Amplification of Wild-Type *RET* Represents a Novel Molecular Subtype of Several Cancer Types With Clinical Response to Selpercatinib

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ABSTRACT

PURPOSE *RET* rearrangements and *RET* activating point mutations represent targetable genomic alterations in advanced solid tumors. However, the frequency and clinicopathologic characteristics of wild-type *RET* amplification in cancer and its potential role as a targetable oncogenic driver are not well-characterized.

METHODS In two institutional cohorts of patients with solid cancers from the Dana-Farber Cancer Institute (DFCI) and Memorial Sloan Kettering Cancer Center (MSKCC) whose tumors underwent next-generation sequencing (NGS), the frequency and clinicopathologic features of wild-type *RET* amplification in the absence of *RET* rearrangements or activating mutations was assessed. The findings were validated using merged data from The Cancer Genome Atlas (TCGA), Genomics Evidence Neoplasia Information Exchange (GENIE), and China Pan-Cancer data sets.

RESULTS The frequency of wild-type *RET* amplification across all solid cancers was 0.08% (26 of 32,505) in the DFCI cohort, 0.05% (26 of 53,152) in the MSKCC cohort, and 0.25% (71 of 28,623) in the cohort from TCGA, GENIE, and China Pan-Cancer. Cancer types with *RET* amplification included non–small-cell lung cancer (NSCLC), hepatobiliary cancer, prostate cancer, breast cancer, and others. The median *RET* copy number in *RET*-amplified cases was 7.5 (range, 6-36) in the DFCI cohort and 5.7 (range, 4-27.7) in the MSKCC cohort. Among 11 *RET*-amplified NSCLCs, eight had no other concurrent driver mutations. Finally, we report on a 69-year-old man with recurrent NSCLC harboring high-level wild-type *RET* amplification (22-28 copies) as the only identified putative genomic driver who experienced both a systemic and intracranial confirmed response to the RET inhibitor selpercatinib.

CONCLUSION Amplification of wild-type *RET* represents a novel, targetable molecular subset of cancer.

ACCOMPANYING CONTENT

Data Supplement

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INTRODUCTION

Genomic alterations in the rearranged during transfection (*RET*) receptor tyrosine kinase gene are targetable oncogenic drivers across multiple cancer types.[1,2] *RET* activating point mutations are common in medullary thyroid cancer while *RET* fusions are found in papillary thyroid cancer (approximately 20%), non–small-cell lung cancer (NSCLC, 1%-2%), and other solid tumors (<1%).[2] Earlier studies with multikinase inhibitors in *RET*-altered cancers were limited by modest efficacy and unfavorable side effect profiles.[3,4] Recently, the selective RET inhibitors selpercatinib and pralsetinib demonstrated higher response rates, more durable efficacy, and fewer toxicities than multikinase RET inhibitors, leading to their approval for cancers with *RET* fusions or activating point mutations.[5-10]

Despite robust characterization of *RET* rearrangements and activating point mutations as oncogenic drivers, whether wild-type *RET* amplification acts as a targetable oncogenic driver is not well-understood. This study investigates the frequency, clinicopathologic characteristics, and genomic

CONTEXT

Key Objective

Although *RET* fusions and activating mutations are well-studied as actionable genomic alterations in multiple solid tumor types, wild-type *RET* amplification remains poorly characterized in cancer. This study examined the frequency and clinicopathologic features of wild-type *RET*-amplified cancer in three pan-cancer cohorts.

Knowledge Generated

Across all solid cancers, the frequency of wild-type *RET* amplification was 0.08%, 0.05%, and 0.25% in the three cohorts, respectively, and was observed in cancer types including non–small-cell lung cancer (NSCLC), hepatobiliary cancer, prostate cancer, and breast cancer. Among *RET*-amplified NSCLCs, 73% had no other concurrent drivers. Finally, we present the first reported case of a response to the RET inhibitor selpercatinib in a patient with *RET*-amplified NSCLC without *RET* fusion or other oncogenic drivers.

Relevance

Wild-type *RET* amplification represents a novel genomic subtype of cancer with susceptibility to targeted therapy, a finding with important implications for targeted treatment strategies in multiple cancer types.

features of wild-type *RET* amplification across solid tumor types. We report the first known case of clinical response to selpercatinib in a patient with metastatic NSCLC with high-level, focal amplification of *RET* without other known *RET* alterations or oncogenic drivers.

METHODS

The frequency of wild-type *RET* amplification in solid tumors was evaluated in three independent pan-cancer cohorts (Data Supplement [Fig A1]). Hematologic malignancies were excluded. For all cohorts, *RET*-amplified cases with a concurrent oncogenic/likely oncogenic *RET* point mutation (per OncoKB[11]) or a concurrent *RET* fusion reported on DNA next-generation sequencing (NGS) were not considered wild-type *RET*-amplified. *RET*-amplified cases identified with sequencing platforms that did not assess for fusions/structural variants (n = 1 case) were also not considered wild-type amplified.

