Progresses Toward Precision Medicine in *RET*-altered Solid Tumors

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ABSTRACT

RET (rearranged during transfection) gene encodes a receptor tyrosine kinase essential for many physiologic functions, but *RET* aberrations are involved in many pathologies. While *RET* loss-of-function mutations are associated with congenital disorders like Hirschsprung disease and CAKUT, *RET* gain-of-function mutations and rearrangements are critical drivers of tumor growth and proliferation in many different cancers. *RET*-altered (RET^+) tumors have been hitherto targeted with multikinase inhibitors (MKI) having anti-*RET* activities, but they inhibit other kinase targets more potently and show limited clinical activities. The lack of target specificity and consequently increased side effects, responsible for dose reduction and drug discontinuation, are critical limitations of MKIs in the clinics. New selective *RET* inhibitors, selpercatinib and pralsetinib, are showing promising activities, improved response rates, and more favorable toxicity profiles in early clinical trials. This review critically discusses the oncogenic activation of *RET* and its role in different kinds of tumors, clinical features of RET^+ tumors, clinically actionable genetic *RET* alterations and their diagnosis, and the available data and results of nonselective and selective targeting of *RET*.

Introduction

RET (rearranged during transfection; ref. 1) gene encodes a receptor tyrosine kinase (RTK) essential for many physiologic functions like early embryogenesis, development of the enteric nervous system, kidney morphogenesis, spermatogenesis, hematopoiesis, and potentially immunomodulation. Unsurprisingly, aberrations of *RET* gene leads to many pathologies. On the one hand, RET loss-of-function mutations are most commonly known genetic cause of Hirschsprung disease, a relatively common congenital hereditary disorder characterized by chronic constipation leading to intestinal obstruction, emesis, and increased risk of enterocolitis. But on the other hand, aberrant *RET* receptor activation via gain-of-function rearrangements and mutations is implicated in many different tumors. In the past, this gene was considered mainly for the early diagnosis of hereditary medullary thyroid cancer (MTC) by detection of germline oncogenic mutations, and prophylactic thyroidectomy for asymptomatic relatives. However, increasing evidences in recent years show aberrant activation of *RET* as a critical driver of tumor growth and proliferation across a broad spectrum of tumors.

RET rearrangements have been frequently found in papillary thyroid cancer (PTC; ref. 2) and non–small cell lung cancer (NSCLC; refs. 3–5), whereas activating point mutations are very common in MTC and multiple endocrine neoplasia 2 (MEN2), where they play pathognomonic role in the latter (6, 7). A study using targeted next-generation sequencing (NGS) on 4,871 patients with diverse tumors found 88 RET^+ cases (1.8%; 88/4,871), with most of them as activating alterations (71.6%; 63/88; ref. 8). In Memorial Sloan Kettering Cancer Center (MSKCC) data (9) of a large cohort of metastatic cancers from cBioPortal (10), we found that there are 2.4% of *RET* alterations (**Fig. 1**).

RET Oncogenic Activation and Prevalence of RET^+ Tumors

The *RET* gene is a single-pass transmembrane RTK located on the long arm of chromosome 10 (10q11.21; **Fig. 2**). Oncogenic activation of *RET* occurs mainly in two different ways: (i) chromosomal rearrangement giving rise to chimeric *RET* fusion genes and (ii) somatic or germline mutations. These gain-of-function alterations lead to the constitutive activation of *RET*, either via ligand-independent dimerization or by aberrant expression or activation of monomeric receptors.

RET rearrangements

RET rearrangements produce chimeras formed by in-frame fusion of 5′-end of partner genes with the 3′-end of *RET* containing its kinase domain. *RET* fusion genes exhibit oncogenic properties by two main mechanisms. First, the upstream partners could contain a dimerization domain that is fused with the kinase domain of *RET*, resulting in ligand-independent dimerization and constitutive activation (11). For example, many upstream fusion partners of *RET* contain the coiled-coil dimerization domain (3, 12). Most of these fusion partners are localized in the cytosol, thus avoiding normal endosomal trafficking and ubiquitin-mediated lysosomal degradation (13).

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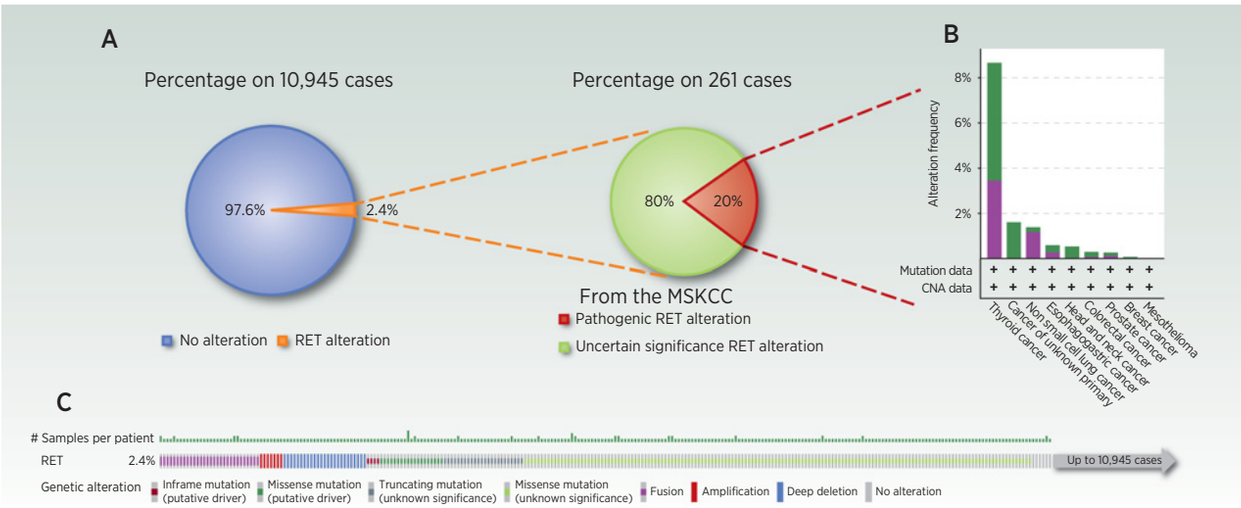

Figure 1.
RET aberrations in different kinds of metastatic tumors obtained from the MSK-IMPACT Clinical Sequencing Cohort (9) available on cBioPortal.org. **A,** Overview of *RET* alterations and their relative frequencies in 10,945 sequenced samples from the MSKCC. **B,** Distribution of putative driver *RET* alterations in different kind of tumors from the MSKCC. **C,** Relative frequency of any *RET* alteration from the MSKCC.

