Clinical Acquired Resistance to KRAS<sub>G12C</sub> Inhibition through a Novel KRAS Switch-II Pocket Mutation and Polyclonal Alterations Converging on RAS–MAPK Reactivation

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ABSTRACT

Mutant-selective KRAS<sup>G12C</sup> inhibitors, such as MRTX849 (adagrasib) and AMG 510 (sotorasib), have demonstrated efficacy in KRAS<sup>G12C</sup>-mutant cancers, including non–small cell lung cancer (NSCLC). However, mechanisms underlying clinical acquired resistance to KRAS<sup>G12C</sup> inhibitors remain undetermined. To begin to define the mechanistic spectrum of acquired resistance, we describe a patient with KRAS<sup>G12C</sup> NSCLC who developed polyclonal acquired resistance to MRTX849 with the emergence of 10 heterogeneous resistance alterations in serial cell-free DNA spanning four genes (KRAS, NRAS, BRAF, MAP2K1), all of which converge to reactivate RAS–MAPK signaling. Notably, a novel KRAS<sup>Y96D</sup> mutation affecting the switch-II pocket, to which MRTX849 and other inactive-state inhibitors bind, was identified that interferes with key protein–drug interactions and confers resistance to these inhibitors in engineered and patient-derived KRAS<sup>G12C</sup> cancer models. Interestingly, a novel, functionally distinct tricomplex KRAS<sup>G12C</sup> active-state inhibitor RM-018 retained the ability to bind and inhibit KRAS<sup>G12C/Y96D</sup> and could overcome resistance.

SIGNIFICANCE: In one of the first reports of clinical acquired resistance to KRAS<sup>G12C</sup> inhibitors, our data suggest polyclonal RAS–MAPK reactivation as a central resistance mechanism. We also identify a novel KRAS switch-II pocket mutation that impairs binding and drives resistance to inactive-state inhibitors but is surmountable by a functionally distinct KRAS<sup>G12C</sup> inhibitor.

INTRODUCTION

The development of compounds that bind covalently to cysteine 12 in KRAS<sup>G12C</sup> cancers has ushered in a new era in efforts to target KRAS directly. Biochemically, these agents lock KRAS in its inactive GDP-bound conformation, thereby inhibiting downstream signaling, leading to preclinical antitumor responses (1–3). The lead clinical compounds sotorasib (AMG 510) and adagrasib (MRTX849) have advanced rapidly and demonstrated tolerability and single-agent activity across KRAS<sup>G12C</sup>-mutant cancers (4, 5). In patients with advanced non–small cell lung cancer (NSCLC) harboring KRAS<sup>G12C</sup> (which comprises approximately 13% of all lung adenocarcinomas), AMG 510 and MRTX849 have...
demonstrated meaningful efficacy with objective response rates of 37% and 45%, as well as disease control rates of 81% and 96%, respectively (6, 7). AMG 510 has recently received Breakthrough Therapy designation from the FDA for the treatment of patients with advanced KRASG12C-mutant NSCLC following at least one prior systemic therapy. Multiple ongoing trials seek to augment responses to KRASG12C inhibitors through combination strategies.

Preclinical studies with MRTX849 and other KRASG12C inhibitors have suggested several mechanisms of up-front resistance, including reactivation of ERK-dependent signaling to bypass KRASG12C blockade (4). Prior work by our group and others has identified adaptive RAS pathway feedback reactivation as a key mechanism of primary resistance to KRASG12C inhibition (8–13). However, the key mechanisms of clinical acquired resistance to KRASG12C inhibitors are currently unknown. Here, as an initial effort to characterize the clinical landscape of potential acquired resistance mechanisms to KRASG12C inhibitors, we present a patient study to reactivate RAS–MAPK signaling, implicating this as a mechanism of resistance. Notably, all putative resistance mutations identified are predicted to converge on reactivation of KRASG12C/Y96D-mediated resistance.