The first pan-cancer cohort consisted of consecutive patients with solid cancers at the Dana-Farber Cancer Institute (DFCI) between 2013 and 2022 whose tumors underwent targeted NGS using the OncoPanel platform,[12] which assesses 277 (version 1, April 2013-July 2014), 302 (version 2, July 2014-September 2016), and 447 (version 3, September 2016-present) cancer-associated genes. *RET* amplification was defined as ≥6 copies. This cutoff is based on an established threshold used for determining amplifications in the OncoPanel platform. Cases with amplification of the entire chromosome 10 (n = 2) rather than having focal *RET* amplification were excluded. Chromosome 10 polysomy was determined by manual visualization of the chromosome 10 copy plots derived from calculated $\log_2$ ratios for all DFCI *RET*-amplified cases (by a dedicated pathologist, M.S.G.),[12] which allowed for easy discernment between focal *RET* amplification versus polysomy (Data Supplement [Fig A2]).

A second institutional analysis was performed in a pan-cancer cohort of patients with solid cancers at the Memorial Sloan Kettering Cancer Center (MSKCC) whose tumors underwent NGS using the MSK-IMPACT platform, which assesses 341 (version 1), 410 (version 2), and 468 (version 3) cancer-associated genes.[13] *RET*-amplified cases from MSKCC were initially identified as those with a *RET* fold change >2, an established threshold used for clinical reporting of amplifications detected using MSK-IMPACT.[13] These identified cases were further evaluated using fraction and allele-specific copy number estimates (FACETS) segmentation methodology, allowing for allele-specific, tumor purity-adjusted determinations of *RET* total copy number and segment size.[14] To ensure high-quality copy number information, samples with an estimated tumor purity <20% or that otherwise failed FACETS quality control were excluded from this further analysis. Ploidy-corrected total copy number was subsequently calculated for samples meeting this requirement; this adjustment allowed for more accurate gene amplification calling since it accounts for the presence of chromosomal aneuploidy or whole-genome doubling. Cases with a ploidy-corrected total copy number ≥4 (an empirical cutoff identified in previous research on detection of amplifications with FACETS)[15] were defined as *RET*-amplified in the MSKCC cohort. For the DFCI and MSKCC cohorts, the thresholds for *RET* amplification were copy number cutoffs specific to the respective unique platforms, which were felt to be more appropriate than arbitrarily introducing a new single threshold across the two cohorts. For both institutional cohorts, patients provided written informed consent to institutional review board–approved protocols at each site.

Finally, analyses using merged data from The Cancer Genome Atlas (TCGA) Pan-Cancer Atlas,[16] Project Genomics Evidence Neoplasia Information Exchange (GENIE) version 13.0[17] (excluding cases from DFCI and MSKCC), and China

Pan-Cancer[18] data sets (all accessed via the cBioPortal for Cancer Genomics[19,20]) were performed to identify the frequency of wild-type *RET* amplification (according to annotations in each data set) in a third pan-cancer cohort.

For all three cohorts, multiple samples from the same patient were only considered as separate cases if they were of different cancer types; for the DFCI cohort, cases from the same patient in which it was ambiguous whether they were of different cancer types were resolved by chart review. Clinicopathologic characteristics of *RET*-amplified cases were abstracted from the medical record or cBioPortal, where available. Given the varied methodologies to evaluate copy count in each cohort, we determined cohort-specific frequencies of *RET* amplification, rather than presenting a single pooled *RET* amplification frequency. For clinicopathologic and genomic features of *RET*-amplified cases, we evaluated both pooled data (especially to allow for exploratory analyses within cancer types, where sample sizes were small) and cohort-specific data.

In the patient case, DNA NGS was performed by OncoPanel.[12] Targeted RNA sequencing was performed by Solid Fusion Assay (Massachusetts General Hospital) using Anchored Multiplex PCR.[21] *RET* fluorescence in situ hybridization (FISH) was performed using a *RET* break-apart probe (Kreatech *RET*, 10q11 Dual Color). Lesions were assessed by computed tomography (CT) and magnetic resonance imaging (MRI) and reviewed by a dedicated radiologist (M.N.) and radiation oncologist (A.A.) according to the RECIST and response assessment in neuro-oncology brain metastases (RANO-BM) criteria, respectively. Consent from the patient for publication of this case was obtained.

RESULTS

RET Amplification Frequency and Clinicopathologic Features Across Cancer Types

To assess *RET* amplification frequency across cancer types and explore associated clinicopathologic and genomic characteristics, two pan-cancer institutional cohorts of solid tumors from DFCI (N = 32,505) and MSKCC (N = 53,152) were assessed. Additionally, merged data of solid cancer cases from the TCGA, GENIE, and China Pan-Cancer data sets (N = 28,623) were evaluated (excluding cases in GENIE from DFCI and MSKCC). Nine cases of *RET* amplification occurring concurrently with *RET* fusion or activating *RET* point mutations were identified (detailed in Data Supplement [Table A1]); these cases were excluded from subsequent analyses to focus on wild-type *RET* amplification. The frequency of wild-type *RET* amplification across all solid cancers was 0.08% (26 of 32,505) in the DFCI cohort, 0.05% in the MSKCC cohort (26 of 53,152), and 0.25% (71 of 28,623) in the cohort from TCGA, GENIE, and China Pan-Cancer (Figs 1A-1C, Data Supplement [Fig A1]). In NSCLC, the frequency of *RET* amplification was 0.08% (4 of 4,778) in the DFCI cohort, 0.04% (3 of 7,139) in the MSKCC cohort, and 0.09% (4 of 4,293) in the TCGA, GENIE, and China Pan-Cancer cohort (Figs 1A-1C). Of the 11 *RET*-amplified NSCLC cases, five were adenocarcinoma, four were squamous cell carcinoma, one was NSCLC favor adenocarcinoma, and one was adenoid cystic carcinoma. Other cancer types with *RET* amplification included breast cancer, hepatobiliary cancer, and prostate cancer, among others. Detailed frequencies of *RET* amplification across cancer types are presented in the Data Supplement (Table A2).

