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Mechanisms of Resistance to Selective RET Tyrosine Kinase Inhibitors in *RET* Fusion-Positive Non-Small Cell Lung Cancer

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Abstract

Background: Rearranged during transfection (*RET*) gene fusions are a validated target in non-small cell lung cancer (NSCLC). RET-selective inhibitors selpercatinib (LOXO-292) and pralsetinib (BLU-667) recently demonstrated favorable antitumor activity and safety profiles in advanced *RET* fusion-positive NSCLC, and both have received approval by the US Food and Drug Administration for this indication. Insights into mechanisms of resistance to selective RET inhibitors remain limited.

Patients and Methods: This study was performed at five institutions. Tissue and/or cell-free DNA was obtained from patients with *RET* fusion-positive NSCLC after treatment with selpercatinib or pralsetinib and assessed by next-generation sequencing (NGS) or *MET* fluorescence in situ hybridization.

Results: We analyzed a total of 23 post-treatment tissue and/or plasma biopsies from 18 *RET* fusion-positive patients who received a RET-selective inhibitor (selpercatinib, n=10; pralsetinib, n=7; pralsetinib followed by selpercatinib, n=1 with biopsy after each inhibitor). Three cases had paired tissue and plasma samples, of which one also had two serial resistant tissue specimens. The median progression-free survival on RET inhibitors was 6.3 months [95% confidence interval (CI), 3.6–10.8 months]. Acquired *RET* mutations were identified in two cases (10%), both affecting the *RET* G810 residue in the kinase solvent front. Three resistant cases (15%) harbored acquired *MET* amplification without concurrent *RET* resistance mutations, and one specimen

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had acquired *KRAS* amplification. No other canonical driver alterations were identified by NGS. Among 16 resistant tumor specimens, none had evidence of squamous or small cell histologic transformation.

Conclusions: *RET* solvent front mutations are a recurrent mechanism of RET inhibitor resistance, although they occurred at a relatively low frequency. The majority of resistance to selective RET inhibition may be driven by RET-independent resistance such as acquired *MET* or *KRAS* amplification. Next-generation RET inhibitors with potency against *RET* resistance mutations and combination strategies are needed to effectively overcome resistance in these patients.

Keywords

RET; non-small cell lung cancer; resistance; tyrosine kinase inhibitor; pralsetinib; selpercatinib

INTRODUCTION

The diagnostic and treatment approach to advanced non-small cell lung cancer (NSCLC) continues to be refined, with a growing number of genetic and molecular markers that guide tailored therapy. The oncogenic rearranged during transfection (*RET*) gene fusions were first identified in NSCLC in 2012.[1–4] Since then, *RET* fusions have been reported in approximately 1–2% of lung cancer, predominantly associated with a never or light smoking history and adenocarcinoma histology.[5] Importantly, lung cancers harboring *RET* fusions are sensitive to tyrosine kinase inhibitors (TKIs) with anti-RET activity, and therefore define a distinct molecular subset.[1, 4, 6]

Initial efforts to target RET in lung cancer involved repurposing readily available multikinase inhibitors (MKIs) with potency against RET such as cabozantinib or vandetanib.[7–12] However, these MKIs were limited by modest efficacy and substantial toxicities. In 2017, two novel, potent RET-selective TKIs, selpercatinib (LOXO-292) and pralsetinib (BLU-667), entered clinical testing in patients with advanced *RET*-altered solid tumors, including *RET* fusion-positive NSCLC.[13, 14] Both RET TKIs demonstrated favorable tolerability and robust efficacy [including in the central nervous system (CNS)] in patients with *RET* fusion-positive lung cancer in registrational phase I/II studies, with objective response rates (ORRs) ranging from 55–64% among platinum chemotherapy-pretreated and 66–85% among treatment-naïve patients, respectively.[15, 16] Durable responses were observed regardless of the *RET* fusion partner or history of prior MKI exposure. On the basis of these data, the US Food and Drug Administration (FDA) recently granted a line-agnostic accelerated approval of selpercatinib and pralsetinib for the treatment of adult patients with metastatic *RET* fusion-positive NSCLC (with selpercatinib also approved for adult and pediatric patients ≥12 years of age with advanced or metastatic *RET*-mutant medullary thyroid cancer or *RET* fusion-positive thyroid cancer who require systemic therapy and are radioactive iodine-refractory).