RESULTS
Heterogeneous Acquired Resistance Alterations Converge on RAS–MAPK Reactivation

A 67-year-old woman with metastatic KRASG12C-mutant NSCLC was treated on the dose-expansion cohort of the phase 1 study of MRTX849 (NCT03785249; Methods; further detailed in Supplementary Methods). Initial scans showed a 32% reduction in tumor size (by RECIST v1.1), but after approximately 4 months of treatment, the patient developed progressive disease, and the patient discontinued therapy at 5.5 months (Fig. 1A). To identify putative mechanisms of acquired resistance to MRTX849 in this patient, we assessed cfDNA using a targeted next-generation sequencing assay (Guardian360; Guardant Health) and droplet digital PCR (ddPCR). Upon development of acquired resistance, the original KRASG12C and TP53E102-I103del variants present in pretreatment cfDNA were again detected in cfDNA but were accompanied by the emergence of 10 distinct mutations affecting RAS–MAPK components KRAS, NRAS, BRAF, and MAP2K1 (which encodes the MEK1 protein) identified across cfDNA specimens obtained after disease progression (Fig. 1B; Supplementary Table S1). The lower allele frequencies of these alterations relative to the truncal KRASG12C and TP53 mutations are consistent with the emergence of these mutations in heterogeneous subclonal populations. These included three activating NRAS mutations (NRASQ61L, NRASG61K, NRASQ61R), which can drive active RAS signaling in a KRAS-independent manner, and BRAFV600E, which can maintain MAPK signaling downstream of KRASG12C in the presence of MRTX849 (Supplementary Fig. S1). Three MAP2K1 mutations (MAP2K1K57N, MAP2K1G58D, MAP2K1E102-I103del) previously demonstrated to be activating and known to be involved in resistance to upstream MAPK pathway inhibitors (i.e., BRAF inhibitors) were also identified (14, 15).

In addition, three KRAS mutations emerged in the postprogression cfDNA. Two of these mutations are the known activating mutations KRASG13D and KRASG12V, and mutant-selective KRASG12C inhibitors have previously been shown to be ineffective against these mutations (4, 16). A deeper analysis of individual sequencing reads from cfDNA suggested that these mutations seemed to occur in trans to the original KRASG12C mutation (Supplementary Fig. S2A and S2B), likely arising in the remaining wild-type copy of KRAS, which appeared to be retained based on pretreatment tumor sequencing (Supplementary Table S2). However, it is not possible from the cfDNA data to confirm that these mutations coexist in cells that also harbor the original KRASG12C mutation. Notably, a single, well-supported family of sequencing reads from the same original template molecule showed the concurrent presence of both nucleotide changes corresponding to KRASG12C and KRASG12V in cis on the same strand, which would encode for a KRASG12C mutation. While it is not possible to confirm the presence of this mutation based on a single read family, this finding raises the possibility that cis mutations resulting in “loss” of the original KRASG12C mutation and conversion to a different KRAS mutation might be another potential mechanism of resistance. Notably, all putative resistance mutations identified are predicted to converge on reactivation of RAS–MAPK pathway signaling, suggesting that this may represent a common primary mechanism of acquired resistance to KRASG12C inhibitors (Fig. 1C).

Interestingly, the third KRAS mutation identified, KRASV96D, represents a novel mutation that is not known to be activating. Notably, although KRAS is the most commonly mutated oncogene in human cancer, a search of two large tumor mutational databases—COSMIC and GENIE, which collectively contain >450,000 molecularly characterized cancers (17, 18)—did not reveal a single previously identified mutation at the KRASV96 locus among >75,000 cases with documented KRAS mutations (Supplementary Table S3). However, the Y96 residue is associated with the switch-II pocket to which MRTX849 and other inactive-state KRASG12C inhibitors bind, suggesting that the previously undescribed Y96D mutation may have a novel and specific role in driving resistance to KRASG12C inhibitors.