In the DFCI cohort, the median *RET* gene copy number among amplified cases was 7.5 (range, 6-36) while in the MSKCC cohort, the median *RET* gene copy number was 5.7 (range, 4-27.7) (Data Supplement [Fig A3A]). Regarding clinicopathologic and genomic features of *RET*-amplified cases, among the 11 *RET*-amplified NSCLCs, eight had no other codriver alterations while three had concurrent driver alterations (*MET* amplification, *KRAS* G12C, and *EGFR* exon 20 insertion) (Data Supplement [Fig A3B]). The median age of patients with *RET*-amplified NSCLC was 69 years and 36.4% were female. In eight *RET*-amplified NSCLC cases with available smoking status, five had a history of tobacco use, two of which had concurrent drivers (*KRAS* G12C and *MET* amplification). Of the 24 *RET*-amplified breast cancer cases, four had concurrent *PIK3CA* mutations, and of the 15 *RET*-amplified breast cancers with immunohistochemistry available, seven were triple-negative, two were triple-positive, three were estrogen receptor–negative (ER–)/progesterone receptor–negative (PR–)/human epidermal growth factor receptor 2–positive (HER2+), two were ER+/PR+/HER2–, and one was ER+/PR–/HER2– (Data Supplement [Fig A3C]). Detailed clinicopathologic and genomic characteristics for the three cohorts are summarized in the Data Supplement (Tables A3-A5).

Case

We present a case of response to selpercatinib in a patient with wild-type *RET*-amplified NSCLC without *RET* fusion or other oncogenic alterations. The patient is a 69-year-old man with a 40 pack-year history of tobacco use who presented with hoarseness and was found to have unresectable stage III NSCLC favor adenocarcinoma, with a 5.6-cm left upper lobe (LUL) lung mass and involvement of 4R and 4L lymph nodes (Data Supplement [Fig A4]). Pathologic analysis showed weak positivity for TTF-1 and INSM1, positive (retained) RB1 expression, and negative p40 and PD-L1 expression (Data Supplement [Fig A5A-C]). Positron emission tomography-CT imaging showed no distant metastases, and a brain MRI was negative for metastatic disease. NGS showed focal *RET* amplification (estimated 22 copies, Data Supplement [Fig A6]) without *RET* fusion or *RET* point mutation and was negative for other oncogenic driver mutations in *KRAS*, *EGFR*, *ALK*, *ROS1*, *BRAF*, *MET*, *NTRK*, *HER2*, and *NRG1*. Targeted RNA sequencing demonstrated no fusions but a high number of *RET* transcripts compared with historical NSCLC controls.