Despite the encouraging efficacy of selective RET TKIs, experience across the targeted therapy paradigm in NSCLC suggests that the eventual development of acquired resistance will limit the duration of benefit from RET-selective inhibitors. As selpercatinib and

pralsetinib are now standard therapies in advanced *RET* fusion-positive lung cancer and will be more widely used, it is paramount to understand the mechanisms of TKI resistance and inform the development of novel therapeutic strategies that can overcome resistance. In one recent study, Solomon and colleagues reported *RET* G810R/S/C/V solvent front mutations that mediated acquired resistance to selpercatinib in three *RET* fusion-positive NSCLC and two *RET*-mutant medullary thyroid cancer (MTC) cases.[17] The frequency of *RET* resistance mutations, however, remains undetermined. Furthermore, outside of this study and one case report of a selpercatinib-resistant NSCLC patient harboring *MET* amplification, [18] insights into mechanisms of resistance to RET-selective TKIs are lacking.

Here, we performed a multi-institutional analysis of repeat tumor or plasma biopsies from a cohort of patients with *RET* fusion-positive NSCLC after treatment with selpercatinib and pralsetinib, in order to systematically characterize acquired resistance mechanisms to these inhibitors.

METHODS

Study Population

Patients were identified at five participating institutions: Massachusetts General Hospital (MGH; n=10), Georgetown University (GU; n=2), National Cancer Centre Singapore (NCCS; n=1), University of California Irvine (UCI; n=1), and University of California San Francisco (UCSF; n=4). Patients were eligible if they had advanced or metastatic NSCLC with *RET* fusion identified by local molecular profiling [e.g., fluorescent in situ hybridization (FISH), DNA-based next-generation sequencing (NGS), or targeted RNA sequencing]. Patients must have received pralsetinib and/or selpercatinib (as any line of systemic therapy) with subsequent resistant tumor or liquid biopsy analyzed by molecular testing. Most of the enrolled patients received pralsetinib or selpercatinib in clinical trials (ClinicalTrials.gov identifier NCT03037385 or NCT03157128), respectively. One patient received selpercatinib through single patient compassionate use access, and one patient received selpercatinib through the expanded access program. The studies were approved by the Institutional Review Board at each participating institution.

Data Collection

Medical records were retrospectively reviewed to extract data on clinical, pathologic, and molecular features. Response to therapy was determined per the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. Progression-free survival (PFS) was measured from the time of therapy initiation to clinical/radiographic disease progression or death. Duration of therapy was measured from the time of therapy initiation to therapy discontinuation. Patients continuing on therapy were censored at last follow-up. All data were updated as of June 10, 2020.

Biopsy Genotyping

All patients included in this study underwent tumor or plasma biopsy after treatment with pralsetinib or selpercatinib and genotyping after providing informed consent. Fifteen tissue biopsies were analyzed using one of the following NGS platforms: the previously described

MGH SNaPshot DNA-based genotyping panel and a separate RNA-based NGS assay (Solid Fusion Assay) for the detection of fusion transcripts (n=10),[19] FoundationOne (n=1; Foundation Medicine, Inc.), Caris MI profile (n=2; Caris Molecular Intelligence), MSK IMPACT (n=1),[20] or UCSF500 (n=1).[21] One tissue specimen was insufficient for NGS but sufficient for analysis by *MET* fluorescence in situ hybridization (FISH). Seven liquid biopsies were analyzed using either the commercially available Guardant 360 cell-free DNA (cfDNA) assay (n=5; Guardant Health, Inc.) or the FoundationACT assay (n=2; Foundation Medicine, Inc.).

MET FISH was performed using formalin-fixed paraffin-embedded tumor specimens and the dual-color FISH assay with 07Q001B550 C-MET (7q31) probe (chromosome 7q31 *MET* locus; Leica Biosystems) and a copy number probe (centromere 7 or *CEP7*; Abbott-Vysis). Signal quantitation of 50 tumor nuclei was used to generate a *MET*/*CEP7* ratio. A ratio greater than 5.0 or clustered *MET* signals too numerous to count were considered highly amplified.

A cell line (MGH9009–1) was developed from the lymph node biopsy of case MGH2, as previously described.[22] *RET* fusion mRNA was PCR-amplified and *RET* kinase domain was sequenced. Primer sequences were: KIAA1468 F 5′- CGAGGTGTCTCGTATTGCAG -3′, RET R 5′- GCATTATTACAGTCCACCAGCG -3′.

Statistical Analysis

The Kaplan-Meier method was used to estimate PFS and duration of therapy medians and probabilities (Stata version 14.2).

RESULTS

Clinical Characteristics

A total of 18 patients with advanced *RET* fusion-positive NSCLC were treated with pralsetinib (n=7), selpercatinib (n=10), or pralsetinib followed by selpercatinib (n=1), and underwent post-treatment biopsies between 2017 and 2020 (Table 1). In the cohort, the median age at diagnosis was 56.5 (range, 30–77). All patients had adenocarcinoma and were never or light smokers. The *RET* fusion partner was known for all patients. The predominant fusion was *KIF5B-RET* (67%), consistent with the literature.[5] Seven patients (39%) had known brain metastases at the time of starting selpercatinib or pralsetinib.