Second, the fusion brings the *RET* kinase at 3′-end directly under the control of the promoter of the upstream partner gene (3, 14). The partner gene could be ubiquitously expressed, consequently overexpressing chimeric *RET* protein triggering activation of multiple downstream pathways involved in cell growth, proliferation, and survival (**Fig. 2**; refs. 11, 15).

RET rearrangements are frequently found in PTC (**Fig. 3**), especially in subjects with previous exposure to ionizing radiation. In particular, 50%–90% of children show *RET* rearrangements in post-Chernobyl PTC (16) as their follicular cells are susceptible to undergo genetic mutations due to high proliferation rate (17). Different studies suggest that the incidence of rearrangements vary widely (2.6%–70%; ref. 18), but more recently The Cancer Genome Atlas consortium found 6.8% of *RET* fusions in a large ($N = 484$) PTC cohort (19). *CCDC6* and *NCOA4* are the two most frequent (>90% of cases) *RET* fusion partners in PTC, with the latter usually associated with bigger tumor size, aggressive behavior, and advanced stage at diagnosis (2).

RET rearrangements are found in around 1%–2% of patients with NSCLC (20) of mainly adenocarcinoma histology, in relatively young (≤60 years) people with minimal or no smoking history, and frequently show brain metastases at the time of diagnosis of advanced disease (21). These cancers are responsive to pemetrexed-based regimens with overall response rate (ORR), median progression-free survival (mPFS), and median overall survival (mOS) not significantly different from *ROS1*- and *ALK*-rearranged tumors (ORR: 45% vs. 78% vs. 50%, $P = 0.30$; mPFS: 19 vs. 23 vs. 19 months, $P = 0.57$; mOS: 24 vs. 24 vs. 37 months, $P = 0.43$; for *RET*-, *ROS1*-, and *ALK*-rearranged tumors, respectively; ref. 22). *RET*-rearranged tumors tend to be mutually exclusive with other major lung cancer drivers such as *KRAS* mutations, *EGFR* mutations, and *ALK* and *ROS1* rearrangements (8). They show low tumor mutation burden and low PD-L1 expression making them "cold" tumors having low response to immunotherapy (23).

KIF5B is the most common fusion partner and lung adenocarcinomas carrying this fusion show a 2- to 30-fold increase in RET expression, but other upstream fusion partners have also been identified (**Fig. 3**; refs. 3, 24).

RET rearrangements are also found in other tumors such as colorectal cancer (25), breast cancer (26), chronic myelomonocytic leukemia (27), spitz tumors (28), medullary thyroid carcinomas (29), and ovarian and salivary gland cancers (8).

RET mutations

Germline RET mutations are pathognomonic hallmark of MEN2 and can be identified in 98%–100% of cases by molecular testing (30). MEN2 is an autosomal dominant multi-tumor syndrome that is further subdivided into MEN2A (>90% of cases), MEN2B, and familial MTC (FMTC; ref. 31). Although, around 200 *RET* variants have been described in MEN2 with approximately half of them annotated to be pathogenic (32), most of the frequent variants are localized in key residues in the extracellular and kinase domains.

Extracellular domain mutations are more commonly observed in MEN2A and FMTC (**Fig. 3**). These missense mutations involve the cysteine-rich domain in the extracellular region and consist of substitution of cysteine (C609, C611, C618, C620, C630, and C634) with other amino acids, thereby disrupting the intramolecular disulfide bonds that determine the three-dimensional structure of the extracellular domain. As a consequence, the nonmutated and unbonded cysteine residues from two different *RET* molecules are free to make intermolecular disulfide bonds. This leads to the ligand-independent dimerization and constitutive activation of *RET* (33, 34). Mutation involving codon 634 (C634R) in exon 11 is the most common substitution identified in patients with MEN2A (85%). In FMTC, the mutations are evenly distributed among the various cysteine residues (C609, C611, C618, C620, and C630; ref. 35).

Mutations in the kinase domain are pathognomonic of MEN2B syndrome with M918T in exon 16 described in 95% of the cases and A883F in exon 15 in further 2%–3% of the cases (36). M918T mutation alters the catalytic core of RET receptor resulting in increased ATP binding and receptor activation regardless of receptor dimerization, and is responsible for increased aggressiveness compared with other *RET* mutations (37, 38).

