MINI REVIEW

Advances in Targeting RET-Dependent Cancers

Vivek Subbiah1,2,3 and Gilbert J. Cote4

ABSTRACT  RET alterations have been characterized as oncogenic drivers in multiple cancers. The clinical validation of highly selective RET inhibitors demonstrates the utility of specific targeting of aberrantly activated RET in patients with cancers such as medullary thyroid cancer or non-small cell lung cancer. The remarkable responses observed have opened the field of RET-targeted inhibitors. In this review, we seek to focus on the impact of therapeutic RET targeting in cancers.

Significance: Successful clinical translation of selective RET inhibitors is poised to alter the therapeutic landscape of altered cancers. Questions that clearly need to be addressed relate to the ability to maintain long-term inhibition of tumor cell growth, how to prepare for the potential mechanisms of acquired resistance, and the development of next-generation selective RET inhibitors.

INTRODUCTION

Genome-driven precision oncology has altered the therapeutic landscape of multiple kinase-driven hematologic and solid malignancies. The use of imatinib in BCR–ABL fusion–positive chronic myelogenous leukemia (1) and the use of crizotinib in ALK fusion– and ROS1 fusion–positive non–small cell lung cancer (NSCLC) are prime examples of transformative first-generation kinase inhibitor therapies (2, 3). Twenty-five years ago, inherited mutations in RET mutations were identified as the cause of multiple endocrine neoplasia type 2 (MEN2; refs. 4–7), which was soon followed by the discovery of somatic activating RET aberrations, genetic fusions, or mutations in diverse malignancies (8–10) warranting their choice as prospective therapeutic targets (Fig. 1). RET fusions are seen in NSCLC (2%) and papillary thyroid cancers (PTC; 10%–20%), whereas somatic (60%–90%) or germline (100%) RET mutations are seen in medullary thyroid cancer (MTC; refs. 11, 12).

Although RET was one of the earliest genes to be cloned, and several multikinase inhibitors (MKI) have RET inhibitory activity, patients with RET-driven malignancies, especially patients with RET fusion–positive NSCLC, have derived only limited benefit from MKIs with secondary RET activity. MKIs such as vandetanib and cabozantinib are FDA-approved in the treatment of advanced MTC and have demonstrated activity in patients with RET fusion–positive NSCLC, but their response rates and duration of response are lower when compared with other selective kinase inhibitors for ALK or ROS1 fusion–driven NSCLC (2, 3, 11, 13, 14). Development of selective RET inhibitors is poised to change this paradigm. In this review, we focus on the impact of RET therapeutic targeting in cancers, within the context of a relatively short-term treatment experience. Questions that need to be further addressed include the ability to maintain long-term inhibition of tumor cell growth, and how to prepare for the potential mechanisms of acquired resistance. We also consider the need for development of second-generation selective RET inhibitors, and finally the potential side effects associated with reduced RET activity in tissues reliant on its expression.

THE DISCOVERY OF THE RET PROTO-ONCOGENE

By 1985, the search for human oncogenes was rapidly advancing. Approximately a dozen or so transforming genes, most notably the RAS family members, had already been identified using a simple assay of transfecting human tumor DNA into NIH 3T3 cells. Serial passaging of the transformed NIH 3T3 cells allowed for these human oncogenes to be cloned through their association with human repetitive DNA sequences. Interestingly, the coincidence of using DNA isolated from a patient with T-cell lymphoma, 3215, led to the discovery of the RET oncogene (15). A single transformed colony was ultimately expanded through secondary and tertiary transfections, providing both confirmation of a transforming oncogene and a DNA source for characterization (15).
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RET fusion partners

KIF5B**
CCDC6**
NCOA4**
TRIM33
CUX1
KIAA1217
FRMD4A
KIAA1468
PRKAR1A
FKBP15
NCOA
GOLGA5
TRIM24
TRIM27
KTN1
RFG9
ER1
HOOK3
PM1
AKAP13
SPEC1L
TBLX1R1
FGFR1OP
EML4
EPHA5
SOSTM1
PARD3
PIPALM
AFAP1L2
PPFIBP2
ACBD5
MYH13

