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*Review*

# RET kinase inhibitors for *RET*-altered thyroid cancers

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***Abstract*:** Precision oncology has opened a new era in cancer treatment focused on targeting specific cellular pathways directly involved in tumorigenesis. The REarrangement during Transfection (*RET*) proto-oncogene is involved in the pathogenesis of various thyroid cancer subtypes. Mutations in *RET* give rise to both hereditary and sporadic medullary thyroid cancer (MTC). *RET* fusions are found in follicular cell-derived thyroid cancers (papillary, poorly differentiated, and anaplastic). Hence, drugs that block the RET tyrosine kinase receptor have been explored in the management of locally advanced or metastatic thyroid cancer. The multikinase inhibitors (MKIs) with nonselective RET inhibition are sorafenib, lenvatinib, vandetanib, cabozantinib, and sunitinib. Although the efficacy of these drugs varies, a major issue is the lack of specificity resulting in a higher rate of drug-related toxicities, leading to dose reduction, interruption, or discontinuation. Moreover, MKIs are subject to drug resistance by *RET* Val804 residue gatekeeper mutations. In phase I/II clinical studies, the highly selective first-generation RET inhibitors, selpercatinib and pralsetinib, demonstrate high efficacy in controlling disease even in the presence of gatekeeper mutations combined with greater tolerability. However, resistance mechanisms such as *RET* solvent front mutations (SFMs) have evolved in some patients, giving the need to develop the selective second-generation RET inhibitors. Although the approval of selpercatinib and pralsetinib in 2020 has profoundly benefited patients with RET-altered thyroid cancer, further research into optimal treatment strategies, mechanisms of drug resistance, long-term consequences of potent RET-inhibition, and development of more effective agents against emergent mutations are much needed.

***Keywords:*** multikinase inhibitor, pralsetinib, RET-altered thyroid cancer, RET inhibitor, selpercatinib, thyroid cancer

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## Introduction

Precision oncology has impacted the therapeutic landscape by identifying targetable oncoproteins. Radioactive iodine ($^{131}$I) is considered as the original molecular-targeted therapy against the sodium/iodide symporter expressed by thyroid follicular cells in the treatment of differentiated thyroid cancer (DTC) [papillary thyroid cancer (PTC), follicular thyroid cancer]. Its efficacy was described by Dr Seidlin, in 1946, when he successfully treated a patient with functional metastatic thyroid cancer with $^{131}$I.[1] In the early 2000s, based on the success of imatinib targeting the KIT kinase receptor in gastrointestinal stromal tumors, there was an exponential development of clinical studies on MKIs, primarily inhibiting vascular endothelial growth factor receptor (VEGFR), in various cancers including thyroid.[2] These trials led to the approval of cabozantinib and vandetanib for advanced and progressive medullary thyroid cancer (MTC) and lenvatinib and sorafenib for radioactive-iodine refractory (RAIR) progressive DTC.[3–6] These agents changed the paradigm of management of our patients with advanced thyroid cancer; however, dose-limiting toxicities associated with treatment would often lead to decreased efficacy and drug cessation.

With the advent of comprehensive next-generation sequencing (NGS) of tumors, identifying

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molecular drivers of tumorigenesis, there has been development of targeted therapies with greater efficacy and less potential off-target adverse events (AEs). Germline activating point mutations of REarrangement during Transfection (*RET*) are present in 25% of MTC cases and give rise to hereditary MTC presenting in the form of multiple endocrine neoplasia (MEN) syndromes types 2A and 2B, whereas 45% of sporadic MTC have a somatic *RET* mutation.[7,8] Although *BRAF* V600E is the most common mutation identified in DTC (59.7%) and found in poorly differentiated thyroid cancer (PDTC, 33%) and anaplastic thyroid cancer (ATC, 45%), these follicular cell-derived thyroid cancers can harbor mutually exclusive fusions associated with *RET* (approximately 6–10% of PTC, 6% of PDTC, rarely in ATC), *BRAF*, *NTRK*, *ALK*, and *PPARG*.[9–11]

In this review, we will describe the history of the *RET* proto-oncogene and its pathogenic role in various subtypes of thyroid cancer, summarize the characteristics of nonselective MKIs approved for thyroid cancer, note the development of novel and highly selective RET-inhibitors for *RET*-altered thyroid cancers, and finally emphasize emergent findings and unmet needs in the management of advanced thyroid cancer.