Outcomes on RET Inhibitors and Patterns of Progression

Fifteen patients (83%) had achieved partial response (PR) per RECIST v1.1 on their first RET-selective inhibitor. The remaining three patients had stable disease as the best overall response. The median PFS on the initial RET-selective TKI was 6.3 months [95% confidence interval (CI), 3.6–10.8 months], and the median duration of therapy was 7.2 months (95% CI, 3.7–19.0 months) (Supplemental Figure 1).

The majority of patients (72%) in this cohort experienced extracranial disease progression. Five patients (28%) had both extracranial and intracranial disease progression.

Summary of Biopsies and Histology

To assess the resistance mechanism to RET inhibitors, tissue biopsies alone were performed in 11 patients (one of whom had a resistant biopsy after pralsetinib and another following selpercatinib), and liquid biopsies alone in four patients. Two patients underwent paired tissue and plasma biopsies. One patient had two serial tumor biopsies of distinct metastatic sites at progression on a RET inhibitor, one of which also had a paired plasma biopsy (summarized in Figure 1 and further delineated in Supplemental Table 1).

In total, therefore, 20 distinct selpercatinib- or pralsetinib-resistant cases were analyzed by molecular testing, 3 of which had paired tissue and plasma (Figure 2).

RET Solvent Front Mutations

The gene alterations detected in the resistant biopsies are summarized in Figure 2. A *RET* resistance mutation was detected in two cases (10%), both affecting the G810 residue in the RET solvent front. In the first patient with *CCDC6-RET* fusion, a *RET* G810S mutation was detected at progression on selpercatinib (case MGH7, previously published).[17] This patient had previously received multiple multikinase inhibitors (e.g., ponatinib, alectinib, vandetanib) as well as pralsetinib, and had a post-pralsetinib/pre-selpercatinib biopsy (MGH1) which did not reveal any *RET* resistance mutations (Supplemental Table 2). Thus, *RET* G810S was most likely acquired on selpercatinib.

A second patient (GU1) with *CCDC6-RET* fusion-positive adenocarcinoma was initially treated with chemoradiation followed by durvalumab for stage 3 disease, with a biopsy at that time demonstrating the *CCDC6-RET* fusion but no evidence of *RET* mutations. This patient subsequently received multiple lines of immunotherapy, chemotherapy, and a multikinase inhibitor (RXDX-105), before enrolling in the clinical trial of selpercatinib. A soft tissue biopsy at progression on selpercatinib obtained after approximately 20 months on therapy did not reveal *RET* resistance mutations (GU1-T1 in Figure 2). She received radiation and continued therapy, but had further disease progression. A repeat biopsy of a progressing liver metastasis approximately 9 months later and paired cfDNA both revealed an acquired *RET* G810C mutation (GU1-T2 in Figure 2).

One case (MGH11) had a *RET* G597V mutation, which lies outside the RET kinase domain and is of unknown functional significance. Of note, this *RET* 597V mutation was also detected in the patient's treatment-naïve plasma sample, and the patient went on to achieve a PR on RET-selective inhibitor with duration of response lasting 16.9 months. Therefore, this *RET* mutation was presumed not to be a driver of resistance. In addition, a *RET* V804 gatekeeper mutation was not detected in this series of post-treatment biopsies.

RET-Independent Resistance

Given the infrequency of on-target molecular mechanisms of resistance, we next investigated potential target-independent mechanisms of resistance. Among a total of 16 selpercatinib- or pralsetinib-resistant tissue biopsies, none had evidence of transformation to small cell or squamous cell histology.

Nineteen of the 20 distinct resistant cases were analyzed by broad NGS-based testing, with the one remaining case analyzed by *MET* FISH only due to insufficient tumor tissue for NGS. *MET* amplification is a recurrent bypass signaling pathway across oncogenic drivers, such as in NSCLC with *EGFR* mutations or *ALK* fusions.[23–26] We identified *MET* amplification in three post-RET TKI cases (15%), none of which harbored a concomitant *RET* resistance mutation (Supplemental Table 2). Two selpercatinib-resistant cases with *KIF5B-RET* fusions (GU2, PFS of 8 months; UCI1, PFS of 7.4 months and previously published[18]) were assessed by cfDNA sequencing and found to have *MET* amplification (plasma copy numbers of 2.7 by Guardant360 and ~17 by FoundationACT, respectively). For both cases, pre-selpercatinib cfDNA analyses did not demonstrate evidence of pre-existing baseline *MET* amplification. Of note, GU2 had a paired selpercatinib-resistant liver tumor biopsy that was also found to harbor *MET* amplification by NGS testing.