Structural Modeling of KRASG12C/Y96D

To understand the significance of the acquired KRASV96D mutation, we performed structural modeling of the G12C-mutant and G12C/Y96D double-mutant KRAS proteins bound to the KRASG12C inhibitors MRTX849, AMG 510, and ARS-1620 (Fig. 2). These three inhibitors bind the GDP state of KRASG12C and exploit a cryptic pocket formed by the central β sheet of RAS and switch-II (first identified by Ostrem and
Clinical Acquired Resistance to KRAS\(^{G12C}\) Inhibition

**Figure 1.** Acquired resistance to KRAS\(^{G12C}\) inhibitor MRTX849 (adagrasib). A, Computed tomography images of the patient’s axillary lymph node metastasis at baseline, during response to MRTX849, and at progression on MRTX849. B, Variant allele fractions of mutations detected in the patient’s serial plasma samples. †, indicates the mutations were detected by ddPCR but not by plasma next-generation sequencing. C, Alterations detected in post-MRTX849 cfDNA include acquired mutations in KRAS as well as multiple components of the MAPK signaling cascade. *, KRAS\(^{G12F}\) represents a potential resistance mechanism supported by limited sequencing reads, as shown in Supplementary Fig. S2.

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**Functional Characterization of KRAS\(^{Y96D}\)**

To assess whether KRAS\(^{Y96D}\) can mediate resistance to MRTX849 and other inactive-state KRAS\(^{G12C}\) inhibitors, we expressed KRAS\(^{G12C}\) or the KRAS\(^{G12C/Y96D}\) double mutant in NCI-H358 (KRAS\(^{G12C}\)-mutant NSCLC), MIA PaCa-2...
Figure 2. Structural basis for resistance to KRAS<sup>G12C</sup> inhibition conferred by KRAS<sup>Y96D</sup>. Shown are the modeled crystal structures of MRTX849 (6UT0), AMG 510 (6OIM), and ARS-1620 (5V9U) bound to KRAS<sup>G12C</sup> (top) and KRAS<sup>G12C/Y96D</sup> (bottom), highlighting the loss of the hydrogen bonds between MRTX849 or AMG 510 and the Y96 residue and the disruption of the switch-II pocket dynamics between ARS-1620 and KRAS<sup>G12C/Y96D</sup>.

(RKAS<sup>G12C</sup>-mutant pancreatic ductal adenocarcinoma), and Ba/F3 cells, which lack endogenous KRAS<sup>G12C</sup> but become oncogene dependent upon withdrawal of IL3. In cell viability assays, relative to KRAS<sup>G12C</sup>-expressing controls, cells expressing KRAS<sup>G12C/Y96D</sup> showed marked resistance to three KRAS<sup>G12C</sup> inhibitors, with IC<sub>50</sub> shifts of >100-fold for MRTX849 and AMG 510 and ~20-fold for ARS-1620 (Fig. 3A; Supplementary Table S4).

Consistent with the effects on cell viability, RAS–MAPK pathway activity, as measured by levels of phosphorylated ERK (pERK) and pRSK, was sustained in KRAS<sup>G12C/Y96D</sup>-expressing cells at even high concentrations of MRTX849, relative to cells expressing KRAS<sup>G12C</sup> alone (Fig. 3B). Similarly, in KRAS<sup>G12C</sup>-mutant NSCLC cells in which p38 signaling is driven by mutant KRAS, including an existing patient-derived model MGH1138-1, persistent pERK and pAKT levels were observed with KRAS<sup>G12C/Y96D</sup> in the presence of MRTX849, relative to KRAS<sup>G12C</sup> expression alone (Fig. 3C; Supplementary Fig. S3). KRAS<sup>G12C/Y96D</sup> also drove marked resistance to MRTX849 in the patient-derived MGH1138-1 model. Furthermore, in 293T cells, which lack endogenous KRAS<sup>G12C</sup> expression, MRTX849 was unable to inhibit pERK levels driven by KRAS<sup>G12C/Y96D</sup> (Fig. 3D). Because MRTX849 and other inactive-state KRAS<sup>G12C</sup> inhibitors bind covalently to KRAS<sup>G12C</sup>, an electrophoretic mobility shift of drug-adducted KRAS<sup>G12C</sup> can be observed upon drug binding due to increased molecular weight. However, this mobility shift was no longer observed when 293T cells expressing KRAS<sup>G12C/Y96D</sup> were treated with MRTX849, suggesting that the Y96D mutation may abrogate inhibitor binding. Notably, KRAS<sup>G12C/Y96D</sup> appeared to have higher basal activation than KRAS<sup>G12C</sup>, as measured by a higher proportion of the active GTP-bound form of KRAS, although activation still appeared to be partly dependent on upstream pathway input (Supplementary Fig. S4A and S4B). Finally, although a decrease in GTP-bound KRAS (representing the active state) was observed in KRAS<sup>G12C</sup>-expressing cells treated with MRTX849, levels of active GTP-bound KRAS were maintained in KRAS<sup>G12C/Y96D</sup>-expressing cells (Fig. 3E; ref. 22). These results suggest that the KRAS<sup>Y96D</sup> mutation disrupts KRAS<sup>G12C</sup> inhibitor binding, leading to sustained KRAS signaling and therapeutic resistance.