## *RET* proto-oncogene

*RET* was isolated and cloned in 1985 by Takahashi *et al.*[12] from transformed mouse NIH/3T3 fibroblast cells which developed a DNA rearrangement with human T-cell lymphoma DNA during transfection (Figure 1). The *RET* proto-oncogene is located on the long arm of chromosome 10 (10q11.2) and encodes the RET tyrosine kinase transmembrane receptor which is a 170-kDa protein monomer.[13–16]

The RET receptor's physiologic signaling process starts with the binding of growth factors to a coreceptor, which in turn causes RET dimerization and phosphorylation of the intracellular kinase domain. This leads to the activation of RAS/MAPK and PI3K/AKT pathways, involved in cell growth, proliferation, differentiation, survival, and migration.[13,17]

The RET receptor's kinase domain consists of an N-terminal lobe (residues 713–805) and a C-terminal lobe (residues 812–1013) connected *via* a hinge/linker (residues 806–811). The N-terminal lobe consists of ß-sheets, whereas the C-terminal lobe contains α-helices. The catalytic cleft is located between the N-terminal lobe and the C-terminal lobe. It is divided into front cleft (containing the ATP binding site), gate area (preceding the hinge region), and back cleft. The catalytic cleft is the focus of kinase inhibitor development. ATP connects to the backbone of the hinge *via* hydrogen bonds. The size of the side chain of Val804 gatekeeper residue controls access to the catalytic pocket (Figure 2).[18,19]

*RET* is important in the normal formation of the kidney, influencing the development of the Wolffian duct and ureteric bud epithelium and the proliferation, differentiation, and survival of neural crest cells. The importance of *RET* became evident in neonatal mice with a homozygous inactivating *RET* mutation that die soon after birth with renal agenesis and absence of enteric neurons in the digestive tract.[20–22] RET signaling also plays a role in the regulation of hematopoietic cells and spermatogenesis.[23,24] During adulthood, RET is mainly present in organs derived from neural crest cells.[25] Loss-of-function *RET* mutations in humans are associated with Hirschsprung disease, congenital malformations of the kidney and urinary tract, and congenital hypoventilation syndrome.[11]

## *RET* activating mutations

### *Germline mutations*

MTC can be inherited in 25% of cases. Germline activating *RET* mutations occur in the hereditary MEN 2 syndrome, which is further classified based on genotype–phenotype correlation as MEN2A and MEN2B.[8]

MEN2A is the most common type accounting for ~95% of MEN2 cases. It is characterized by MTC in all cases, pheochromocytoma (PHEO) in ~50% of cases, primary hyperparathyroidism ~20–30% of cases. About 95% of mutations in MEN2A occur in the cysteine-rich domain of RET extracellular region (codon 634 in exon 11 accounts for ~85% of cases). These mutations substitute cysteine with another amino acid and result in the formation of disulfide-bonded RET homodimers with subsequent ligand-independent constitutive activation of the kinase region.[8,26]

MEN2B corresponds to ~5% of MEN2 cases. It is the most aggressive type with early onset of MTC. It is characterized by MTC in all cases,

