence of MRTX849. MIA PaCa-2 cells stably expressing BRAF (V600E)-V5 were generated with pMXs-Puro-BRAF (V600E)-V5 plasmid as described in the Methods. MIA PaCa-2 parental cells and those stably expressing BRAF (V600E)-V5 were treated with MRTX849 at the indicated concentrations for 4 hours and subjected to Western blot analysis.



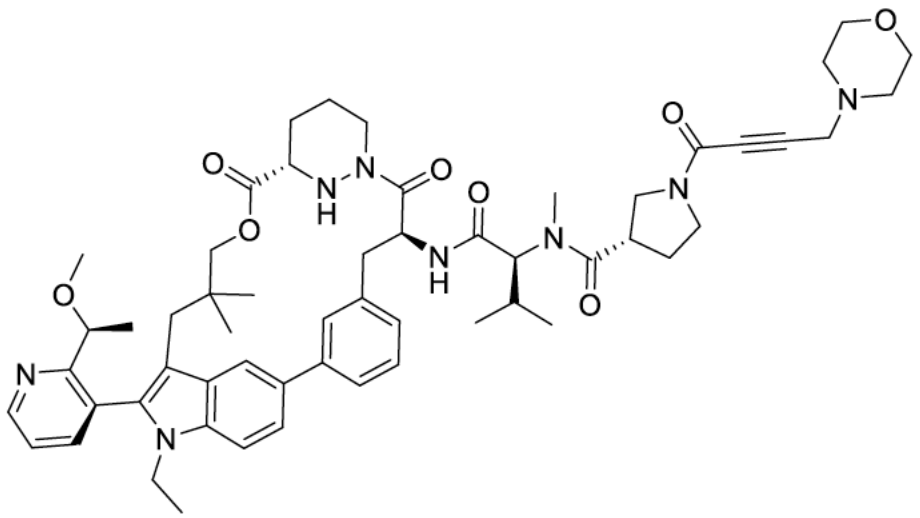
Supplementary Figure S2. Phasing of *KRAS* G12 and G13 mutations based on sequencing reads from the post-treatment cell free DNA (cfDNA). The sequencing read pile-up demonstrates $KRAS^{G13D}$ (A) and $KRAS^{G12V}$ (B) mutations occurring *in trans* to $KRAS^{G12C}$. Of note, in a single family of reads from the same original template molecule, nucleotide changes corresponding to $KRAS^{G12C}$ and $KRAS^{G12V}$ occurred *in cis*, encoding for $KRAS^{G12F}$ (B).



Supplementary Figure S3. Effect of MRTX849 in LU-65 KRAS^{G12C} NSCLC cells. Western blot of LU-65 cells transiently expressing KRAS^{G12C} or KRAS$^{G12C/Y96D}$ after treatment with MRTX849 for 4 hours.



Supplementary Figure S4. KRAS G12C/Y96D displays greater basal activity that is targetable by upstream inhibition of SHP2. **A,** Densitometry analysis of KRAS-GTP levels of untreated HEK293T stably expressing $KRAS^{G12C}$ and $KRAS^{G12C/Y96D}$ from Figure 3, (n=2). **B,** Western blot analysis of HEK293T stably expressing KRAS mutants treated with indicated inhibitors for 4h. RMC = RMC-4450 (SHP2 inhibitor).



Supplementary Figure S5. Structure of RM-018

SUPPLEMENTARY METHODS

Detailed patient history

A 67-year-old female former light smoker was diagnosed with stage IV lung adenocarcinoma. Molecular testing of her primary lung tumor at initial diagnosis ('pre-MRTX849 tissue', 23.9 months prior to initiating MRTX849) revealed low-level PD-L1 expression (tumor proportion score of 20%, E1L3N antibody) and *KRAS* G12C mutation, concomitant with *STK11* splice region variant (c.734+5G>C), *TP53* insertion/deletion (F338fs), *RB1* splice region variant (c.1695+5_1695+15del), and *FBXW7* loss. She was treated with first-line carboplatin, pemetrexed, pembrolizumab followed by maintenance pemetrexed and pembrolizumab for a total of approximately 15 months with stereotactic radiosurgery (SRS) to brain metastases, and then received a second-line investigational agent (an antibody drug conjugate) for 8.5 months before discontinuing for extracranial disease progression. The pre-MRTX849 cfDNA was collected prior to starting on the second-line investigational agent (18.2 months prior to initiating MRTX849).

The patient then enrolled in a dose expansion cohort of the phase 1 trial of adagrasib (MRTX849; KRYSTAL-1). She was treated with 600 mg twice daily dosing. The first restaging computed tomography (CT) after 6 weeks of treatment demonstrated a 32% reduction in tumor burden (per RECIST v1.1). Repeat imaging after 4 months of treatment showed progressive disease with increased right upper lobe lung mass, nodal metastases (axillary, anterior diaphragmatic, mediastinal, and internal mammary), and subcentimeter brain metastasis. She underwent biopsy of resistant plasma and SRS to the progressing brain lesion and continued to receive MRTX849 for clinical benefit. Six weeks later, CT scans confirmed further extracranial disease progression. MRTX849 was therefore discontinued. Serial plasma samples were collected at the time of MRTX849 discontinuation, 9 days post-discontinuation, and 51 days post-discontinuation (of note, the patient received 13 days of an investigational SHP2 inhibitor between the 9-day and 51-day timepoints).