The Active State KRAS<sup>G12C</sup> Inhibitor RM-018 Overcomes KRAS<sup>G12C/Y96D</sup>

As KRAS<sup>G12C/Y96D</sup> conferred resistance to multiple KRAS<sup>G12C</sup> inhibitors currently in clinical development, suggestive of shared vulnerability for this class of inhibitors, we sought to identify whether a structurally and functionally distinct KRAS<sup>G12C</sup> inhibitor might retain potency against this resistance mutation. RM-018 is a novel KRAS<sup>G12C</sup> inhibitor that binds specifically to the GTP-bound, active ["RAS(ON)""] state of KRAS<sup>G12C</sup>. RM-018 is a “tricomplex” KRAS inhibitor, which exploits a highly abundant chaperone protein, cyclophilin A, to bind and inhibit KRAS<sup>G12C</sup>, as previously described (Fig. 4A; structure shown in Supplementary Fig. S5; refs. 23, 24). Briefly, upon entering the cell, RM-018 forms a “binary complex” with cyclophilin A. This binary complex can associate with the active state of KRAS<sup>G12C</sup>, aided by protein–protein surface interactions between cyclophilin A and KRAS, and forms a covalent bond with KRAS<sup>G12C</sup> in a mutant-selective manner. This resultant “tricomplex” inhibits KRAS<sup>G12C</sup> through binding of cyclophilin A, leading to steric occlusion and preventing the association of downstream effector proteins. Given the markedly different mechanism of action of this class of inhibitor, we hypothesized that the inhibitory activity of RM-018 might be differentially affected by KRAS<sup>Y96D</sup> compared with inactive-state KRAS<sup>G12C</sup> inhibitors.

RM-018 demonstrated selectivity for KRAS<sup>G12C</sup>-driven cells, exhibiting low nanomolar potency in KRAS<sup>G12C</sup>-mutant H358 cells while not impairing the viability of cells driven by KRAS<sup>G12D</sup>, BRAF<sup>V600E</sup>, or RTK-driven signaling through wild-type RAS (Fig. 4B). Interestingly, although KRAS<sup>G12C/Y96D</sup> expression led to marked IC<sub>50</sub> shifts of >100-fold for
Figure 3. Cellular characterization of KRAS<sup>G12D</sup> in KRAS<sup>G12C</sup>-mutant models. A, Cell viability assays performed with NCI-H358, MIA PaCa-2, and Ba/F3 cells infected with retrovirus packaging KRAS (G12C or G12C/Y96D). Cell lines were treated with indicated drugs for 72 hours and the viabilities were measured with CellTiter-Glo. B, Western blot analysis was performed after treating MIA PaCa-2 cells stably expressing KRAS G12C or KRASG12C/Y96D with MRTX849 for 4 hours. C, MGH1138-1 cells expressing KRASG12C or KRASG12C/Y96D were treated with MRTX849 for 4 hours and subjected to Western blot analysis (left) and cell viability assay following 72 hours of treatment with the indicated concentrations of MRTX849 (right). D, Western blot analysis of HEK293T cells transiently expressing KRAS mutants after treatment with MRTX849 for 4 hours. E, RAS-GTP pulldown was performed after treating HEK293T stably expressing KRAS mutants with MRTX849.
Figure 4. Novel KRAS inhibitor RM-018 overcomes KRASG12C/Y96D. A, Mechanism of action of RM-018. B, RM-018 selectively inhibits cell viability in cells harboring KRASG12C. C, Cell viability assays performed with NCI-H358, MIA PaCa-2, Ba/F3, and MGH1138-1 cells stably infected with KRASG12C or KRASG12C/Y96D treated for 72 hours with RM-018. D and E, Western blot analysis performed in MIA PaCa-2 stably expressing KRASG12C or KRASG12C/Y96D (D) and HEK293T cells transiently expressing KRAS mutants (E) after treatment of RM-018 for 4 hours. F, Western blot analysis of MGH1138-1 cells transiently expressing KRASG12C or KRASG12C/Y96D after treatment with RM-018 for 4 hours. G, HEK293T cells transiently expressing KRAS mutants were treated with the indicated drug at 100 nmol/L each for 4 hours and then subjected to Western blot analysis.