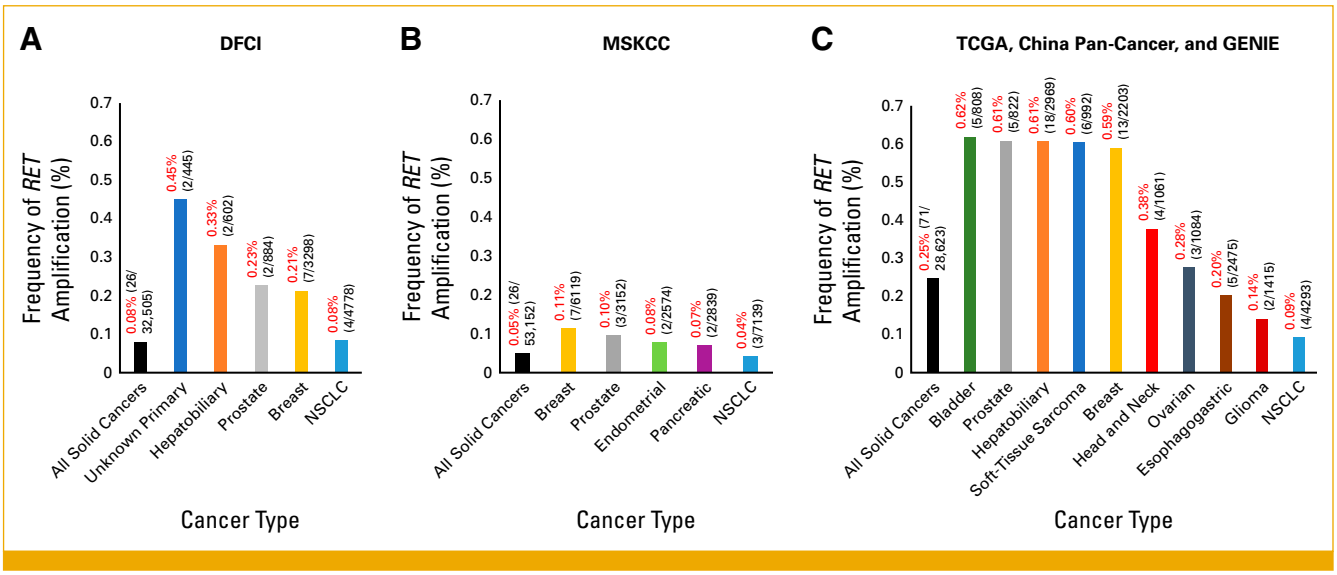

FIG 1. (A) Frequency of wild-type *RET* amplification overall and by individual cancer type in a pan-cancer cohort of 32,505 sequenced solid tumor cases from DFCI. (B) Frequency of wild-type *RET* amplification overall and by individual cancer type in a pan-cancer cohort of 53,152 solid tumor cases from MSKCC. (C) Frequency of wild-type *RET* amplification overall and by individual cancer type in 28,623 solid tumor cases from the TCGA Pan-Cancer Atlas, GENIE (excluding cases from DFCI and MSKCC), and the China Pan-Cancer data sets. For all three bar graphs (A-C), cancer types displayed are those with a frequency of >1% of the cohort and those in which >1 *RET*-amplified case was identified. Data Supplement (Table A2) summarizes frequencies across all observed cancer types. DFCI, Dana-Farber Cancer Institute; GENIE, Genomics Evidence Neoplasia Information Exchange; MSKCC, Memorial Sloan Kettering Cancer Center; NSCLC, non–small-cell lung cancer; TCGA, The Cancer Genome Atlas.

The patient was initially treated with cisplatin plus pemetrexed and concurrent radiation followed by durvalumab consolidation, with a decrease in the LUL mass. Subsequent imaging approximately 3 months after durvalumab initiation showed increased right axillary lymphadenopathy, and a biopsy of this lymph node revealed recurrent NSCLC with similar morphology and immunohistochemical phenotype to the prior (Data Supplement [Fig A5D-F]). Additionally, a brain MRI demonstrated a new enhancing right frontal lobe metastasis. Genomic sequencing of the recurrent axillary lymph node redemonstrated *RET* amplification (28 copies), without other oncogenic alterations (Fig 2A). Additionally, *RET* FISH on the axillary recurrence showed no split signals or isolated 3′ signals to indicate a *RET* rearrangement but did show marked 5′ *RET* signal amplification (5′ signals with >25 copies per cell and an intact number of normal fused 5′ and 3′ signals, Fig 2B), supportive of focal *RET* amplification rather than chromosome 10 polysomy. Repeat targeted RNA sequencing again showed no oncogenic fusions but increased *RET* transcript levels compared with historical NSCLC controls, similar to the previous analysis (Fig 2C).

Given the progression after recent platinum-doublet chemotherapy and PD-L1 immunotherapy, along with the finding of high-level *RET* amplification, the patient was started on off-label selpercatinib at the standard dose of 160 mg twice daily. Stereotactic radiosurgery to the brain metastasis was considered in multidisciplinary discussion with radiation oncology but deferred given the lesion's close proximity to the optic nerve. Subsequent serial imaging while on selpercatinib demonstrated an ongoing confirmed objective response, with a complete response in the right axillary lymph node achieved at approximately 6 weeks (ongoing at approximately 5 months; Fig 2D, Data Supplement [Fig A7]) and a partial response in the brain metastasis achieved at 3 months (ongoing at approximately 6 months; Fig 2E, Data Supplement [Fig A7]). Selpercatinib was tolerated with an adverse effect profile consistent with that previously published.[8] The patient experienced transient grade 1 transaminase elevation that subsequently resolved as well as intermittent abdominal pain, indeterminate for causal relationship to selpercatinib, or baseline chronic diverticulitis.

DISCUSSION

Selective targeting of RET has become the standard of care for patients with *RET* fusion–positive cancer or *RET* activating point mutations. Here, we demonstrate that amplification of wild-type *RET* represents a novel, targetable, albeit rare molecular subtype across cancer types, and to our knowledge, we report the first case of clinical response to the RET inhibitor selpercatinib in a patient with advanced NSCLC with *RET* amplification and no other known oncogenic alterations.

RET amplification was first described in thyroid cancer and has subsequently been identified in various cancer types. In a previous pan-cancer cohort (N = 4,871), 0.5% had *RET*