Another patient (MGH2) had received pralsetinib after prior chemotherapy, achieving RECIST PR. He had disease progression after 5.3 months, and a biopsy was performed of the resistant retroperitoneal lymph node. Tissue proved insufficient for NGS analysis. Sanger sequencing of the cDNA extracted from the corresponding patient-derived cell line did not reveal *RET* resistance mutations. Given the finding of *MET* amplification in other specimens, we pursued *MET* FISH testing, which demonstrated a high-level focal amplification of *MET* with *MET*/*CEP7* ratio of greater than 25:1 (Figure 3). NGS and *MET* FISH analysis of the treatment-naïve tumor from this patient did not detect evidence of *MET* amplification.

We identified a *MET* single nucleotide variant in two cases: *MET* M822I (NCCS1) and *MET* S108L (UCSF-339) (Figure 2). These mutations reside outside the MET kinase domain and are of unclear functional significance.

KRAS amplification is another genetic event which has been reported as a driver of resistance to targeted therapies in multiple contexts, including EGFR-directed therapies in colorectal cancer, and MET TKIs or ALK TKIs in NSCLC with *MET* exon 14 skipping or *ALK* fusions, respectively.[27–29] We detected *KRAS* amplification in a post-selpercatinib bone biopsy from a patient with *KIF5B-RET* fusion-positive NSCLC who had PR on selpercatinib and subsequently had disease progression after 16.7 months (UCSF-347, Figure 2; Supplemental Table 2). This resistant specimen was also found to have *FGFR2* amplification, *CCNE1* amplification, *LRP1B* deletion, and variants in *TP53* and *KMT2A*. By comparison, a treatment-naïve lymph node specimen from this patient harbored *CCNE1* amplification, *LRP1B* deletion, and *TP53* variant, but no evidence of *KRAS* or *FGFR2* amplification.

We did not identify acquired oncogenic mutations in other canonical drivers previously implicated in targeted therapy resistance, such as *EGFR* or *ERBB2* (Figure 2, Supplemental Table 3). One pralsetinib-resistant case (MGH6 in Figure 2) had a *PIK3CA* H1047R mutation detected in post-treatment lung biopsy, which had not been detected in the TKI-naïve bone biopsy. *BRAF* N236S and *ROS1* D2213E variants, both of unknown functional significance, were noted in one case each (UCSF-346 and NCCS1, respectively, in Figure 2).

De novo oncogenic fusions involving *ALK*, *ROS1*, *NTRK1*-3, *BRAF*, *NRG1*, or *MET* genes were not detected.

DISCUSSION

In this multi-institutional study, we examined a total of 23 tumor and liquid biopsies derived from advanced *RET* fusion-positive NSCLC patients who were treated with RET-selective inhibitors pralsetinib and selpercatinib. To our knowledge, this is the largest study to date to examine mechanisms of resistance to RET-selective inhibitors. We identified *RET* solvent front mutations and *MET* amplification as recurrent mechanisms of resistance, and additionally identified *KRAS* amplification in one resistant case.

Solvent front mutations in the target kinase are known to confer on-target resistance in other fusion oncogene-driven lung cancers. For example, *ALK* G1202R and *ROS1* G2032R are refractory solvent front mutations that cause resistance to a number of available TKIs in *ALK* or *ROS1* fusion-positive NSCLC, respectively.[30, 31] Earlier this year, Solomon and colleagues reported *RET* G810 solvent front mutations as a mechanism of resistance to selpercatinib in five patients with *RET* fusion-positive NSCLC and *RET*-mutant MTC, predicted to hinder drug binding based on structural modeling.[17] However, the frequency of these *RET* mutations remained unknown. Here, we detected the *RET* G810C and G810S mutations in two cases (10%), supporting solvent front mutations as a recurrent mechanism of resistance to RET inhibitors and underscoring the importance of developing next-generation RET TKIs with potency against these mutations.

Overall, *RET* resistance mutations were detected at a low frequency in this cohort, and other, non-solvent front *RET* mutations including gatekeeper mutations affecting the V804 residue (known to confer resistance to MKIs such as vandetanib[32]) did not emerge in our series. The relatively low prevalence and narrow spectrum of *RET* mutations may reflect the high anti-RET potency of selpercatinib and pralsetinib, although our findings will require validation in larger cohorts. Interestingly, despite the potency of pralsetinib and selpercatinib against the gatekeeper *RET* V804 mutations based on preclinical studies,[13, 14] the study by Solomon et al. identified *RET* V804 and G810 mutations *in trans* in two selpercatinib-resistant cases and *in cis* in a minority of reads in one selpercatinib-resistant case.[17] Further studies are needed to elucidate whether the gatekeeper mutations can confer resistance to selpercatinib and/or pralsetinib despite the preclinical evidence, and whether the spectra of *RET* resistance mutations (and non-*RET* resistance alterations) differ between selpercatinib and pralsetinib.