MRTX849 and AMG 510 and ~20-fold for ARS-1620 (Fig. 3A) relative to KRASG12C expression alone, the efficacy of RM-018 on cell viability was largely unaffected by KRASG12C/Y96D expression, with IC_{50} shifts of only ~2-fold (Fig. 4C; Supplementary Table S4). In addition, RM-018 was able to inhibit pERK and pRSK levels with similar potency in the presence of KRASG12C or KRASG12C/Y96D expression in MIA PaCa-2, 293T cells, and the patient-derived KRASG12C-mutant NSCLC cell line MGH1138-1 (Fig. 4D–F). Inhibition of cell viability by RM-018 was also unaffected by KRASG12C/Y96D.
expression in the patient-derived MGH1138-1 model. Furthermore, the KRAS mobility shift induced by covalent binding of RM-018 was observed in both cell lines in the presence of either KRASG12C or KRASG12C/Y96D expression, suggesting that binding of RM-018 to KRAS is not abrogated by the KRASY96D mutation. Indeed, although a KRAS mobility shift due to covalent drug binding was observed in 293T cells expressing KRASG12C for MRTX849, AMG 510, and RM-018, only RM-018 exhibited this same mobility shift and was able to inhibit downstream signaling in the presence of the KRASG12C/Y96D mutation (Fig. 4G). Taken together, these data suggest that RM-018 retains the ability to bind and inhibit KRASG12C/Y96D and may represent a potential therapeutic strategy to overcome this acquired resistance mechanism.

**DISCUSSION**

The arrival of covalent KRASG12C-selective inhibitors in the clinic and early signs of activity demonstrated by MRTX849 and AMG 510 have generated great enthusiasm (4, 5). However, our experiences across targeted therapies in lung cancer and other cancers collectively demonstrate that acquired resistance to the KRASG12C inhibitors will represent an inevitable challenge going forward. Although preclinical studies have nominated putative mechanisms of up-front resistance, including RAS–MAPK pathway reactivation (4, 8), mechanisms of acquired resistance to MRTX849 or AMG 510 causing disease relapse in patients remain unknown.

The acquired resistance demonstrated in this patient is instructive in highlighting several points. First, 10 distinct resistance alterations arose in this patient, all converging on the spectrum of potential acquired resistance mechanisms. However, in the context of the reactivation of RAS–MAPK signaling, suggesting that this may be a central common mechanism of acquired resistance. Nonetheless, in light of our observations, efforts toward rational design of next-generation inhibitors.

**METHODS**

**Patient Treatment and Specimen Collection**

The patient was treated with MRTX849 dosed 600 mg twice daily on the phase I study (KRYSTAL-1) after providing written informed consent (ClinicalTrials.gov identifier: NCT03785249). She had received two prior lines of therapy. All pre- and posttreatment biopsies and genotyping were performed in accordance with the Massachusetts General Hospital (MGH) institutional review board–approved protocol and in accordance with the Declaration of Helsinki. The pretreatment tumor specimen was analyzed using the MGH SNaPshot next-generation sequencing assay (25). All cfDNA samples were sequenced using the commercially available Guardant360 assay (GuardantHealth). Detailed patient history is available in the Supplement.