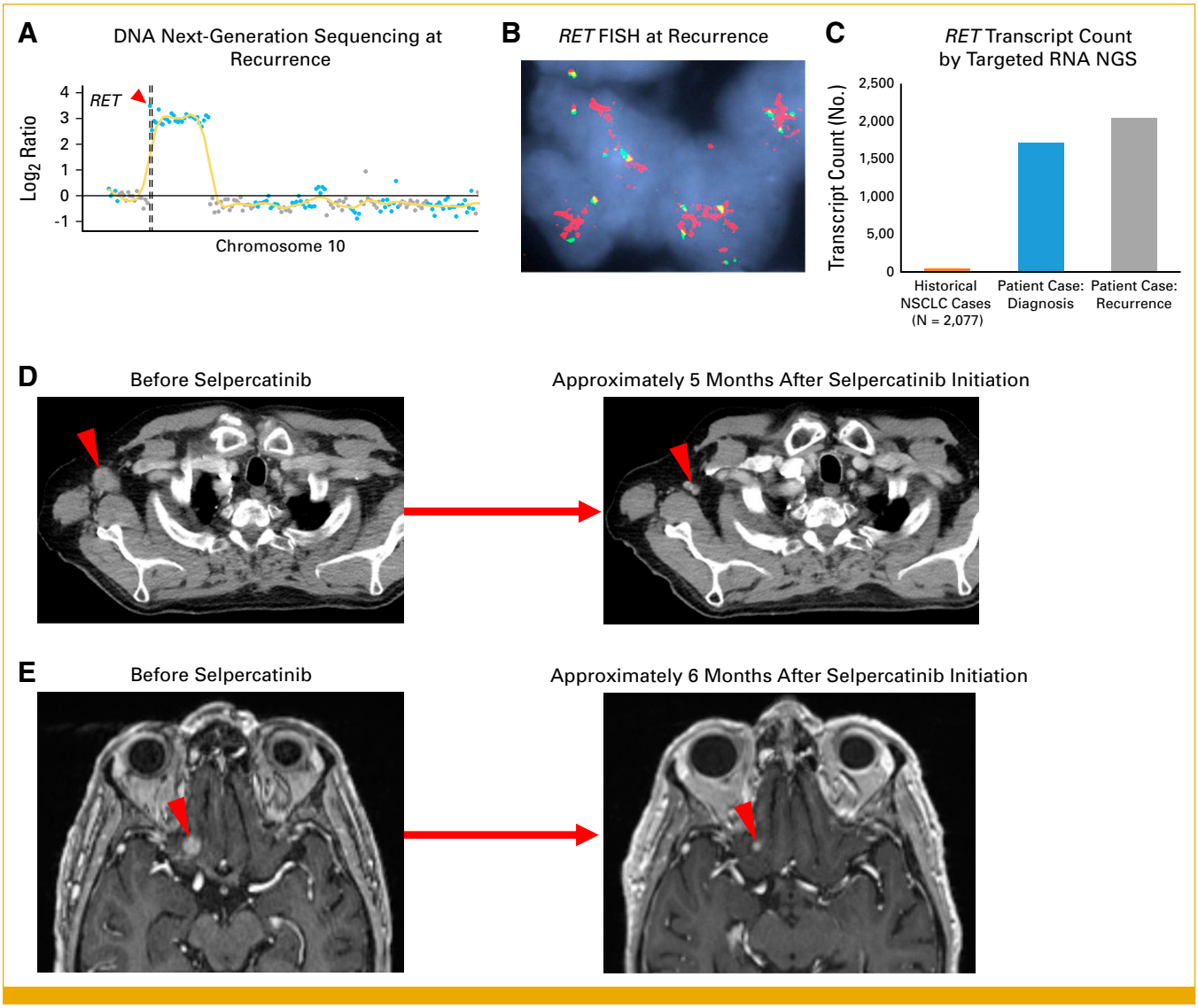

FIG 2. (A) Copy number plot from NGS of the right axillary lymph node recurrence showing high-level *RET* amplification. The red arrow denotes the position of the *RET* gene along chromosome 10. The vertical dashed line represents the centromere position. (B) FISH of formalin-fixed paraffin-embedded tissue from the right axillary lymph node recurrence. The red 5′ probe covers most of the *RET* gene and sequences upstream of *RET* while the green 3′ probe is outside and downstream to *RET*. (C) Quantification of total *RET* intragenic transcript count (ie, all splice variants) using a targeted RNA NGS fusion assay by anchored multiplexed PCR in the patient case at diagnosis and recurrence compared with the mean intragenic transcript count from a historical cohort of NSCLC controls between 2015 and 2022. (D) CT imaging demonstrates a response to selpercatinib in the right axillary lymph node. (E) Contrast-enhanced brain MRI shows a response to selpercatinib in a right inferior frontal lobe metastasis. CT, computed tomography; FISH, fluorescence in situ hybridization; MRI, magnetic resonance imaging; NGS, next-generation sequencing; NSCLC, non–small-cell lung cancer; PCR, polymerase chain reaction.

amplification (with an incidence of 0.48% in NSCLC)[22]; in a separate breast cancer cohort (N = 9,693), the frequency was 0.84%.[23] To our knowledge, our analyses provide the largest pan-cancer assessment of *RET* amplification to date. We found that *RET* amplification frequency across all solid cancer types was 0.08% in the DFCI cohort, 0.05% in the MSKCC cohort, and 0.25% in the TCGA, GENIE, and China Pan-Cancer cohort and that the frequency in NSCLC was 0.08%, 0.04%, and 0.09% in these three cohorts, respectively. Our observed frequencies are lower than previously reported, which may, in part, be due to the larger cohort size in our study. Additionally, in the DFCI cohort, cases with amplification of the entire chromosome 10 rather than focal *RET* amplification were excluded while in the MSKCC cohort, FACETS was used to correct for tumor ploidy as previously described[14]; these measures may contribute to a lower, more accurate representation of the frequency of focal *RET* amplification (information on ploidy was not available in the TCGA, GENIE, and China Pan-Cancer cohort). Differing amplification thresholds may additionally account for some of the

differences in frequency estimates between our cohorts. Given the unique methodologies in determining copy number count and distinct amplification thresholds in the DFCI and MSKCC platforms, we used these cohort-specific cutoffs, rather than impose a new single arbitrary cutoff across cohorts. Varied methodologies between institutions in evaluating copy count pose inherent limitations, an area that merits future work.