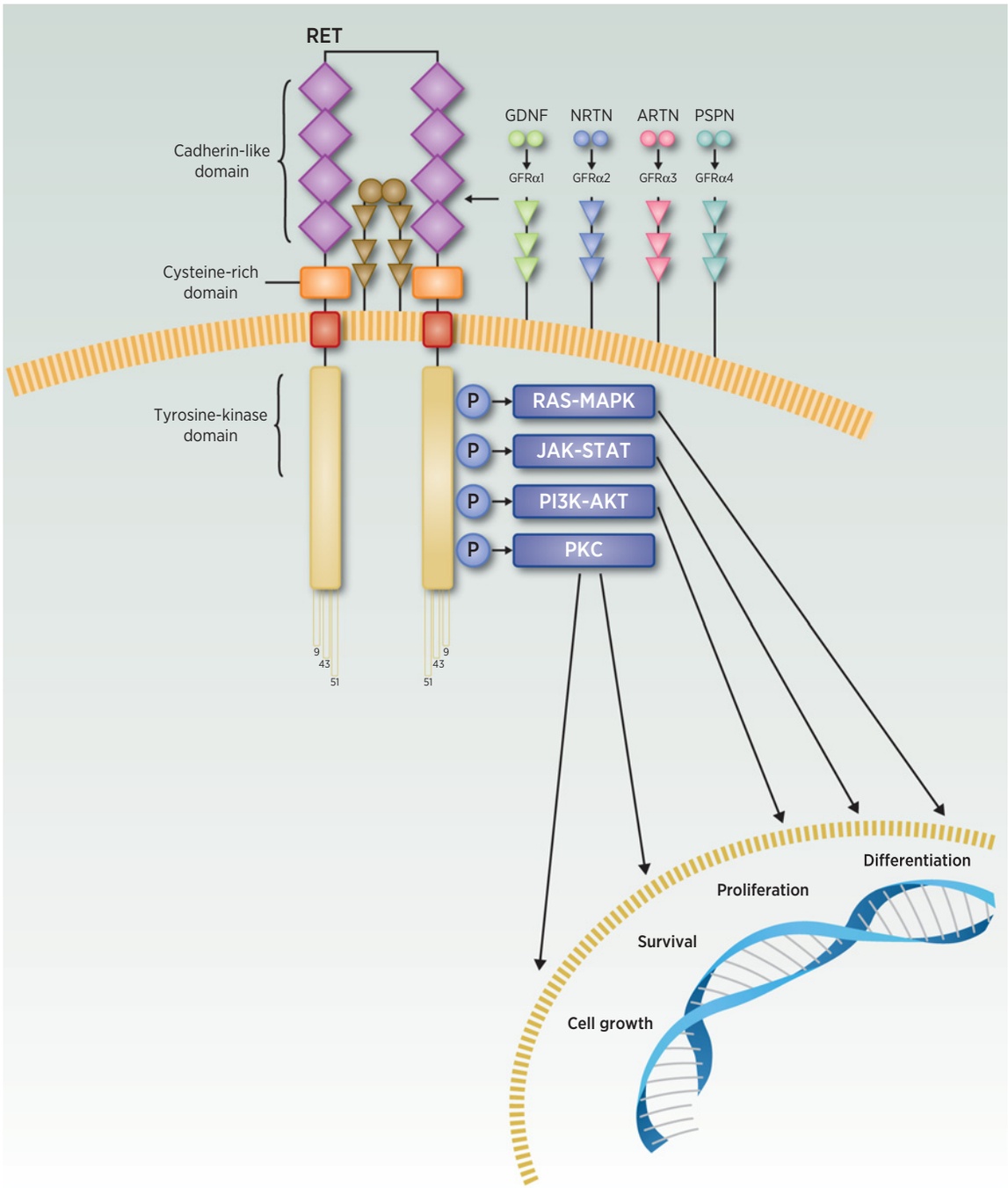

Figure 2.

RET receptor and signaling pathways activated by *RET*. The *RET* gene encodes a single-pass transmembrane glycoprotein receptor, consisting of a large extracellular domain, a transmembrane domain, and an intracellular tyrosine kinase domain. The N-terminal extracellular region contains four cadherin-like repeats (CDH1–4) and a membrane-proximal cysteine-rich domain, which is crucial for protein conformation and ligand binding. The C-terminal intracellular domain consists of a tyrosine kinase domain and isoform-specific tails. There are three isoforms of *RET* formed by alternative splicing: RET9, RET43, and RET51, containing 9, 43, and 51 C-terminal amino acids, respectively. *RET* receptor's ligands belong to the glial cell line–derived neurotrophic factor (GDNF) family ligands, and consist of GDNF, neurturin (NRTN), artemin (ARTN), and persephin (PSPN). A peculiar characteristic of RET receptor, which distinguishes it from other RTKs, is that it does not bind directly to its ligands (the GFLs), but it needs a cofactor, a protein belonging to the growth factor receptor-alfa (GFRα) family. The GFRα family receptors GFRα1, GFRα2, GFRα3, and GFRα4 bind with high affinity and specificity to *RET* ligands GDNF, NRTN, ARTN, and PSPN, respectively. The GFRα family coreceptors bind to GFLs forming a binary complex, which then recruits *RET* allowing its dimerization and autophosphorylation on intracellular tyrosine residues with subsequent activation of multiple downstream pathways like RAS-MAPK, PI3K-AKT, JAK-STAT, and PKC involved in cell proliferation, growth, differentiation, and survival.