Importantly, our findings indicate that the majority of cases progressing on RET-selective inhibitors are likely driven by off-target, RET-independent mechanisms of resistance. Indeed, the preponderance of resistant cases without *RET* resistance mutations is striking when compared to *EGFR*-mutant or *ALK* fusion-positive NSCLC, where approximately 50–60% of resistance to next-generation TKIs is driven by target-independent mechanisms.[25, 30] This observation highlights the importance of identifying putative potentially targetable RET-independent resistance drivers, with the ultimate goal of designing new treatment approaches.

We identified *MET* gene amplification as a recurring RET-independent resistance mechanism in *RET* fusion-positive lung cancer, observed in 15% of cases in this cohort. *MET* amplification is an established mechanism of resistance to EGFR inhibitors in *EGFR*-mutant NSCLC and has been identified in up to 20% of EGFR TKI-resistant biopsies.[23–25] Notably, a combination of EGFR and MET inhibitors, such as osimertinib plus savolitinib or osimertinib plus capmatinib, is able to effectively overcome this MET-driven resistance in clinic.[33, 34] Similarly, *MET* amplification can mediate resistance to next-generation ALK inhibitors in *ALK* fusion-positive lung cancer.[26] Within the framework of this collective knowledge, our findings support *MET* amplification as a recurring, clinically relevant driver of resistance across multiple distinct subsets of oncogene-driven lung cancer.

Furthermore, our findings naturally raise the question of whether combined RET and MET inhibition could represent a viable therapeutic strategy to target resistance in a subset of patients progressing on selpercatinib or pralsetinib. Certainly, studies evaluating combinations of a RET-selective inhibitor with a MET inhibitor will be required in order to explore this possibility. Multikinase inhibitors with activity against both MET and RET (e.g., cabozantinib) may represent an alternative and perhaps more readily accessible option, though likely less desirable in terms of potency and tolerability. The identification of potentially targetable resistance gene alterations, such as *MET* amplification or *RET* solvent front mutations in this study, implies that repeat biopsies will have clinical value in patients progressing on RET inhibitors.

Finally, it is worth noting that over a quarter of patients in our cohort had both intracranial and extracranial disease progression, despite the known favorable CNS activity of selpercatinib and pralsetinib.[15, 30, 35] This observation serves to emphasize that CNS penetration and efficacy should be an integral feature of next-generation RET inhibitors. If successfully developed, next-generation RET TKIs could enable a sequential treatment paradigm in *RET* fusion-positive disease, reminiscent of that seen in *ALK* or *ROS1* fusion-positive lung cancer.

This study had several important limitations. First, although this is the largest study to date to analyze a series of selective RET TKI-resistant biopsies, the cohort remains small in size, and the possibility of ascertainment bias cannot be excluded. Second, various NGS platforms including cfDNA assays were used to detect gene alterations, with no standardized definition for calling gene amplification (such as *MET* amplification). This was a limitation inherent to the retrospective analysis of real-world, clinical genotyping results. Second, it is plausible that the spectrum and relative frequencies of detected resistance alterations may vary with longer follow-up, particularly if certain alterations are associated with an earlier versus more delayed onset of resistance. It should be noted that the median PFS and duration of therapy in this cohort were 6.3 months and 7.2 months, respectively, which are shorter than has been reported from the phase I/II trials of selpercatinib and pralsetinib.[15, 16] Thus, this cohort may have been biased towards early progressors, and further, larger studies are needed with additional resistant biopsies and functional studies. Our analysis was also limited to genetic alterations detected through different assays and did not assess for non-genetic mechanisms of resistance that may additionally have a role in *RET* fusion-positive lung cancer. While histologic transformation—such as from adenocarcinoma to squamous

cell or small cell histology as identified in resistant *EGFR*-mutant or *ALK* fusion-positive lung cancer[24, 25, 36–39]—was not observed in our series, we speculate that this was likely due to the relatively low frequency of such events and a small number of cases analyzed herein. Despite these limitations, our study offers important early insights into the relative prevalence and spectrum of mechanisms of resistance to RET-selective inhibitors.

In summary, we demonstrated that *RET* resistance mutations, though recurrent, are identified in a low frequency of *RET* fusion-positive NSCLC after progression on selpercatinib or pralsetinib. The majority of resistance appears to be driven by RET-independent mechanisms, such as *MET* amplification or *KRAS* amplification detected in our series. Moving forward, it will be important to continue to assess and validate mechanisms of resistance in larger cohorts of *RET*-altered solid tumors. Our findings should help inform the development of next-generation RET inhibitors and other treatment approaches such as combination strategies, with the goal of overcoming resistance and improving outcomes in patients with *RET* fusion-positive lung cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HIGHLIGHTS

- Resistance is a major challenge in *RET* fusion-positive lung cancer treated with RET tyrosine kinase inhibitors (TKIs).
- *RET* mutations involving the solvent front residue G810 are a recurrent yet infrequent mechanism of resistance to RET TKIs.
- The majority of resistance to selective RET inhibition is driven by RET-independent resistance, such as *MET* amplification.
- RET TKIs with potency against *RET* solvent front mutations and combination strategies are needed to overcome resistance.