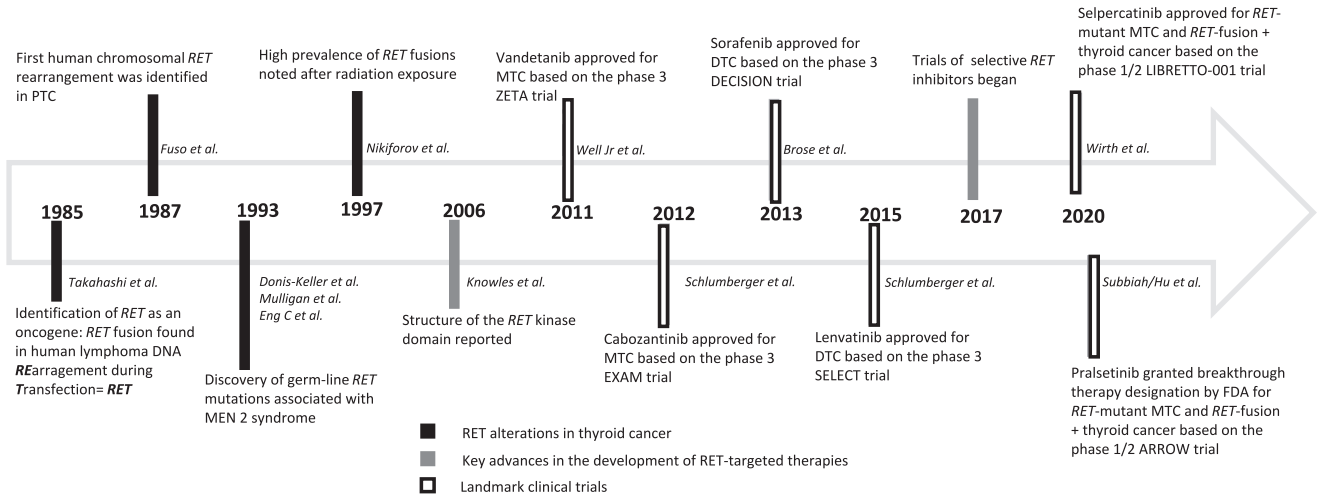


**Figure 1.** RET proto-oncogene: historical background.

PHEO in ~50% of cases, and unique physical features like ganglioneuromas and marfanoid habitus. About 95% of mutations in MEN2B occur in codon M918T of exon 16 which corresponds to the most aggressive form of disease. M918T increases the ATP binding affinity to RET monomers with resultant autophosphorylation without the need of dimerization.[8,26] All patients with MTC should undergo genetic testing for a *RET* mutation, because 1–7% of apparently sporadic MTC are due to *de novo* germline mutations.[8]

### *Somatic mutations*

MTC can be sporadic in 75% of cases. Up to 45% of these patients have somatic-activating *RET* mutations. M918T is the most common reported *RET* somatic mutation, but somatic mutations have been identified involving codons 634, 804, and many others. Deletions and duplications in *RET* have also been found on NGS tumor testing. Mutually exclusive point mutations of *RAS* have been reported in sporadic MTC but with less frequency (approximately 15%).[7]

## *RET* rearrangements or fusions

In addition to activating germline and somatic mutations of *RET*, oncogenesis can be mediated by the development of a chromosomal rearrangement that encodes a protein that fuses the RET kinase domain to a protein partner harboring the dimerization domain. The molecular mechanism responsible for *RET* fusions is thought to be a mistake in repairing DNA double-strand breaks. Chromosomal breakpoints lead to the fusion of the 3′ sequence of *RET* encoding the kinase domain to the 5′ sequence of another upstream partner gene, encoding a dimerization and localizing domain, which in turn produces an active RET fusion protein.[26–30] The malignant potential of RET fusion proteins is determined by two mechanisms: (1) ligand-independent constitutive proliferative signaling and (2) impaired RET inactivation by endocytosis and recruitment of membrane-associated ubiquitin ligases.[31,32]

The first human chromosomal RET rearrangement was identified in PTC by Fuso et al. in 1987. They noted a fusion of the RET tyrosine kinase domain with the 5′ terminal region of CCDC6. This chromosomal rearrangement was named RET-PTC1.[33] Although RET fusions genes identified with PTC were labeled historically in numeric order (e.g. *RET-PTC1*, *RET-PTC2*, *RET-PTC3/4*), the preferred current nomenclature includes the fusion-partner gene name (e.g. *CCDC6-RET*, *PRKAR1A-RET*, and *NCOA4-RET*). Multiple *RET* rearrangements have been described and are cell-specific somatic fusions; no germline *RET* fusions have been identified (Table 1).[9,33–46] *RET* fusions are found in ~6–10% of PTC, 6% of PDTC and are less frequently in ATC.[9,10,47,48] The prevalence of *RET* fusions is higher (approximately 60–80%) in radiation-induced thyroid cancer as evidenced after the Chernobyl nuclear accident and the atomic bomb in Japan.[49–51] *RET* fusions are seen more often in children and young adults diagnosed with thyroid cancers.[52–57]