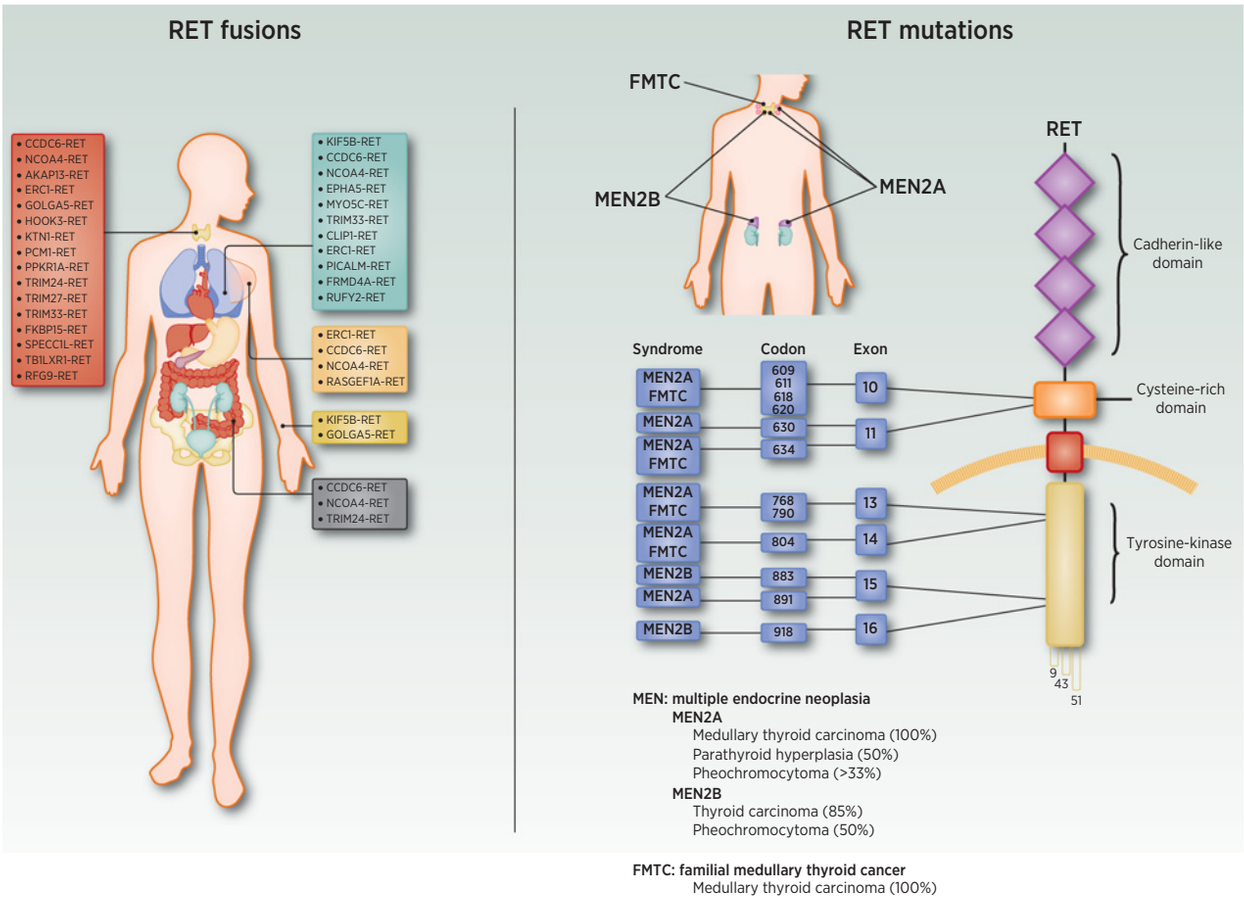

Figure 3.

Mutations and fusions in *RET* gene in solid tumors (6–8, 24–29, 32–36). RET-fusions associated with the tumors of various organs (left). The RET mutational landscape in MEN2 syndromes and FMTC, where they occur very frequently (right). The sites of the occurrences of MEN2 and FMTC are shown in the top, left of the right panel.

Mutations in tyrosine kinase domain have also been described in MEN2A and FMTC (E768D and L790F in exon 13, and V804L and V804M in exon 14) and are generally associated with more indolent disease or later disease onset (**Fig. 3**). Because of the overlapping mutational patterns in the extracellular and kinase domains between these two diseases, FMTC is also viewed as a phenotypic variant of MEN2A with decreased penetrance.

Somatic *RET* mutations are also found in other thyroid malignancies such as anaplastic thyroid carcinoma (4.3%), melanoma (6.6%), desmoplastic melanoma (20%), cutaneous squamous cell carcinoma (10%), colorectal cancer (3.6%–6.9%), paraganglioma, breast cancer, and ureter urothelial carcinoma (39).

RET overexpression and amplification

Overexpression of wild-type *RET* receptor has been found in many tumors (40). *ERα*$^{+}$ breast cancers express *RET* protein in 30%–70% of the cases, with higher expressions correlating with aggressive behavior, metastasis, and resistance to endocrine therapy (41). There is a cross-talk between *RET* and *ERα* pathway and targeting *RET* sensitizes breast cancer cells to tamoxifen (42). Nevertheless, targeting *RET* pathway in breast cancer has been limited to only preclinical studies as of now (43).

RET and its ligands are expressed in 40%–65% of pancreatic ductal adenocarcinomas and they are associated with tumor regional invasion, perineural spread, poor prognosis, and reduced overall survival (11, 40). *RET* is overexpressed in patients with surgically resected pancreatic cancer and correlates with lymphatic invasion and poor survival (44).

RET expression has been found in several other tumor types including prostate cancer, colorectal cancer, myeloid tumors, melanoma, renal cell carcinoma, and head and neck tumors, although their clinical relevance is still not known (40).

RET copy-number gains or amplifications are found in different tumors (45), in particular in lung cancer (46, 47), but it does not correlate with overall *RET* expression (45). Its clinical significance remains unclear as a retrospective study done on four randomized phase III NSCLC trials did not find any difference in objective response in terms of *RET* copy-number gains (48).

Diagnosis of *RET* Alterations

RET alterations have been traditionally found by analyzing tumor biopsies using classical methods such as RT-PCR, IHC, and break-apart FISH (20, 49). Liquid biopsy coupled with NGS represents the modern noninvasive alternative, which can detect multiple genomic alterations (mutations, indel, amplification, and rearrangement) simultaneously (50). Recently, *KIF5B-RET* rearrangement was

detected from liquid biopsy of 2 patients with lung cancer previously tested negative for driver mutations (51), and this technique was found to be 100% specific and 98% concordant with tissue-based testing in a larger cohort (52). Liquid biopsy has been successfully used to detect *RET*-M918T mutation with prognostic significance in circulating cell-free DNA (cfDNA; ref. 53). Another analysis conducted on 32,989 patients with advanced tumor using cfDNA assay detected 176 somatic activating *RET* alterations including 143 fusions and 33 missense mutations (54). cfDNA assays represent a promising alternative to invasive biopsies as a surrogate marker for multiple indications in cancer including diagnosis, prognosis, and monitoring (55).

RET-Targeted Therapies

A variety of multikinase inhibitors (MKI), which show anti-*RET* activities, but more potently inhibit other kinase targets such as *VEGFR2, KIT, PDGFR, EGFR, MET*, and *BRAF*, have been tested in RET^{+} solid tumors, in particular in NSCLC and thyroid cancers. Because of their multi-target inhibition, it is very difficult to establish whether their antitumor activity was primarily induced by *RET* inhibition or by inhibition of other kinase targets. These drugs, in fact, were not designed to target *RET*, but showed biochemical activities against it (**Table 1**; ref. 56) and thus were repurposed.

MKIs in RET-rearranged NSCLC

MKIs cabozantinib, vandetanib, lenvatinib, and RXDX-105 have been evaluated in phase II studies in pretreated *RET*-rearranged NSCLC patients (57–61). These drugs have shown modest clinical activities with ORR, mPFS, and mOS ranging from 16%–47%, 4.5–7.3 months, and 9.9–11.6 months, respectively, in these studies (**Table 2**). These results are better than those observed with single-drug chemotherapy administered in unselected patients with advanced NSCLC after failure from initial platinum-based doublet therapy, but they are far inferior to the data obtained with *EGFR, ALK*, and *ROS1* inhibitors with an attested ORR of 72%–83% and mPFS of 19–35 months (62).