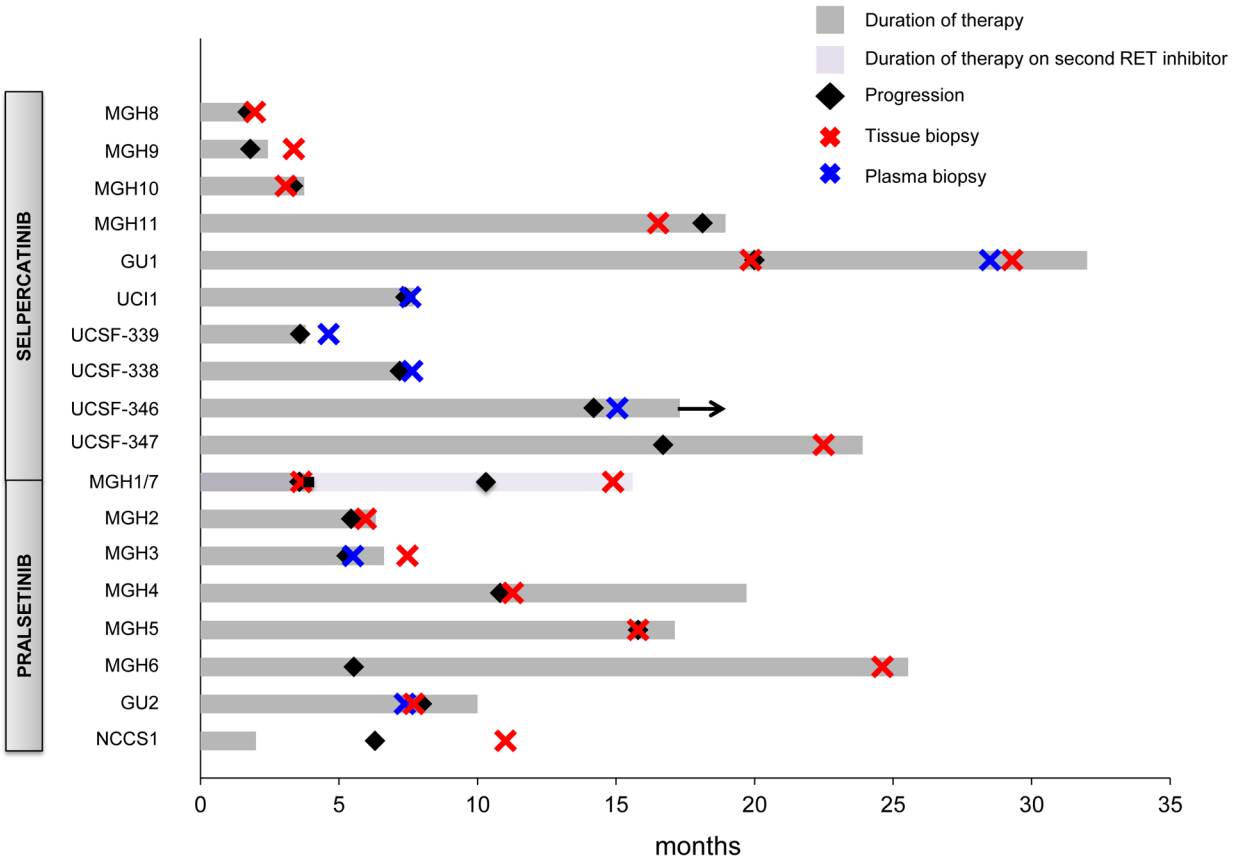

Figure 1.

Duration of RET inhibitor treatment and timing of biopsies in the cohort. Arrow indicates ongoing therapy with a RET inhibitor at the time of this analysis. MGH1 and MGH7 biopsies were derived from the same patient (labeled here as MGH1/7), who first had disease progression on pralsetinib and underwent a tissue biopsy, followed by treatment with selpercatinib, again with disease progression and a repeat tissue biopsy.