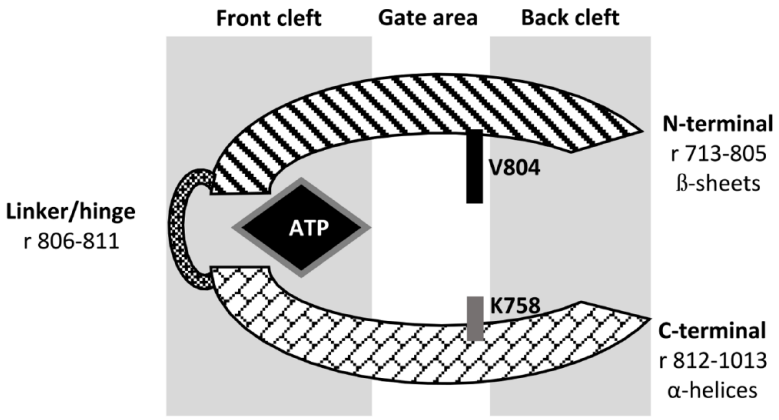


**Figure 2.** RET receptor kinase domain catalytic cleft.
r, residues; V804 is the gatekeeper residue.

## Testing strategies for *RET*

Germline *RET* testing is the standard of care for all patients diagnosed with MTC to evaluate for hereditary MTC in the context of MEN2 syndromes. This is regardless of personal or family history of other MEN2-related neoplasias or MTC, as 1–6% of apparently sporadic MTC will harbor a germline *RET* mutation.[8]

Somatic testing for driving mutations in sporadic MTC will be able to identify targetable mutation of RET, clarify if the cancer harbors a resistance mechanism such as the gatekeeper 804, or identify a currently nontargetable mutation of RAS or others. Testing should be done with NGS, PCR, FISH, or liquid biopsy platforms that are validated.[58]

## RET inhibitors

As *RET* mutations and fusions result in improper RET kinase domain activation, the ATP binding pocket of the kinase domain is a key target for treatment of the associated cancers. MKIs function as ATP-competitive inhibitors and are categorized into five types: type I competes with ATP when the kinase is in its activated form (i.e. vandetanib, sunitinib, pazopanib); type II binds to the ATP binding site and an adjacent hydrophobic/allosteric site only available when the kinase is inactivated and maintains the inactive conformation of the kinase (i.e. cabozantinib, sorafenib, ponatinib); types III and IV are noncompetitive selective inhibitors that bind to an allosteric site distal to the ATP binding site and the hinge; and type V selectively and irreversibly binds to the active kinase site by forming covalent bonds.[59,60]

## Nonselective multikinase inhibitors

MKIs have nonselective RET inhibition, targeting a spectrum of kinases besides RET often with greater potency. The primary therapeutic target of MKIs is VEGFR-2 to inhibit angiogenesis. The inhibition of VEGFR-2 has been implicated in many of the dose-limiting AEs of these agents, such as hypertension, thrombosis, hemorrhage, and problems with wound healing.[61,62] The inferior pharmacokinetic properties contribute to less potent RET inhibition and nonselective targeting of other kinases facilitate drug-related AEs, which can in turn result in dose reduction, interruption, and discontinuation, which limit the efficacy of these drugs (Table 2).[27] Besides having off-target side effects which can be dose limiting, MKIs are ineffective against *RET* V804 gatekeeper mutations.[63]

### *Approved nonselective MKIs for DTC: sorafenib and lenvatinib*

Sorafenib and lenvatinib are MKIs with strong VEGFR blockade. DECISION (NCT00984282 for sorafenib) and SELECT (NCT01321554 for lenvatinib) were the phase III clinical trials that led to approval by the US Food and Drug Administration (FDA) and by the European Union European Medical Agency (EMA) for the treatment of locally recurrent or metastatic progressive RAIR DTC. Keeping in mind that these clinical trials are not comparable to each other, sorafenib showed an increase of 5 months and lenvatinib of 14.7 months in the median progression-free survival (PFS) compared with placebo (primary end point in both trials). Sorafenib showed an overall response rate (ORR) (all PRs)