Some of these studies also evaluated the clinical responses according to the fusion partners. The most common fusions described were *KIF5B-RET* followed by *CCDC6-RET*. In vandetanib trial (59), response rate was much better in *CCDC6-RET*–fused tumors compared with *KIF5B-RET* (83% vs. 20%), which was also reflected in better mPFS (8.3 vs. 2.9) and mOS (not reached vs. 11.1 months). Also, RXDX-105 (61) trial showed better response in non-*KIF5B-RET* subgroup (ORR: 67% vs. 0%). Preclinically, it has been shown that *KIF5B-RET* fusion is responsible for 2- to 30-fold increase in *RET* expression that needs to be blocked by kinase inhibitors (3).

Another retrospective study involving global multicenter registry evaluated the clinical activities of different MKIs in patients with *RET*-rearranged NSCLC. The results of this study were similar to the data obtained with the same MKIs tested in phase II trials in terms of clinical outcome. Partial responses (PR) were obtained by cabozantinib (26%), vandetanib (18%), sunitinib (22%), and lenvatinib (50%; 1/2 patient), whereas complete responses were very few [cabozantinib (5%) and nintedanib (50%; 1/2 patient)]. mPFS and mOS were 2.3 months [95% confidence interval (CI), 1.6–5.0 months] and 6.8 months (95% CI, 3.9–14.3 months), respectively (63). The clinical outcomes were not statistically different according to the different upstream partners (*KIF5B, CCDC6*, and *EPHA5*). However, the presence of *KIF5B* fusion partner was more commonly observed in patients with brain metastases (21, 63).

Overall, MKIs show modest clinical benefit in *RET*-rearranged NSCLC. Moreover, the lack of target specificity is responsible for increased toxicity with the consequence of drug dose reduction or treatment discontinuation. In fact, the dose reduction rate ranged from 23% to 79% and the treatment discontinuation rate was from 8% to 21% in these trials (57–61, 63). However, these results should be further investigated as the small sample sizes of these studies and the retrospective nature of one of the studies do not allow to make definitive conclusions.

MKIs in thyroid cancers

Several MKIs showing anti-*RET* activities have also been tested in clinical trials in patients with thyroid cancer. However, unlike NSCLC, the patient selection criteria were not based on aberration in *RET* gene in these trials. Thus, it is not possible to get a definitive answer about their activities and clinical efficacies. Nevertheless, some of RET^{+} patients were also enrolled in these trials, which allowed *post hoc* subgroup analyses giving some clue regarding the MKIs' activities in RET^{+} thyroid cancers.

Cabozantinib and vandetanib, two of the MKIs also tested in NSCLC, are approved for first-line treatment in MTC based on the results of EXAM (64, 65) and ZETA (66) trials, respectively (**Table 2**); although the drug approval was independent from *RET* alteration status. In the EXAM trial, cabozantinib increased the ORR (28% vs. 0%; $P < 0.001$) and mPFS compared with placebo (11.2 vs. 4.0 months; HR, 0.28; 95% CI, 0.19–0.40; $P < 0.001$) with a nonsignificant increase in overall survival (OS; 26.6 vs. 21.1 months; HR, 0.98; 95% CI, 0.63–1.52). Retrospective analysis of EXAM trial data (65, 67) showed that the presence of *RET* M918T mutation compared with the absence of this alteration was associated with a benefit in terms of ORR (34% vs. 2%), progression-free survival (PFS; 14.2 vs. 5.8 months), and OS (44.3 vs. 20.2 months; **Table 2**). The presence of M918T mutation results in increased ATP binding and receptor activation (37, 38), and cabozantinib as well as vandetanib are shown to be effective against M918T in biochemical assays (**Table 1**).

ZETA (66) trial demonstrated that vandetanib prolonged the mPFS compared with placebo (30.5 vs. 19.3 months; $P = 0.001$) and also demonstrated a statistically significant improvement in ORR (45% vs. 13%; $P < 0.001$). In this clinical trial, there were a large number of patients with unknown *RET* mutational status due to insufficient biological material useful to allow this analysis. Nevertheless, in the subgroup analysis, the presence of M918T mutation was again

Table 1. Biochemical IC_{50} values (in nmol/L) of MKIs and new selective *RET* inhibitors.

Drug	RET	VEGFR2	V804L	V804M	M918T	CCDC6-RET
Cabozantinib	11	2	45	162	8	34
Vandetanib	4	4	3,597	726	7	20
Pralsetinib	0.4	35	0.3	0.4	0.4	0.4
Selpercatinib	0.2–12.5	100	—	0.8	0.7	—

Table 2. Clinical trials with MKIs in lung and thyroid cancer.