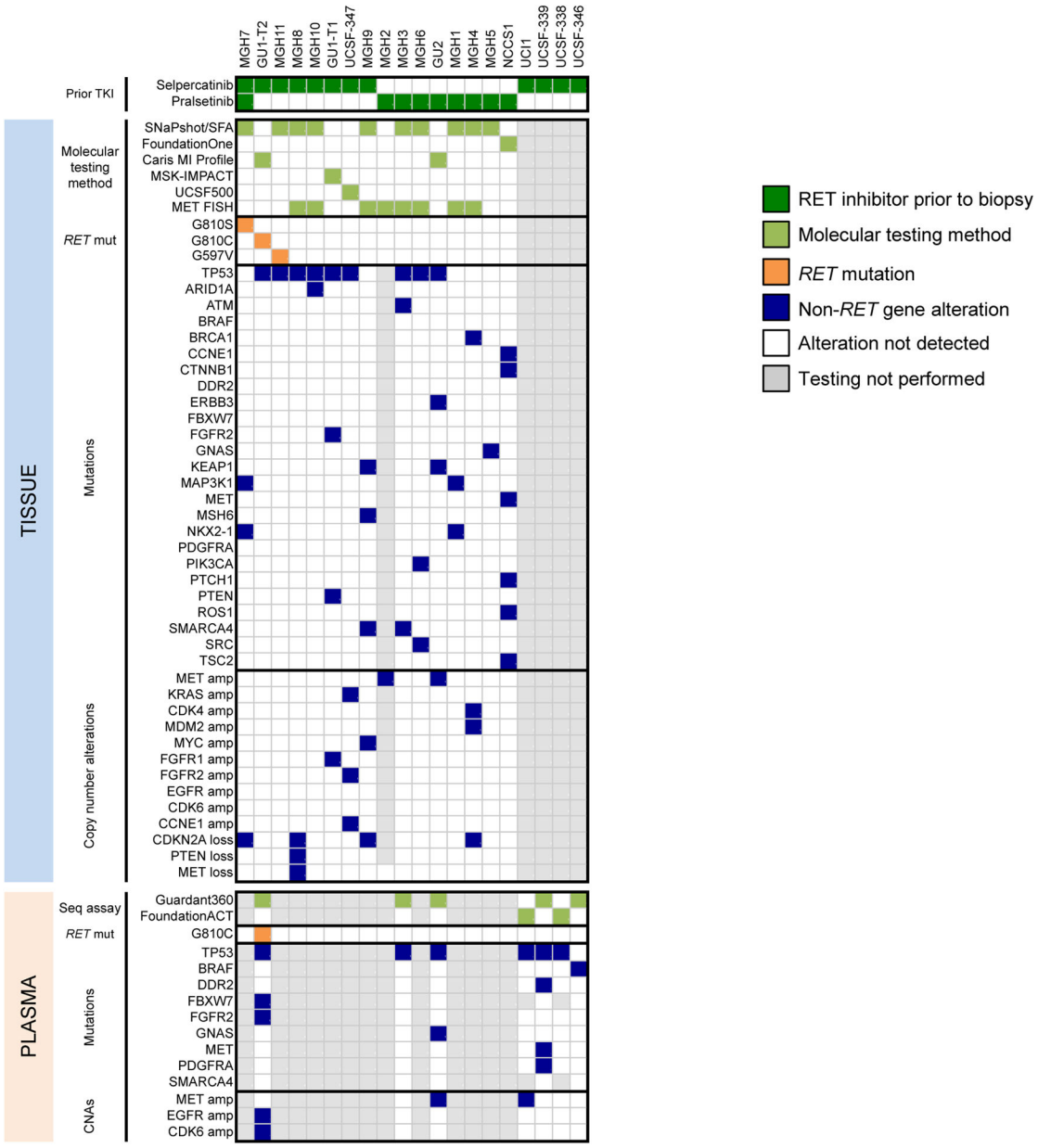

Figure 2.

Summary of gene alterations in *RET* fusion-positive NSCLC resistant to selective RET inhibition. The heatmap summarizes findings from tissue (top) and cell-free DNA (bottom) analysis after treatment with selpercatinib or pralsetinib. Only those genes included in the MGH SNaPshot assay are shown. TKI, tyrosine kinase inhibitor; SFA, solid fusion assay; mut, mutation; seq, sequencing; CNA, copy number alteration.