Our analyses also shed light on the clinicopathologic and genomic features of *RET*-amplified tumors. A disproportionate number of *RET*-amplified breast cancer cases were triple-negative (7 of 15 cases with available immunohistochemistry); although the sample size is small, this finding is intriguing and is in line with prior evidence that *RET*-amplified breast cancer tends to be ER-negative and *ERBB2* nonamplified.[23] This observation must be interpreted in the context of the fact that breast cancer in general, and *TP53*-mutant triple-negative breast cancer in particular, tends to be characterized by genomic instability with multiple amplification events.[24] Regarding NSCLC, compared with previous real-world reports on clinical characteristics of NSCLC harboring a *RET* fusion, the 11 *RET*-amplified NSCLC cases in this study were slightly older (median age 69 years, compared with a median age of 56-65 years in previous studies of *RET*-fused NSCLC) and more male-predominant (36.4% female, compared with 45%-56% female in previous studies of *RET*-fused NSCLC).[25-28] A history of smoking was present in 62.5% of *RET*-amplified NSCLC cases, with available smoking status slightly higher than the historical rate of tobacco use (31%-49.1%) observed among *RET*-fused NSCLC in previous studies.[25-28] Additionally, 36.4% of *RET*-amplified NSCLC cases were squamous cell carcinoma, higher than the frequency (0%-1.7%) seen previously in *RET*-fused NSCLC.[25-28] These conclusions are limited by the small sample size of our *RET*-amplified NSCLC subset. There was not sufficient data on PD-L1 and tumor mutational burden (TMB) in our *RET*-amplified NSCLC cases to allow for meaningful comparisons. Finally, a key finding from our exploration of genomic features of *RET*-amplified tumors is that 8 of the 11 *RET*-amplified NSCLC cases did not have a concurrent driver alteration, suggesting that *RET* amplification may be the sole potential oncogenic driver in a subset of lung cancers.

The ability of *RET* amplification to serve as a potential oncogenic alteration vulnerable to targeted therapy is supported by our case of a patient with *RET*-amplified NSCLC without other known drivers who responded to selpercatinib. Three previous case reports observed efficacy of multikinase inhibitors in *RET*-amplified tumors: one case described response to sunitinib in treatment-refractory *RET*-amplified germ cell tumor,[29] the second documented response to cabozantinib + nivolumab in a patient with hepatocellular carcinoma harboring *RET* amplification, high TMB, and positive PD-L1 expression (although whether the kinase inhibitor or the immunotherapy drove this response is unclear),[30] and the third reported stable disease in a patient with *RET*-amplified adenocarcinoma of the tongue (thought to have originated in a minor salivary gland) treated with sunitinib, with 22% shrinkage of lung metastases.[31] Importantly, the first two cases did not pursue RNA NGS or FISH testing and thus may have overlooked the presence of *RET* fusion since *RET* fusion and amplification can cooccur. In contrast to the activity of multikinase inhibitors in these cases, another study observed no difference in response rate among 24 patients with *RET*-amplified NSCLC who received vandetanib versus a comparator arm in four phase III trials of vandetanib, although conclusions from this study are limited by the small sample size and the modest impact of multikinase inhibitors even in *RET* fusion-positive cases.[32] With the advent of selective RET inhibitors, whether these therapies are effective against *RET*-amplified tumors is of interest. One report documented response to pralsetinib in a patient with NSCLC with a novel intergenic *RET* fusion and *RET* amplification, although whether the fusion or amplification accounted for the response is unclear.[33] Notably, a recent case report observed a response to selpercatinib in *RET*-amplified glioblastoma.[34] This report together with our case of response to selpercatinib in wild-type *RET*-amplified NSCLC provide compelling motivation for further investigation of *RET* amplification as a possible novel targetable oncogenic driver across cancer types. Indeed, the potential oncogenicity and targetability of *RET* amplification is supported by preclinical studies showing that *RET* amplification promotes transformation of nontumorigenic mammary cells in vitro and that overexpression or amplification of wild-type *RET* in mice induces formation of mammary tumors that are susceptible to RET inhibitors.[23,35] The possibility of *RET* amplification serving as a targetable oncogenic driver is further supported by analogous situations that exist for *HER2* and *MET* amplification in NSCLC.[36-38] Together, the body of preclinical data supporting wild-type *RET* amplification as an oncogenic driver, our case and prior cases of response of wild-type *RET*-amplified cancer to selective RET inhibition, and the parallels with *HER2* and *MET* amplification offer important evidence that wild-type *RET* amplification may serve as a novel driver alteration.