MKIs	Study phase	All pts	Treatment group	ORR (%)[a]	mPFS (mos.)	mOS (mos.)	TRAEs all grades (%)[b]
			Lung cancer				
Vandetanib (57)[c]	II	18	Vandetanib	3/17 (18)	4.5	11.6	Hypertension 16/18 (89) Rash 13/18 (72)
Cabozantinib (58)	II	26	Cabozantinib	7/25 (28)	5.5	9.9	ALT increased 25/26 (96) AST increased 19/26 (73)
Vandetanib (59)[c]	II	34	Vandetanib	9/19 (47)	4.7	11.1	Hypertension 16/34 (84) Diarrhea 15/34 (79)
Lenvatinib (60)	II	25	Lenvatinib	4/25 (16)	7.3	na	Hypertension 17/25 (68) Nausea 15/25 (60)
RXDX-105 (61)	II	81	RXDX-105	6/31 (19)	nr	nr	Fatigue 38/152 (25) Diarrhea 37/152 (24)
			Thyroid cancer				
Sorafenib (68)	II	21	Sorafenib	1/15 (6)	17.9	nr	HFSR 19/21 (90) Rash (non-HFSR) 18/21 (86)
Lenvatinib (69)	II	59	Lenvatinib	21/59 (36)	9.0	16.6	Diarrhea 44/59 (75) Proteinuria 35/59 (59)
Sunitinib (70)	II	71	Sunitinib	19/71 (27)	na	na	Asthenia/fatigue 59/71 (83) Mucosal AE 46/71 (65)
Dovitinib (71)	II	40	Dovitinib	8/39 (20)	5.4	nr	Diarrhea 21/39 (54) Anorexia (36)
Motesanib (72)	II	91	Motesanib	2/91 (2)	12	na	Thyroid dysfunctions 76/91 (83) Diarrhea 37/91 (41) Fatigue 37/91 (41)
Vandetanib (66)	III	331	Vandetanib	104/231 (45)	30.5	na	Diarrhea 130/231 (56)
			Placebo	13/100 (13)	19.3		Rash 104/231 (45)
Cabozantinib (64, 65, 67)	III	330	Cabozantinib	58/208 (28)	11.2	26.6	Diarrhea 135/214 (63)
			Placebo	0/104 (0)	4.0	21.1	PPE 107/214 (50)
			Mutational subgroups				
			Cabozantinib				
			RET MUT+	32/101 (32)	14	—	
			RET MUT−	7/32 (22)	5.8	—	
			RET M918T+	26/77 (34)	14.2	44.3	
			RET M918T−	14/69 (2)	5.8	20.2	
			Placebo				
			RET M^{918T+}	—	—	18.9	
			RET M^{918T-}	—	—	21.5	

Abbreviations: AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HFSR, hand-foot-skin reaction; mos, months; MUT, mutation; na, not available; nr, not reached; Pts, patients.

[a]Data refers only to patients evaluable for response.

[b]Data refers to the top two TRAEs (in terms of %). In RXDX-105 trial, TRAEs refer to all population included in the study (lung cancer, thyroid cancer, and gastrointestinal cancer). In dovitinib trial, they were evaluated in a total of 39 patients because 1 patient withdrew before study drug administration.

[c]The two vandetanib trials are different in terms of methods employed to detect *RET* alteration. In the Korean study by Lee and colleagues (57), FISH was used, while RT-PCR followed by FISH was used in LURET study (59). In the Korean study, the prevalence of *RET* rearrangement was much higher than that reported in patients with NSCLC. Consequently, it is possible that some false positive patients were included that can explain the lower response rate observed in this study.

associated with a better ORR compared with the group without this alteration (54.5% vs. 30.9%).

Other MKIs with anti-*RET* activities like sorafenib, lenvatinib, sunitinib, dovitinib, and motesanib have also been tested in phase II clinical trials in thyroid cancers (68–72). In the global population, regardless of the *RET* alteration status, ORR and mPFS ranged from 2% to 36% and 5.4 to 17.9 months, respectively (**Table 2**). Some of these studies did perform *post hoc RET* subgroup analyses, but no significant correlation with tumor response was found in these subgroups of patients (68, 69, 72). In motesanib trial (72), which contained the highest number of RET^{+} patients, the ORR was 0% in 33 RET^{+} tumors compared with 8% in 13 *RET* wild-type tumors.

In conclusion, cabozantinib and vandetanib seem to give better results for some specific RET^{+} cases in thyroid cancer, but this hypothesis needs to be verified in prospective trials in RET^{+} selected patients. It is also noteworthy that the side effects recorded with these drugs are not negligible and are similar to NSCLC trials. The treatment discontinuation rate was 12% with vandetanib and 16% with cabozantinib, while 35% treated with vandetanib and 79% treated with cabozantinib required dose reduction because of adverse events.

New Selective *RET* Inhibitors in Clinical Trials (Selpercatinib and Pralsetinib)

The limited activities and increased toxicities of MKIs in RET^{+} cancers, responsible for dose reduction and/or treatment discontinuation, in part, are explained by their off-target activities. New selective

Table 3. Clinical trials with new selective *RET* inhibitors in lung and thyroid cancer.

RET inhibitors	Tumor type	All pts	Fusion or mutation types (%)	ORR (%)[a]	TRAEs all grades (%)[b]
Selpercatinib (73)	Lung	253	KIF5B 149/253 (59) Non-KIF5B 76/253 (30) Unknown 28/253 (11)	Treatment naïve 33/39 (85) Pretreated 67/105 (64)	nr[c]
Pralsetinib (75)	Lung	120	KIF5B 79/120 (66) Non-KIF5B 18/120 (15) Unknown 23/120 (19)	Treatment naïve 19/26 (73) Pretreated 49/80 (61)	Neutropenia 31/120 (26) AST increased 24/120 (20)
Selpercatinib (73)	Thyroid	226	M918T 129/226 (57) Non-M918T 97/226 (43)	Treatment naïve 64/88 (73) Pretreated 38/55 (69)	nr[c]
		27	CCDC6 14/27 (52) Non-CCDC6 13/27 (48)	Treatment naïve 8/8 (100) Pretreated 15/19 (79)	
Pralsetinib (76)	Thyroid	64	M918T 36/64 (56) Non-M918T 28/64 (44)	18/32 (56)	Hypertension 19/64 (30) Neutropenia 15/64 (23)
		9	CCDC6 4/9 (44) Non-CCDC6 5/9 (56)	5/6 (83)	

Abbreviations: ALT, alanine aminotransferase increased; AST, aspartate aminotransferase; mos, months; nr, not reached; Pts, patients.
[a]Data refer only to patients evaluable for response. For pralsetinib study, the data refer to date cutoff of November 18, 2019 for lung cancer group and of April 28, 2019 for thyroid cancer group, after confirmed by central independent blinded review.
[b]TRAEs were presented for the safety evaluable cohort of patients. In the table, we report TRAEs recorded in ≥20% of patients.
[c]Not reported for patients' subgroups, but for the total populations. Most commons TRAEs included increased AST (51%), increased ALT (45%), dry mouth (39%), diarrhea (37%), increased creatinine (37%), hypertension (35%), fatigue (35%), peripheral edema (33%), and constipation (25%).