**Most common fusions or mutations

Somatic RET fusions and mutations associated with oncogenesis

Meningioma (5.6%)
PTC (10% to 20%)
NSCLC (1% to 2%)

MTC (~60% to 90%)

Malignant (0.7%) and basal cell carcinoma (12.5%)

Esophageal adenocarcinoma (1.4%)
Breast carcinoma (0.2%)
Gastric adenocarcinoma (0.7%)
Ureter urothelial carcinoma (16.7%)
Colorectal adenocarcinoma (0.7%)

Germline RET mutation associated with oncogenesis

C515
C609
C611
C618
C620
C630
E632
C634**(MEN2A)
V648
K666
E768
L790
Y791
V804M*
V804L
Y806C
A883
S891
S904
M918**(MEN2B)

Figure 1. Frequency and distribution of RET fusions and RET mutations across malignancies. Visual art © 2019 The University of Texas MD Anderson Cancer Center. Red text indicates the most prevalent RET-dependent malignancies. CMML, chronic myelomonocytic leukemia.

However, when the 3215 transforming DNA was compared with normal human DNA, a discontinuity was discovered, leading to the hypothesis that the oncogene was derived from recombination of two unlinked segments. The authors proposed a mechanism of REarrangement during Transfection that ultimately led to the naming of the RET oncogene. Molecular analysis of the RET transforming gene determined the fusion partner to be an upstream ring finger domain (originally unrelated to genes identified at the time) and a downstream transmembrane linked to a tyrosine kinase domain (16). As other tyrosine kinases had previously been linked to oncogenic transformation, it was ultimately the gene encoding this domain that was given the name “RET proto-oncogene.” Despite the belief that the RET oncogene was created through an experimental artifact, the same NIH 3T3 transformation assay was able to subsequently demonstrate the frequent occurrence of RET gene fusions in papillary thyroid cancers (17) and to confirm the transforming ability of MEN2-associated RET-activating mutations (18).

WHY IS RET AN ONCOGENE?

In the decades that have passed since the discovery of RET, much has been uncovered related to its role in cancer. To date, three general mechanisms of aberrant RET activation have been reported in cancer: in-frame RET gene fusions (15, 16), targeted mutation of the RET gene itself (4–6), and finally aberrant overexpression of the RET gene (19, 20). What the three mechanisms appear to share in common is the inappropriate activation of the tyrosine kinase, most commonly in the complete absence of ligand. The multifunctional docking sites at phosphoryrosine 1062 (pY1062) and pY1096 serve as the primary RET signaling hubs (reviewed in refs. 22, 23). Activation of RAS–MAPK and PI3K–AKT signaling pathways results from adaptor protein binding to these docking sites (Fig. 2; refs. 22, 23).

MECHANISMS OF RET ACTIVATION, SIMILARITIES AND DIFFERENCES

Targeted mutation of RET results in aberrant activation through three broad mechanisms—dimerization through the formation of intermolecular cysteine disulfide bonds, impacting of the ATP-binding domain, and finally enhancement of the kinase domain activity (Fig. 2). Because germline RET activating mutations occur in patients with hereditary MEN2, we know that tumorigenesis is primarily limited to
a subpopulation of cells of neuroendocrine origin, most notably thyroid C cells and cells within the adrenal medulla. Although it has been argued that the specificity of tumor formation derives from the high level of RET expression in these cell types, this is clearly an oversimplification. RET is known to be more broadly expressed and has clear roles in mediating progenitor stem-cell function.