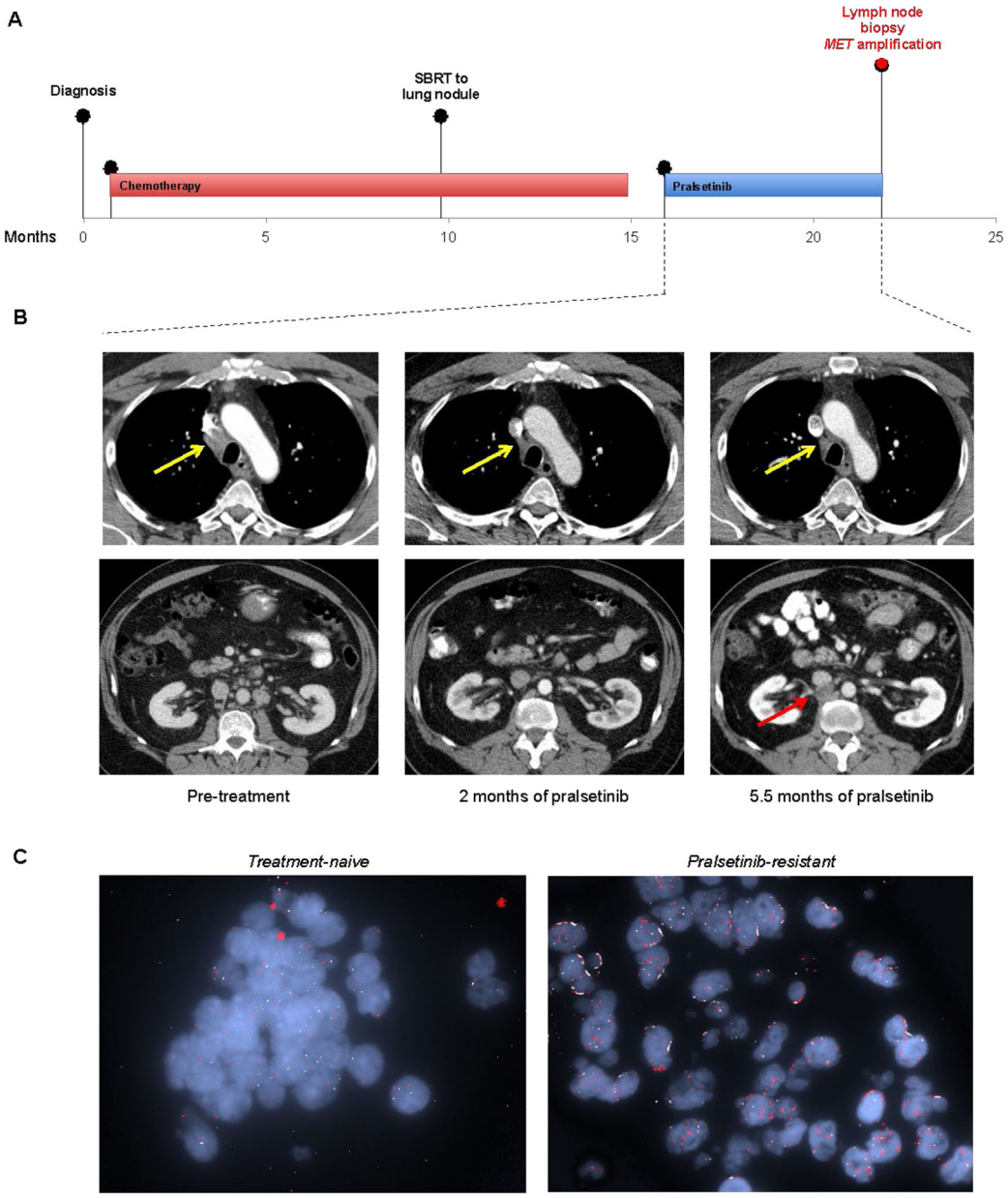

Figure 3.

Emergence of high-level focal *MET* amplification after selective RET inhibition. (A) Treatment and biopsy timeline of MGH2 who had *RET* fusion-positive NSCLC with disease progression on pralsetinib. (B) Representative axial computed tomography images at baseline, 2 months, and 5.5 months after starting pralsetinib. Yellow arrows indicate interval response in the mediastinal lymph node. Red arrow indicates progression of a retroperitoneal lymph node on therapy, which was biopsied. (C) Fluorescence in situ hybridization images capture the *de novo* increase in *MET* copy number at resistance.

Table 1.

Baseline characteristics of the RET inhibitor-resistant cohort with *RET* fusion-positive lung cancer.

Characteristic	n (%), N = 18
Age at diagnosis, median (range)	56.5 (30–77)
Female	10 (56)
Never or light smoker	18 (100)
Adenocarcinoma	18 (100)
RET fusion	
KIF5B-RET	12 (67)
CCDC6-RET	4 (22)
Other	2 (11)
RET inhibitor prior to biopsy	
Selpercatinib	10 (56)
Pralsetinib	7 (39)
Pralsetinib, then selpercatinib	1 (6)*
Prior lines of therapy	
0	3 (17)
1	10 (56)
2 or more	5 (28)
Prior platinum chemotherapy	13 (72)
Prior multikinase inhibitor with anti-RET activity	4 (22)

*One patient underwent a repeat biopsy at resistance to pralsetinib, then started selpercatinib and had another biopsy at resistance to selpercatinib.