RET inhibitors, selpercatinib and pralsetinib, might solve off-target toxicity problems as they inhibit more potently and selectively both wild-type *RET* and *RET*$^+$ cancer cell lines in biochemical assays (**Table 1**; ref. 56). These data formed the basis for the first-in-human phase I/II trial of these drugs in *RET*$^+$ tumors.

Selpercatinib (retevmo or LOXO-292)

A phase I/II, open-label, first-in-human study (LIBRETTO-001), is evaluating selpercatinib in patients with *RET*$^+$ advanced solid tumors (ClinicalTrials.gov Id: NCT03157128). On the basis of the early impressive data of this trial, selpercatinib has been granted "accelerated approval" by FDA on May 8, 2020 for metastatic *RET*-fusion$^+$ NSCLC and MTC and *RET*-mutant MTC.

In 105 patients with *RET*-fusion$^+$ NSCLC pretreated with platinum chemotherapy, many of whom were heavily pretreated with other agents, the ORR was 64% (95% CI, 54%–73%) according to the data presented to the FDA (73). Responses were observed regardless of previous MKI or immune checkpoint inhibitor therapy. The median duration of response (DoR) was 17.5 months (95% CI, 12–NE months; NE, not estimable). In 39 treatment-naïve patients, the ORR was 85% (95% CI, 70%–94%), and the median DoR was not reached. Selpercatinib was also effective in patients with advanced and metastatic thyroid cancer. In 55 patients with *RET*-mutant MTC who previously received cabozantinib and/or vandetanib, the ORR was 69% (95% CI, 55%–81%). In 88 treatment-naïve patients, the ORR was 73% (95% CI, 62%–82%) and the median DoR was 22.0 months (95% CI, NE–NE). The ORR in 19 evaluable previously treated *RET*-fusion$^+$ thyroid cancer patients was 79% (95% CI, 54%–94%), whereas the ORR in 8 systemic therapy–naïve patients was 100% (95% CI, 63–100%). The median DoR was 18.4 months (95% CI, 7.6–NE months) in the former and it was not reached in the latter.

The LIBRETTO study also evaluated plasma clearance of *RET* alterations using circulating cfDNA in 34 patients with a detectable pretreatment *RET* alteration in plasma. The *RET* alteration allele frequency in plasma decreased by a median of 96% with complete clearance in 15 patients (44%; ref. 74).

In the larger safety dataset of 531 patients who received selpercatinib regardless of cancer type or *RET* alteration status, treatment emergent adverse events were mostly of grade 1–2 with a discontinuation rate of 5% (73).

Pralsetinib (BLU-667)

Preliminary results of phase I/II ARROW trial (NCT03037385) conducted with pralsetinib in *RET*-altered tumors showed that pralsetinib is more active and tolerable compared with MKIs in NSCLC and thyroid cancer (refs. 75, 76; **Table 3**). A recent update of NSCLC cohort (data cut-off date of November 18, 2019) reconfirmed the activity and tolerability of this drug. Patients with *RET* fusion$^+$ NSCLC were treated with pralsetinib at the recommended phase II dose of 400 mg once daily. Pralsetinib was effective in treatment-naïve patients (ORR, 73%) as well as patients pretreated with platinum-based chemotherapy (ORR, 61%; **Table 3**). Importantly, pralsetinib appeared active regardless of prior immunotherapy, kind of *RET* fusion partner, and presence of intracranial metastases. Furthermore, 78% (7/9) of patients with *RET* fusion$^+$ NSCLC with measurable baseline brain metastases at study entry demonstrated shrinkage of intracranial metastases, suggesting penetration of this agent into the central nervous system. Data on DoR and PFS have not been reported to date. Pralsetinib was also effective in removing circulating tumor DNA (ctDNA) from plasma. After 8 weeks of treatment with pralsetinib, *RET* ctDNA was reduced ≥50% for 90% of patients with NSCLC (77). Considering these encouraging results, pralsetinib is currently being evaluated in the phase III clinical trial AcceleRET (NCT04222972) in patients with *RET*-fusion$^+$ metastatic NCLSC.

In addition to NSCLC, pralsetinib also demonstrated promising clinical activity in *RET*$^+$ thyroid cancers (**Table 3**; ref. 76).

Sixty-four patients with advanced MTC harboring different *RET* mutations and 9 patients with *RET* fusion$^+$ PTC received pralsetinib 400 mg once daily. The majority of patients with MTC (67%) received cabozantinib and/or vandetanib as previous therapy. Among 32 response evaluable MTC patients (as per data cutoff of April 28, 2019), the confirmed ORR was 56%. Among 6 response evaluable

patients with *RET* fusion$^+$ PTC the ORR was 83% (76). Importantly, responses occurred regardless of prior MKI therapy (prior cabozantinib/vandetanib: 63% confirmed ORR) or RET genotype (PR in 2/3 evaluable patients with V804M). All responding patients remained on treatment at the time of data cutoff (median DoR not yet reached). *RET* ctDNA was reduced ≥50% for 83% of patients with MTC harboring somatic *RET* mutations after 8 weeks of treatment with pralsetinib (77).

Among those patients treated at recommended phase II dose, treatment-related adverse events (TRAE) were generally low grade and reversible (**Table 3**). Across the entire study ($N = 354$) population, the rate of discontinuation due to TRAEs was relatively low at 4% (75).

The FDA has granted "breakthrough therapy designation" to pralsetinib based on these data.

Resistance to anti-*RET* Therapy and Future Perspectives in Drug Development

The mechanisms of acquired resistance to first-generation selective *RET* inhibitors is not yet fully known. Preclinical studies have shown that the acquired gatekeeper mutation V804L is associated with resistance to anti-*RET* MKI therapy and selpercatinib was specifically designed to overcome that (78). Other studies show that *RET* solvent front mutations (SFM) (G810A/S, G810R, and G810C) are responsible for the acquired resistance to vandetanib and selpercatinib (79, 80).

The next-generation *RET* inhibitors are being designed not only to overcome the acquired resistances but also to inhibit *RET* more potently and selectively. TPX-0046 is a potent and selective next-generation *RET/SRC* inhibitor with activities toward different *RET* mutations including the G810R SFM (79). BOS172738 is another novel RET inhibitor with nanomolar potency against *RET* and approximately 300-fold selectivity against VEGFR2 (81).