To date, germline RET fusions have not been observed, only cell-specific somatic fusions. They were initially thought to be limited to thyroid follicular cell tumors, but have more recently been identified in 1% to 2% of NSCLCs, and at a <1% frequency in a range of tumor types, including colorectal cancer, breast cancer, and pancreatic cancer (11, 12). There are numerous RET fusion partners, all of which appear to provide dimerization domains (Fig. 1). A survey of datasets for cancer types within cBioPortal finds that approximately 10% of reported RET mutations are fusions, with approximately 10% of those comprising tumor types other than thyroid or lung. RET fusions are the most commonly observed aberration in tumors that are not of neuroendocrine origin. RET fusions are thought to be oncogenic for two reasons. First, fusion provides a mechanism to aberrantly express RET in a cell type where it is normally transcriptionally silent. Second, in all cases the extracellular domain is replaced with a protein dimerization domain. The outcome is the production of an intracellular RET tyrosine kinase domain capable of ligand-independent activation. Interestingly, despite the absence of a transmembrane domain, the RET fusion proteins remain capable of MAPK pathway and PI3K-AKT activation. However, beyond the examination of proliferative signaling, the cellular functions of RET fusion have not been extensively studied. It is also important to point out that key regulatory mechanisms of RET inactivation, such as endocytosis and recruitment of membrane-associated ubiquitin ligases, do not appear to affect the fusion proteins, which additionally may enhance their oncogenicity (24, 25). Furthermore, when localized to the cytoplasm or nucleus, monomeric RET has additional functions, such as regulation of ATF4, that could certainly be affected (26). These circumstances seemingly predict a greater oncogenic potential for RET fusions compared with activating mutations that remains to be fully addressed. Direct comparisons of RET fusions with full-length RET containing activating mutations are limited, but it is important to note that experimental inclusion of RET-activating mutations into fusion constructs does not increase tumorigenicity in xenograft models (27, 28). Given these differences, we are largely left to speculate on the precise impact of targeted inhibition on oncogenesis driven by these two mechanisms of aberrant RET activation. As a result, our understanding of targeting RET-driven cancer continues to evolve largely through clinical trials (27, 28).

**DOES TARGETING RET WORK?**

The original concept of therapeutically targeting RET largely stems from MTC studies. Activating germline mutations cause MTC, somatic activating mutations are found in sporadic MTC, and these same mutations are capable of inducing cellular transformation in the NIH 3T3 cell
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Dose-reduction rate and drug-discontinuation rates

Together with nonselectivity for RET and patients can tolerate, leading to drug discontinuation or dose reduction that limit use in some patients or limit the dose that clinical side effects such as nausea, diarrhea, rash, and hyperactivity leads to significant and sometimes prohibitive “off-target” discovery of their inhibitory actions on RET. This MKI activity (axitinib) have been shown to be effective in RET-cabozantinib, lenvatinib, and sorafenib) function primarily as VEGFR2 and MET, but were repurposed because of the RET activity that have been approved by the FDA for MTC. (33).

However, as vandetanib is a repurposed VEGFR inhibitor, it has never been definitively established whether its antitumor activity (axitinib) have been shown to be effective in RET-cabozantinib, lenvatinib, and sorafenib) function primarily as VEGFR2 and MET, but were repurposed because of the RET activity that have been approved by the FDA for MTC. (33).

The first proof-of-concept came when it was demonstrated that ribozyme-mediated cleavage of mutant RET mRNA inhibited MTC tumor cell growth in vitro (29). Subsequently, it was demonstrated that overexpression of a dominant-negative RET was capable of inhibiting human MTC cell line growth in vitro (30) and in xenograft models (31). Neither approach was clinically viable, however, thereby opening the door for small-molecule inhibitors.

MKIs

Vandetanib/ZD6474 was among the first drugs demonstrated to inhibit the activity of both oncogenic RET fusions as well as RET activated through the M918T mutation (32). However, as vandetanib is a repurposed VEGFR inhibitor, it has never been definitively established whether its antitumor actions (and those of similar multikinase-targeting drugs like cabozantinib, lenvatinib, and sorafenib) function primarily through inhibition of RET, particularly as MKIs without RET activity (axitinib) have been shown to be effective in RET-altered thyroid cancers (33).