**Table 1.** *RET* fusions.

| Study | RET/PTC type – historical nomenclature | Partner gene | Tumor type |
|---|---|---|---|
| Grieco *et al.*[33] | RET/PTC1 | *CCDC6* | PTC, NSCLC, CRC |
| Bongarzone *et al.*[34] | RET/PTC2 | *PRKAR1A* | PTC |
| Santoro *et al.*[35]<br>Fugazzola *et al.*[36] | RET/PTC3<br>RET/PTC4 | *NCOA4* | PTC, NSCLC, CRC |
| Klugbauer *et al.*[37] | RET/PTC5 | *GOLGAS* | PTC |
| Klugbauer and Rabes[38] | RET/PTC6 | *TRIM24* | PTC |
| Klugbauer and Rabes[38] | RET/PTC7 | *TRIM33* | PTC, NSCLC |
| Nakata *et al.*[39] | ELKS-RET | *ELKS* | PTC |
| Salassidis *et al.*[40] | RET/PTC8 | *KTN1* | PTC |
| Klugbauer *et al.*[41] | RET/PTC9 | *RFG9* | PTC |
| Corvi *et al.*[42] | PCM1-RET | *PCM1* | PTC |
| Saenko *et al.*[43] | ΔRFP-RET | *TRIM27* | PTC |
| Ciampi *et al.*[44] | HOOK3-RET | *HOOK3* | PTC |
| Cancer Genome Atlas Research Network[9] | ERC1-RET | *ERC1* | PTC |
| Cancer Genome Atlas Research Network[9] | AKAP13-RET | *AKAP13* | PTC |
| Cancer Genome Atlas Research Network[9] | TBL1XR1-RET | *TBL1XR1* | PTC |
| Cancer Genome Atlas Research Network[9] | FKBP-RET | *FKBP* | PTC |
| Cancer Genome Atlas Research Network[9] | SPECC1L-RET | *SPECC1L* | PTC |
| Cancer Genome Atlas Research Network[9] | RET-ANK3 | *ANK3* | PTC |
| Hamatani *et al.*[45] | ACBD5/RET | *ACBD5* | PTC |
| Grubbs *et al.*[46] | MYH13-RET | *MYH13* | MTC |

CRC, colorectal cancer; NSLC, non-small cell lung cancer; PTC, papillary thyroid cancer.

of 12.2% and lenvatinib of 64.8% (CR 1.5% and PR 63.2%). There was no difference in overall survival (OS) between the sorafenib and placebo groups; however, this was confounded by the fact that 71.4% of the placebo-randomized patients crossed over to sorafenib treatment on disease progression. An updated survival analysis found that lenvatinib led to an increase in OS – median OS not reached after 34 months of treatment in the lenvatinib group compared with 19.1 months in the placebo crossover arm (HR = 0.53; $p = 0.0051$).[67]

In general, the most common AEs to both drugs were hypertension, diarrhea, skin/hair/mucous membranes alterations [hand–foot–skin reaction (HFSR), rash, desquamation, alopecia, mucositis], fatigue, decreased appetite, weight loss, and nausea. Common reported laboratory abnormalities were elevation in serum thyroid-stimulating hormone (TSH), hypocalcemia, and proteinuria. AEs prompted dose interruptions (66.2%), reductions (64.3%), or withdrawals (18.8%) in patients receiving sorafenib. HFSR was the most common reason for these to occur. Lenvatinib led to a dose interruption, reduction, and discontinuation in 82.4%, 67.8%, and 14.2% of patients, respectively. Asthenia and hypertension were the most frequent AEs leading to dose discontinuation (each in 1.1% of patients); while diarrhea

**Table 2.** Common adverse events (AEs) associated with tyrosine kinase inhibitors approved for thyroid cancer, in order of frequency. For further description of grading of AEs, recommend referring to the published trial results.[4–6,64–66].