**Cell Lines and Reagents**

Ba/F3 cells were obtained from the RIKEN BRC Cell Bank (RIKEN BioResource Center). MGH1138-1 cells were generated from a patient with KRASG12C-mutant NSCLC using methods that have been previously described (26). Prior to cell line generation, the patient provided written informed consent to participate in a DanaFarber/Harvard Cancer Center institutional review board–approved protocol giving permission for research to be performed on their sample. The remaining cell lines were obtained from ATCC or the Center for Molecular Therapeutics at the MGH Cancer Center, which routinely performs cell line authentication testing by SNP and short tandem repeat analysis. HEK293T cells were maintained in DMEM supplemented with 10% FBS. Mia PaCa-2 and NCI-H358 cells were maintained in DMEM/F12 supplemented with 10% FBS. LU-65 and MGH1138-1 cells were maintained in RPMI supplemented with 10% FBS. Ba/F3 cells were maintained in DMEM supplemented.
with 10% FBS and 10 ng/mL IL3. The KRAS (G12C or G12C/Y96D) gene was inserted in pMXs-Puro Retroviral Expression Vector, which was purchased from Cell Biolabs. Retrovirus packaging mutated KRAS genes were produced with HEK293T cells. After concentration of virus with Retro-Concentin Retro Concentration Reagent (System Biosciences), MIA PaCa-2, NCI-H358, and Ba/F3 cells were infected with the virus packaging either the KRASG12C or KRASG12C/Y96D gene. After 48 hours of incubation, the cells were treated with puromycin (1-2 μg/mL) for another 48 hours. IL3 was withdrawn to select for Ba/F3 cells dependent on mutant KRAS signaling after 48 hours of puromycin treatment. The remaining cells were maintained in media supplemented with puromycin. For transient expression experiments, a day after seeding the cells, pMXs-Puro-KRASG12C or pMXs-Puro-KRASG12C/Y96D vectors were induced with Lipofectamine 2000 Transfection Reagent (ThermoFisher Scientific) following the manufacturer’s protocol. After 16 to 24 hours of incubation, cells were treated with inhibitors for 4 hours. AMG 510 was purchased from MedChemExpress. MRTX849 and ARS-1620 were purchased from Selleck Chemicals. RM-018 was provided by Revolution Medicines, and details of the chemical synthesis can be found in International Patent Application No. PCT/US2020/058841.

**Cell Viability Assays**

Cell lines were seeded in a 96-well plate at 2 to 10 × 10^3 cells/well depending on cell lines and after 24 hours treated with a serial dilution of drugs and incubated for 72 hours. Cell viability was measured with CellTiter-Glo (Promega).

**Western Blot Analysis**

Cell lines were treated with MRTX849, AMG 510, or RM-018 for 4 hours and lysates were prepared as described previously (27). All antibodies were diluted in 5% bovine serum albumin as follows: KRAS (Sigma), pERK (Thr202/Tyr204, 1:1,000; Cell Signaling Technology), p44/42 MAPK (ERK1/2), (1:1,000; Cell Signaling Technology), phospho-RSK1 (T359+S363, 1:1,000; Abcam), phospho-AKT (Ser473, 1:1,000; Cell Signaling Technology), AKT (1:1,000; Cell Signaling Technology), and GAPDH (1:1,000; MilliporeSigma).

**RAS-GTP Pulldown**

After indicated inhibitor treatment, RAS activity was assessed by GST-RAF-RBD pulldown (Cell Signaling Technology), followed by Western blot analysis with pan-RAS or RAS isoform-specific antibodies. Pulldown samples and whole-cell lysates were resolved on 4% to 12% Bis-Tris gels, and Western blotting was performed using antibodies against KRAS (Sigma) and pan-RAS (Cell Signaling Technology).