Vandetanib and cabozantinib are MKIs with nonselective RET activity that have been approved by the FDA for MTC. They were originally designed to target other kinases, such as VEGFR2 and MET, but were repurposed because of the discovery of their inhibitory actions on RET. This MKI activity leads to significant and sometimes prohibitive “off-target” clinical side effects such as nausea, diarrhea, rash, and hypertension that limit use in some patients or limit the dose that patients can tolerate, leading to drug discontinuation or dose reduction (Table 1). Together with nonselectivity for RET and inferior pharmacokinetic properties, these MKIs prevented potent RET inhibition.

SELECTIVE RET INHIBITORS

BLU-667 (pralsetinib) and LOXO-292 (selpercatinib) are two highly potent and selective RET inhibitors designed to offset the weaknesses of MKIs (refs. 11, 27, 28; Table 1). High potency and RET selectivity were confirmed in robust preclinical models using multiple in vitro and in vivo RET-dependent tumor models (27, 28). In addition, favorable pharmacokinetic properties, including high bioavailability, predictable exposure, and minimal potential for drug–drug interactions, were confirmed as well. On-target acquired resistance with tyrosine kinase inhibitors (TKI) is always an issue in developmental therapeutics, specifically a mutation at the gatekeeper position that sterically hinders inhibitor binding, as seen in chronic myeloid leukemia (BCR-ABL T315I ) or EGFR-mutant NSCLC (EGFR T790M ). Similarly, the RET gatekeeper mutations V804L and V804M have been described and have been shown to be acquired resistance mechanism to MKIs (28, 34). Interestingly, these gatekeeper aberrations have been seen as germline mutations in patients presenting with MTC, and although cabozantinib and vandetanib do not cover these mutations adequately, the selective RET inhibitors BLU-667 and LOXO-292 were specifically designed to inhibit these mutations in subnanomolar concentrations (27, 28, 34).

Following preclinical validation, early clinical studies with BLU-667 showed remarkable responses in MKI-naive and MKI-refractory patients with RET-rearranged NSCLC and patients with RET-mutant advanced MTC (27). Contemporaneously, utilizing rapid dose titration guided by real-time pharmacokinetic assessments to achieve meaningful clinical

<table>
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<tr>
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<tr>
<td></td>
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<td>36%</td>
<td>16%</td>
</tr>
<tr>
<td>Selpercatinib (LOXO-292)</td>
<td>78% (RET+ thyroid cancer)</td>
<td>68%</td>
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<tr>
<td></td>
<td>59% (MTC)</td>
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<tr>
<td>Pralsetinib (BLU-667)</td>
<td>56% (MTC)</td>
<td>58%</td>
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Abbreviations: DDR, drug-discontinuation rate; DRR, dose-reduction rate; ORR, objective response rate.

aRelatively high dose reduction and discontinuation rates in MKIs due to drug-related adverse events like hypertension, diarrhea, rash, fatigue, hand-foot syndrome, proteinuria, hypopigmentation, QT prolongation, thrombosis, and hemorrhage preclude effective long-term use of MKIs in thyroid cancer.

bORRs, dose-reduction rates, and drug-discontinuation rates of MKIs versus selective RET inhibitors

Table 1.

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exposures safely and rapidly, a patient with RETM918T-mutant MTC and an acquired RETV804M gatekeeper resistance mutation, previously treated with six MKI regimens, was treated with LOXO-292 and achieved a rapid tumor response (28). A second patient with symptomatic brain metastases who was treated similarly experienced rapid tumor regression in the brain metastases. The mature clinical data that we currently have for selective RET inhibitors is mainly from RET fusion–positive NSCLC and RET-mutant MTC. Patients harboring RET-fusion NSCLC or PTC respond regardless of fusion partner, and patients with RET-mutant MTC respond regardless of germline or somatic mutation, including patients harboring germline V804M. Below we discuss the emerging clinical data for the selective RET inhibitors.