Another intriguing question is whether *RET* amplification can serve as a mechanism of acquired resistance to targeted therapies for other driver alterations. Our *RET*-amplified cases with available treatment course information either already had *RET* amplification at baseline or did not have paired pretreatment and post-treatment genomics available to examine this question. However, *RET* amplification was previously reported as a potential mechanism of acquired resistance to HER2-targeted therapy in a patient with breast cancer.[23] Further research is warranted to probe this question in greater depth.

Overall, this study provides evidence that amplification of wild-type *RET* represents a novel, actionable, rare genomic subset of NSCLC and other cancers. This finding underscores the importance of broad next-generation sequencing to identify rare but actionable alterations that can profoundly influence patients' lives and motivates future work in larger *RET*-amplified cohorts to help inform targeted treatment strategies across cancer types.

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M.M.A. and J.R. are senior authors and contributed equally to this work.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Research Funding: Foundation Medicine
Patents, Royalties, Other Intellectual Property: Wolters Kluwer (Royalties for Pocket Oncology), Osimertinib Selpercatinib
Other Relationship: Merck, GlaxoSmithKline, Teva, Taiho Pharmaceutical, Pfizer, PharmaMar, Puma Biotechnology, Pfizer, Merus, Boehringer Ingelheim

Lynette Sholl
Stock and Other Ownership Interests: Moderna Therapeutics
Consulting or Advisory Role: Genentech (Inst), Lilly (Inst), AstraZeneca
Research Funding: Roche/Genentech (Inst), Bristol Myers Squibb (Inst)

Mark M. Awad
Consulting or Advisory Role: Merck, Pfizer, Bristol Myers Squibb, Foundation Medicine, Novartis, Gritstone Bio, Mirati Therapeutics, EMD Serono, AstraZeneca, Instil Bio, AstraZeneca, Regeneron, Janssen, Affini-T Therapeutics, Affini-T Therapeutics
Research Funding: Genentech/Roche (Inst), Lilly (Inst), AstraZeneca (Inst), Bristol Myers Squibb (Inst), Amgen (Inst)
Travel, Accommodations, Expenses: Bristol Myers Squibb Foundation
Open Payments Link: https://openpaymentsdata.cms.gov/physician/1127368

Julia Rotow
Honoraria: AstraZeneca
Consulting or Advisory Role: AstraZeneca, Gritstone Bio, AbbVie, Lilly, Takeda, Guardant Health, Sanofi/Regeneron, Genentech, Janssen, Bioatla, Bioatla, G1 Therapeutics, Jazz Pharmaceuticals, Amgen, Amgen
Travel, Accommodations, Expenses: AstraZeneca