**Structural Modeling**

Publicly available crystal structures of KRASG12C in complex with MRTX849 (PDB:6UT0), AMG 510 (PDB:6O1M), and ARS-1620 (PDB:5V9U) were downloaded from the RCSB Protein Data Bank (PDB; ref. 28). Structures were rendered in PyMol (The PyMOL Molecular Graphics System) and analyzed for hydrogen bonds and other molecular interactions between the KRASG12C inhibitors and the KRAS protein. Structures of Y96 amino acid mutation were generated by Protein Mutagenesis Wizard implemented in PyMol, with one of the backbone-dependent rotamers manually selected.

**cfDNA Extraction and ddPCR**

Whole blood was collected by routine phlebotomy in two 10-ml. Streck tubes. Plasma was separated within 1 to 4 days of collection through two different centrifugation steps (the first at room temperature for 10 minutes at 1,600 × g and the second at 3,000 × g for the same time and temperature). Plasma was stored at −80°C until cfDNA extraction. cfDNA was extracted from plasma using the QIAamp Circulating Nucleic Acid Kit (QIAGEN) with 60 minutes of proteinase K incubation at 60°C. All other steps were performed according to the manufacturer’s instructions. For ddPCR experiments, DNA template (up to 10 μL, with a total of 20 ng) was added to 12.5 μL ddPCR Supermix for Probes (Bio-Rad) and 1.25 μL custom primer/probe mixture. This reaction mix was added to a DG8 cartridge together with 60 μL Droplet Generation Oil for Probes (Bio-Rad) and used for droplet generation. Droplets were then transferred to a 96-well plate (Eppendorf) and then thermal cycled with the following conditions: 5 minutes at 95°C, 40 cycles of 94°C for 30 seconds, 55°C (with a few grades of difference among assays) for 1 minute, followed by 98°C for 10 minutes (Ramp Rate 2°C/s). Droplets were analyzed with the QX200 Droplet Reader (Bio-Rad) for fluorescent measurement of FAM and HEX probes. Gating was performed based on positive and negative controls, and mutant populations were identified. The ddPCR data were analyzed with QuantaSoft analysis software (Bio-Rad) to obtain fractional abundance of the mutant DNA alleles in the wild-type/normal background. The quantification of the target molecule was presented as the number of total copies (mutant plus wild-type) per sample in each reaction. Allelic fraction was calculated as follows: AF% = [(Nmut/Nmut + Nwt) × 100], where Nmut is the number of mutant alleles and Nwt is the number of wild-type alleles per reaction. ddPCR analysis of normal control plasma DNA (from cell lines) and no DNA template controls was always included. Probe and primer sequences are available upon request.

**Authors’ Disclosures**

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Clinical Acquired Resistance to KRAS<sub>G12C</sub> Inhibition

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Authors’ Contributions

N. Tanaka: Conceptualization, data curation, formal analysis, investigation, writing–original draft, writing–review and editing. J.J. Lin: Conceptualization, data curation, formal analysis, investigation, writing–original draft, writing–review and editing. M.B. Ryan: Conceptualization, data curation, formal analysis, investigation, writing–original draft, writing–review and editing. M. Sakhi: Conceptualization, formal analysis, investigation, writing–original draft, writing–review and editing. L.A. Kiedrowski: Conceptualization, formal analysis, writing–review and editing. A.G. Michel: Formal analysis, investigation, writing–review and editing. M.U. Syed: Formal analysis, investigation, writing–review and editing. K.A. Fella: Formal analysis, investigation, writing–review and editing. M. Sahki: Formal analysis, investigation, writing–review and editing. J. Baiev: Formal analysis, investigation, writing–review and editing. D. Juric: Conceptualization, formal analysis, investigation, writing–review and editing. J. F. Gainor: Conceptualization, formal analysis, investigation, writing–review and editing. L. Bar-Peled: Conceptualization, formal analysis, investigation, writing–review and editing. A.N. Hata: Conceptualization, resources, data curation, formal analysis, supervision, funding acquisition, validation, investigation, writing–original draft, writing–review and editing. R.S. Heist: Conceptualization, resources, data curation, formal analysis, supervision, funding acquisition, validation, investigation, writing–original draft, writing–review and editing. R.B. Corcoran: Conceptualization, resources, data curation, formal analysis, supervision, funding acquisition, validation, investigation, writing–original draft, writing–review and editing.

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