**PRLSETINIB (BLU-667)**

ARROW, a phase I clinical trial designed to evaluate the safety, tolerability, and efficacy of BLU-667 in multiple ascending doses in adults with RET-altered NSCLC, MTC, and other advanced solid tumors showed that the recommended phase II dose was 400 mg every day. As per the data presented at the American Society of Clinical Oncology 2019 annual meeting, 48 patients with RET-fusion NSCLC were evaluable for response assessment, including 35 patients previously treated with platinum-based chemotherapy (35). Nearly all patients (90%) had radiographic tumor reductions. The objective response rate (ORR) was 60% [one complete response and 20 partial responses (PR); all responses were confirmed], and the disease control rate (DCR) was 100% in the patients previously treated with platinum-based chemotherapy. Remarkably, pralsetinib was highly active regardless of RET fusion partner, including RET–KIF5B and RET–CCDC6. Thirty-two patients with RET-mutant MTC were evaluable for response assessment, including 16 patients previously treated with the MKIs cabozantinib or vandetanib (36). The ORR was 63% [nine confirmed PRs, one PR pending confirmation] and the DCR was 94%. On the basis of these data, pralsetinib has been demonstrated that the RET V804M/L modification or bypass signaling (41, 42). Indeed, the earliest preclinical studies demonstrated that the RET V804M/L mutation functioned as a vandetanib gatekeeper, severely limiting its efficacy (43). More recent studies have demonstrated that V804M/L provides a gatekeeper function in RET-driven cancers, as the vast majority of patients continue to show tumor regression or remain progression-free (38).

**SELPERCATINIB (LOXO-292)**

LIBRETTO-001 is a global phase I/II trial of selpercatinib (LOXO-292) in RET-altered cancers. On the basis of early promising data, selpercatinib received FDA breakthrough designations for RET fusion–positive NSCLC, RET mutation–positive MTC, and RET fusion–positive PTC. In the registration dataset consisting of the first 105 enrolled patients with RET fusion–positive NSCLC with prior platinum-based chemotherapy, selpercatinib treatment resulted in a 68% ORR [95% confidence interval (CI), 58%–76%; ref. 37]. These patients received a median of three prior systemic regimens (55% previously treated with an anti–PD-1/PD-L1 antibody and 48% previously treated with at least one MKI) and ORR was similar regardless of prior therapy. Up to 50% of RET fusion–positive NSCLCs can metastasize to the brain, and in the subset of patients with brain metastases in the registration dataset, selpercatinib treatment demonstrated a central nervous system ORR of 91% (95% CI, 59%–100%). As of the data cutoff date of June 17, 2019, median duration of response (DOR) was 20.3 months (95% CI, 13.8–24.0) and median progression-free survival (PFS) was 18.4 months (95% CI, 12.9–24.9; ref. 37). In a safety analysis of all 531 patients enrolled to LIBRETTO-001, selpercatinib was well tolerated, with only 9 patients (1.7%) discontinuing therapy due to treatment-related toxicity.

In the RET-mutant MTC registration dataset (38) consisting of the first 55 enrolled patients with prior cabozantinib and/or vandetanib therapy, selpercatinib treatment resulted in a 56% ORR (95% CI, 42%–70%). ORR was similar regardless of prior MKI therapy, and 53% of patients were previously treated with ≥2 prior MKI. As of the data cutoff date of June 17, 2019, median DOR was not reached [95% CI, 11.1–not estimable (NE)] and median PFS was not reached (95% CI, 11.3–NE). Interestingly, in 76 cabozantinib/vandetanib (MKI)–naïve patients with RET-mutant MTC, selpercatinib treatment resulted in a 59% ORR (95% CI, 47%–70%; ref. 38). In 26 patients with RET fusion–positive thyroid cancer (PTC, Hürthle cell, poorly differentiated, and anaplastic) 62% ORR (95% CI, 41%–80%) was reached. Median DOR and PFS were not reached in the MKI-naïve or RET fusion–positive thyroid cancer cohorts, as the vast majority of patients continue to show tumor regression or remain progression-free (38).