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REFERENCES

1. Shabbir A, Kojadinovic A, Shafiq T, et al: Targeting RET alterations in cancer: Recent progress and future directions. Crit Rev Oncol Hematol 181:103882, 2023
2. Subbiah V, Cote GJ: Advances in targeting RET-dependent cancers. Cancer Discov 10:498-505, 2020
3. Drilon A, Rekhtman N, Arcila M, et al: Cabozantinib in patients with advanced RET-rearranged non-small-cell lung cancer: An open-label, single-centre, phase 2, single-arm trial. Lancet Oncol 17: 1653-1660, 2016
4. Gautschi O, Milia J, Filleron T, et al: Targeting RET in patients with RET-rearranged lung cancers: Results from the Global, Multicenter RET Registry. J Clin Oncol 35:1403-1410, 2017
5. Wirth LJ, Sherman E, Robinson B, et al: Efficacy of selpercatinib in RET-altered thyroid cancers. N Engl J Med 383:825-835, 2020
6. Subbiah V, Hu MI, Wirth LJ, et al: Pralsetinib for patients with advanced or metastatic RET-altered thyroid cancer (ARROW): A multi-cohort, open-label, registrational, phase 1/2 study. Lancet Diabetes Endocrinol 9:491-501, 2021
7. Griesinger F, Curigliano G, Thomas M, et al: Safety and efficacy of pralsetinib in RET fusion-positive non-small-cell lung cancer including as first-line therapy: Update from the ARROW trial. Ann Oncol 33:1168-1178, 2022
8. Drilon A, Oxnard GR, Tan DSW, et al: Efficacy of selpercatinib in RET fusion-positive non-small-cell lung cancer. N Engl J Med 383:813-824, 2020
9. Subbiah V, Cassier PA, Siena S, et al: Pan-cancer efficacy of pralsetinib in patients with RET fusion-positive solid tumors from the phase 1/2 ARROW trial. Nat Med 28:1640-1645, 2022
10. Subbiah V, Wolf J, Konda B, et al: Tumour-agnostic efficacy and safety of selpercatinib in patients with RET fusion-positive solid tumours other than lung or thyroid tumours (LIBRETTO-001): A phase 1/2, open-label, basket trial. Lancet Oncol 23:1261-1273, 2022
11. Chakravarty D, Gao J, Phillips SM, et al: OncoKB: A precision oncology knowledge base. JCO Precis Oncol 10.1200/PO.17.00011
12. Garcia EP, Minkovsky A, Jia Y, et al: Validation of OncoPanel: A targeted next-generation sequencing assay for the detection of somatic variants in cancer. Arch Pathol Lab Med 141:751-758, 2017
13. Cheng DT, Mitchell TN, Zehir A, et al: Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT): A hybridization capture-based next-generation sequencing clinical assay for solid tumor molecular oncology. J Mol Diagn 17:251-264, 2015
14. Shen R, Seshan VE: FACETS: Allele-specific copy number and clonal heterogeneity analysis tool for high-throughput DNA sequencing. Nucleic Acids Res 44:e131, 2016
15. Repetto M, Richards AL, Chen MF, et al: MYC amplification segment-size mutual exclusivity analysis with other oncogenic events in a pan-cancer sequencing cohort. J Clin Oncol 41, 2023 (suppl 16; abstr 3073)
16. Weinstein JN, Collisson EA, Mills GB, et al: The Cancer Genome Atlas Pan-cancer analysis project. Nat Genet 45:1113-1120, 2013
17. André F, Arnedos M, Baras AS, et al: AACR Project GENIE: Powering precision medicine through an International Consortium. Cancer Discov 7:818-831, 2017
18. Wu L, Yao H, Chen H, et al: Landscape of somatic alterations in large-scale solid tumors from an Asian population. Nat Commun 13:4264, 2022
19. Cerami E, Gao J, Dogrusoz U, et al: The cBio cancer genomics portal: An open platform for exploring multidimensional cancer genomics data. Cancer Discov 2:401-404, 2012
20. Gao J, Aksoy BA, Dogrusoz U, et al: Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 6:pl1, 2013
21. Zheng Z, Liebers M, Zhelyazkova B, et al: Anchored multiplex PCR for targeted next-generation sequencing. Nat Med 20:1479-1484, 2014
22. Kato S, Subbiah V, Marchlik E, et al: RET aberrations in diverse cancers: Next-generation sequencing of 4,871 patients. Clin Cancer Res 23:1988-1997, 2017
23. Paratala BS, Chung JH, Williams CB, et al: RET rearrangements are actionable alterations in breast cancer. Nat Commun 9:4821, 2018
24. Duijf PHG, Nanayakkara D, Nones K, et al: Mechanisms of genomic instability in breast cancer. Trends Mol Med 25:595-611, 2019
25. Aldea M, Marinello A, Duruisseaux M, et al: RET-MAP: An International multicenter study on clinicobiologic features and treatment response in patients with lung cancer harboring a RET fusion. J Thorac Oncol 18:576-586, 2023
26. Lee J, Ku BM, Shim JH, et al: Characteristics and outcomes of RET-rearranged Korean non-small cell lung cancer patients in real-world practice. Jpn J Clin Oncol 50:594-601, 2020
27. Hess LM, Han Y, Zhu YE, et al: Characteristics and outcomes of patients with RET-fusion positive non-small lung cancer in real-world practice in the United States. BMC Cancer 21:28, 2021
28. Drilon A, Lin JJ, Filleron T, et al: Frequency of brain metastases and multikinase inhibitor outcomes in patients with RET-rearranged lung cancers. J Thorac Oncol 13:1595-1601, 2018
29. Subbiah V, Meric-Bernstam F, Mills GB, et al: Next generation sequencing analysis of platinum refractory advanced germ cell tumor sensitive to Sunitinib (Sutent®) a VEGFR2/PDGFRβ/c-kit/FLT3/RET/CSF1R inhibitor in a phase II trial. J Hematol Oncol 7:52, 2014
30. Yang X, Shi J, Chen X, et al: Efficacy of cabozantinib and nivolumab in treating hepatocellular carcinoma with RET amplification, high tumor mutational burden, and PD-L1 expression. Oncologist 25:470-474, 2020
31. Jones SJ, Laskin J, Li YY, et al: Evolution of an adenocarcinoma in response to selection by targeted kinase inhibitors. Genome Biol 11:R82, 2010
32. Platt A, Morten J, Ji Q, et al: A retrospective analysis of RET translocation, gene copy number gain and expression in NSCLC patients treated with vandetanib in four randomized Phase III studies. BMC Cancer 15:171, 2015
33. Wang SS, Wang F, Zeng Z, et al: Case report: A novel intergenic MIR4299/MIR8070-RET fusion with RET amplification and clinical response to pralsetinib in a lung adenocarcinoma patient. Front Oncol 12:929763, 2022
34. Czech C, Chen A, Morgan KP, et al: Response to selpercatinib in a patient with recurrent glioblastoma and RET amplification. J Natl Compr Canc Netw 20:966-971, 2022
35. Gattelli A, Garcia Sola ME, Roloff TC, et al: Chronic expression of wild-type Ret receptor in the mammary gland induces luminal tumors that are sensitive to Ret inhibition. Oncogene 37:4046-4054, 2018
36. Remon J, Hendriks LEL, Mountzios G, et al: MET alterations in NSCLC-current perspectives and future challenges. J Thorac Oncol 18:419-435, 2023
37. Noonan SA, Berry L, Lu X, et al: Identifying the appropriate FISH criteria for defining MET copy number-driven lung adenocarcinoma through oncogene overlap analysis. J Thorac Oncol 11: 1293-1304, 2016
38. Riudavets M, Sullivan I, Abdayem P, et al: Targeting HER2 in non-small-cell lung cancer (NSCLC): A glimpse of hope? An updated review on therapeutic strategies in NSCLC harbouring HER2 alterations. ESMO Open 6:100260, 2021