A further therapeutic strategy being studied in *RET*$^+$ tumors is the combination of anti-*RET* therapy with other targeted agents. Preclinical studies show that adding mTOR inhibitor, everolimus, to MKIs reduces cell growth in *RET*-altered solid tumors by inhibiting PI3K-AKT, which is a downstream pathway that could confer resistance to these drugs (82, 83). It has also been shown that *EGFR* tyrosine kinase inhibitor, gefitinib, resensitizes cancer cells to *RET* inhibitors, even in the presence of *EGF* (84). Finally, the combination of an anti-*MDM2* agent with *RET* inhibitors could be explored because MDM2 amplification, which blocks the activity of tumor suppressor gene p53, has been observed in tumor sample of patients treated with *RET* inhibitors (85).

Conclusion

In recent years, *RET* proto-oncogene is gaining increasing clinical relevance. In fact, it has moved forward from being an orphan receptor to an important candidate for highly selective targeted therapy. In the past, this gene was considered mainly for the early diagnosis of hereditary MTC by detection of germline oncogenic mutation. But increasing evidences show that aberrantly activated *RET* is a critical driver of tumor growth and proliferation across a broad spectrum of tumors, with mutations and fusions as the most common alterations responsible for its oncogenic activation. MKIs previously achieved suboptimal outcomes in patients with *RET*$^+$ cancers, reflecting the lack of target specificity and consequently increased side effects that made chronic dosing challenging. With the advent of new highly selective *RET* inhibitors, pralsetinib and selpercatinib, which achieved improved response rates, more durable disease control, and favorable safety profile compared with MKIs, *RET* alterations have become increasingly more relevant in the clinics. The highly selective targeting of *RET* using precision medicine is setting a new paradigm for personalized cancer care in *RET*$^+$ tumors.

Disclosure of Potential Conflicts of Interest

J.F. Gainor reports personal fees from Blueprint, Loxo/Lilly, Bristol-Myers Squibb, Merck, Genentech/Roche, Takeda, Oncorus, Regeneron, Gilead, AstraZeneca, Pfizer, Agios, Amgen, and Array, grants from Novartis, and other from Ironwood (immediate family member–employee) outside the submitted work. F. Piha-Paul reports personal fees from Lilly and grants and personal fees from Illumina during the conduct of the study, and Bayer and Pfizer (outside the submitted work), as well as grants, personal fees, and nonfinancial support from AstraZeneca, Bristol-Myers Squibb, MSD, and Roche, and personal fees and nonfinancial support from Novartis outside the submitted work. V. Subbiah reports grants from LOXO/Eli Lilly (clinical trials support), Blueprint Medicines (clinical trials support), Turning Point Therapeutics (clinical trials support), Roche/Genentech (clinical trials support), Novartis (clinical trials support), Bayer (clinical trials support), GlaxoSmithKline (clinical trials support), Nanocarrier (clinical trials support), Vegenics (clinical trials support), Dragonfly (clinical trials support), Boston Pharmaceuticals (clinical trials support), and Helsinn (clinical trials support) during the conduct of the study; other from LOXO Oncology (advisory board) and Novartis (advisory board) outside the submitted work, as well as other research funding and grant support from Celgene, Northwest Biotherapeutics, Berg Health, Incyte, Fujifilm, PharmaMar, D3, Pfizer, MultiVir, Amgen, AbbVie, Alfa-sigma, Agensys, Boston Biomedical, Idera Pharmaceuticals, InhibRx, Exelixis, Medimmune, Altum, Takeda, National Comprehensive Cancer Network, NCI-CTEP, and UT MD Anderson Cancer Center, travel support from Novartis, PharmaMar, ASCO, ESMO, Helsinn, and Incyte, consultant/advisory board relationships with Helsinn, LOXO Oncology/Eli Lilly, R-Pharma US, INCYTE, QED Pharma, Medimmune, and Novartis, and other remuneration from Medscape. A. Drilon reports personal fees from Loxo/Lilly (honoraria), Blueprint (honoraria), and Exelixis (honoraria) during the conduct of the study, and Ignyta/Genentech/Roche (honoraria/ad board), Bayer (honoraria/ad board), Takeda/Ariad/Millenium (honoraria/ad board), TP Therapeutics (honoraria/ad board), AstraZeneca (honoraria/ad board), Pfizer (honoraria/ad board), Helsinn (honoraria/ad board), BeiGene (honoraria/ad board), BerGenBio (honoraria/ad board), Hengrui (honoraria/ad board), Tyra (honoraria/ad board), Verastem (honoraria/ad board), MORE Health (honoraria/ad board), AbbVie (honoraria/ad board), 14ner/Elevation Oncology (honoraria/ad board/SAB), Remedica Ltd. (honoraria/ad board), ArcherDX (honoraria/ad board), and Monopteros (honoraria/ad board) outside the submitted work, as well as reports a patent for Pocket Oncology and UpToDate (Wolters Kluwer) with royalties paid from Wolters Kluwer, other from Merck (food/beverage), Puma (food/beverage), Merus (food/beverage), and Boehringer Ingelheim (food/beverage), and CME honoraria from Medscape, OncLive, PeerVoice, Physicians Education Resources, Targeted Oncology, Research to Practice, Axis, PeerView Institute, Paradigm Medical Communications, and WebMD. F. Andrè reports speaker/advisor (compensated to institution) for AstraZeneca, Novartis, Pfizer, Daiichi Sankyo, Lilly, and Roche Genentech, research grants from AstraZeneca, Novartis, Pfizer, Daiichi Sankyo, Lilly, and Roche Genentech, and is a founder of Pegacsy. G. Curigliano reports other from Blueprint Medicines (institutional grant for clinical trial at IEO) during the conduct of the study, as well as other from Bristol-Myers Squibb (steering committee member), AstraZeneca (steering committee member), Roche (advisory board), Ellipsis (advisory board), Novartis (advisory board), Lilly (advisory board), and Pfizer (advisory board) outside the submitted work. No potential conflicts of interest were disclosed by the other authors.

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