**MECHANISMS OF ON-TARGET AND OFF-TARGET DRUG RESISTANCE**

The treatment of MTC with vandetanib represented the first attempt to target oncogenic RET. Despite a high DCR (73%), resistance developed with a median PFS of 27.9 months (39). With nearly a decade of clinical use, the mechanisms of vandetanib resistance remain largely unknown (40). This has largely been complicated by two factors. First, vandetanib is an MKI with antiangiogenic and anti-RET activity. As such, both pathways could be subject to resistance mechanisms. Second, the tools to examine resistance mechanisms have only recently begun to be applied to study RET-driven cancer. From clinical trial data, it is clear that both primary and acquired resistance mechanisms exist. Furthermore, similar to other oncogenic receptor tyrosine kinases, acquired resistance is expected to occur primarily through either target modification or bypass signaling (41, 42). Indeed, the earliest preclinical studies demonstrated that the RETV804M/L mutation functioned as a vandetanib gatekeeper, severely limiting its efficacy (43). More recent studies have demonstrated that V804M/L provides a gatekeeper function in oncogenic RET fusions, as well as limiting efficacy of other MKIs such as cabozantinib (44, 45). For this reason, the selective RET inhibitors have been screened and designed for activity against gatekeeper mutations.

It is only recently that an acquired RETV804I mutation has been demonstrated following resistance to multi-TKI treatment (28). The gatekeeper was detected in the circulating cell-free DNA (cfDNA) of a patient with MTC following treatment with consecutive TKIs over a 5-year time frame. In follow-up studies examining a small cohort of patients with MTC
with RET<sup>M918T</sup> somatic driver mutations, 75% of patients had detectable circulating RET<sup>900M</sup> cfDNA after development of resistance to vandetanib or cabozantinib (46), suggesting that acquisition of a RET gatekeeper mutation is a common mechanism of resistance in MTC. Acquired RET gatekeeper mutations on treatment with MKIs and successful treatment with the selective RET inhibitor selpercatinib has been reported for NSCLC as well (34). In the LURET patient cohort of vandetanib-treated patients, only a single case was observed to acquire a RET mutation after development of resistance (47, 48). This patient, with a CCDC6–RET fusion, developed a novel RET<sup>900F</sup> activation loop mutation shown to reduce drug binding. Additional studies will be needed to clarify the frequency of acquired RET mutation in these two cancer types and whether target modification is a less-favored mechanism of resistance for oncogenic fusions.

Evidence also exists for bypass mechanisms of resistance to RET inhibition. Notably, resistance to cabozantinib has been associated with acquisition of MET<sup>II238V</sup> in NSCLC (49) and MET amplification in colorectal cancer (50). In support of these findings, preclinical models have demonstrated that RET inhibition can be overcome through activation of MET or EGFR family members, as well as through activating RAS mutations (51–53).

In the near future, studies of acquired resistance in patients treated with selective RET inhibitors are expected, but as of now, unlike with EGFR- and ALK-driven cancers, we do not yet have vast patient experience to draw upon (54). However, what we have learned is that many of the discoveries of resistance mechanisms initially made in preclinical studies found themselves duplicated in patient studies, and later addressed by new clinical approaches. If we apply a similar approach to treatment of RET-driven cancer, then identification of the mechanism(s) of resistance becomes a critical step in providing an effective treatment. First, it is clear that the current generation of RET inhibitors provides the ability to use a higher targeting dose and is largely insensitive to acquisition of gatekeeper mutations. Thus, these inhibitors provide a logical treatment choice for TKI resistance in MTC, where acquired gatekeeper mutation frequency is high, or as a first-line therapy where the use of higher pharmacologic dosing is largely insensitive to acquisition of RET M918T somatic driver mutations, 75% of patients had detectable circulating RET<sup>900M</sup> cfDNA after development of resistance to vandetanib or cabozantinib (46), suggesting that acquisition of a RET gatekeeper mutation is a common mechanism of resistance in MTC. Acquired RET gatekeeper mutations on treatment with MKIs and successful treatment with the selective RET inhibitor selpercatinib has been reported for NSCLC as well (34). In the LURET patient cohort of vandetanib-treated patients, only a single case was observed to acquire a RET mutation after development of resistance (47, 48). This patient, with a CCDC6–RET fusion, developed a novel RET<sup>900F</sup> activation loop mutation shown to reduce drug binding. Additional studies will be needed to clarify the frequency of acquired RET mutation in these two cancer types and whether target modification is a less-favored mechanism of resistance for oncogenic fusions.

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**OTHER SELECTIVE RET INHIBITORS IN DEVELOPMENT**

Multiple resistance mutations have been reported from preclinical studies, including the gatekeeper mutation V804L to cabozantinib and solvent front mutations (SFM) G810A/S to vandetanib (61). Although the first-generation selective RET inhibitors are designed to cover the gatekeeper mutation, they do not adequately cover the SFMs. Emergence of SFMs has been reported preclinically (62). Recently, RET G810R, S, and C SFMs as acquired resistance mechanisms have been demonstrated in RET-aberrant patients who progressed on selective RET inhibitors (63). Second-generation RET inhibitors are in development that may have different properties. TPX-0046, a next-generation RET inhibitor that is structurally differentiated and potent against a broad range of mutations, has currently entered first-in-human studies (ClinicalTrials.gov Identifier: NCT04161391) after investigational new drug–enabling preclinical studies. Other selective RET inhibitors in development include BOS-172738 (NCT03780317) and TAS0953/HM06 (in preclinical development).

**CONCLUSION**

The considerable enthusiasm in the precision oncology of RET-dependent cancers including RET fusion–positive NSCLC, RET fusion–positive PTC, and RET-mutant MTC stems from the successful clinical trial results of selective RET inhibitors and offers a tantalizing array of opportunities in RET-dependent cancer research. The fewer off-target side effects, more effective control of growth, and sustained anti-tumor activity compared with MKIs opens up a new era of precision oncology in RET-driven cancers. Approval of these selective RET inhibitors will further the use of the drugs in the community. Next steps include clinical trials with these drugs earlier in the disease course, and combination therapy trials. Current RET inhibitors are a proof of principle for selective RET inhibition, but we need to know when we will get the most benefit from this specific targeting, and we clearly need to be prepared for addressing acquired resistance and escape from RET targeting.

**Disclosure of Potential Conflicts of Interest**

V. Subbiah is a consultant/advisory board member at LOXO Oncology/Eli Lilly, Helsinn, R-Pharma US, Incyte, QED, Novartis, and Medimmune, and reports receiving commercial research grants from LOXO Oncology/Eli Lilly, Blueprint Medicines, Fujifilm, Pharmamar, D3, Pfizer, Multivir, Amgen, AbBvie, Agensys, Boston Bio-medical, Idera, Turning Point Therapeutics, Exelixis, Inhibrx, Altim, Medimmune, Dragonly Therapeutics, Takeda, Roche/Genentech, Novartis, Bayer, GSK, Nanocarrier, BergeHealth, Incyte, and North-west Biotherapeutics. No potential conflicts of interest were disclosed by the other author.

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REFERENCES

42. Wells SA Jr, Gonsnell JE, Gagel RF, Moley J, Pfister D, Sosa JA, et al. Vandetanib for the treatment of patients with locally advanced or
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Advances in Targeting RET-Dependent Cancers

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