| Sorafenib | Lenvatinib | Vandetanib | Cabozantinib | Selpercatinib | Pralsetinib |
|---|---|---|---|---|---|
| Hand foot skin (76%) | Hypertension (68%) | Diarrhea (57%) | Diarrhea (63%) | Dry mouth (39%) | Leukopenia (34%) |
| Diarrhea (69%) | Diarrhea (59%) | Rash (53%) | Stomatitis (51%) | Hypertension (30%) | Neutropenia (34%) |
| Alopecia (67%) | Fatigue (59%) | Dermatitis acneiform/acne (35%) | Hand foot skin (50%) | Increased AST (28%) | Increased AST (34%) |
| Rash/desquamation (50%) | Decreased appetite (50%) | Nausea (33%) | Weight loss (48%) | Increased ALT (26%) | Hypertension (33%) |
| Fatigue (50%) | Weight loss (46%) | Hypertension (33%) | Decreased appetite (46%) | Fatigue (26%) | Anemia (29%) |
| Weight loss (47%) | Nausea (41%) | Headache (26%) | Nausea (43%) | Peripheral edema (18%) | Constipation (28%) |
| Hypertension (41%) | Stomatitis (36%) | Fatigue (24%) | Fatigue (41%) | Diarrhea (17%) | Asthenia (26%) |
| Anorexia (32%) | Hand foot skin (32%) | Decreased appetite (21%) | Dysgeusia (34%) | Constipation (16%) | Increased ALT (23%) |
| Oral mucositis (23%) | Proteinuria (31%) | Abdominal pain (21%) | Hair color changes (34%) | Nausea (15%) | Hyperphosphatemia (22%) |
| Pruritus (21%) | Vomiting (28%) | Dry skin (15%) | Hypertension (33%) | Increased creatinine (14%) | Lymphopenia (20%) |
| Nausea (21%) | Headache (28%) | Vomiting (15%) | Constipation (27%) | Headache (13%) | Increased creatinine (18%) |
| Headache (18%) | Dysphonia (24%) | QT prolongation (14%) | Abdominal pain (27%) | QT prolongation (13%) | Muscle/joint pain (18%) |
| Cough (15%) | Arthralgia (18%) | Photosensitivity reaction (13%) | Vomiting (24%) | | Dysgeusia (16%) |
| Constipation (15%) | Dysgeusia (17%) | Hypocalcemia (11%) | Dysphonia (20%) | | Diarrhea (16%) |
| | | | | | Low platelets (15%) |
| | | | | | Edema (15%) |
| | | | | | Headache (13%) |
| | | | | | Dry mouth (12%) |

(22.6%), hypertension (19.9%), proteinuria (18.8%), and decreased appetite (18.0%) were the most common reasons for lenvatinib treatment interruption or reduction.[4,64]

### *Approved nonselective MKIs for MTC: vandetanib and cabozantinib*

Vandetanib and cabozantinib are MKIs with predominant VEGFR blockade; however, vandetanib also inhibits epidermal growth factor receptor (EGFR) while cabozantinib targets mesenchymal epithelial transition factor (MET). ZETA (NCT00410761 for vandetanib) and EXAM (NCT00704730 for cabozantinib) were the phase III clinical trials that led to approval by the FDA and EMA for the treatment of progressive, unresectable, locally advanced, or metastatic MTC, regardless of tumor genotype.[5,6] Vandetanib showed an increase of 11.2 months and cabozantinib of 7.2 months in median PFS compared with placebo. Vandetanib showed an ORR of 45% (all PRs) and cabozantinib of 28% (all PRs). Responses were seen regardless of *RET* mutation status; however, a subgroup analysis in the vandetanib trial showed a PFS statistical benefit in

MTC harboring the somatic *RET* M918T mutation compared with placebo; and a similar observation was appreciated with cabozantinib in germline/somatic *RET* M918T mutation. The cabozantinib trial also suggested a PFS benefit in *RAS*-mutated MTC when compared with placebo but this was not statistically significant due to the small number of patients analyzed. Both trials are not comparable to each other, and it is important to highlight that the cabozantinib trial required radiological progression before enrollment, whereas the vandetanib study did not. Both drugs led to a biochemical response rate of calcitonin reduction in more than 45% from baseline with a similar trend in carcinoembryonic antigen (CEA). At the study cut-off date, the median OS calculation for vandetanib was not reached; however, it is likely to be confounded due to crossover from placebo to the active treatment in progressing patients. There was a 5.5 month improvement in median OS with cabozantinib over placebo (26.6 *versus* 21.1 months) that was not statistically significant.[68] Patients receiving placebo were not allowed to cross over to cabozantinib at the time of progression in the trial.

In general, the most common AEs to both drugs were diarrhea, stomatitis, rash, palmar-plantar erythrodysesthesia (PPE), decreased weight and appetite, nausea, vomiting, abdominal pain, fatigue, hypertension, and headache. Potential laboratory abnormalities consisted of elevated liver function tests (LFTs), electrolyte disorders (hypocalcemia, hypophosphatemia, hypomagnesemia, hypokalemia, hyponatremia), alterations in complete blood count (lymphopenia, neutropenia, thrombocytopenia), proteinuria, and elevated TSH. AEs prompting dose reductions or withdrawals occurred in 35% and 12% of patients receiving vandetanib, respectively. Asthenia (1.7%) and rash (1.3%) were the most frequent AEs leading to discontinuation of vandetanib. QTc prolongation was present in 8% of patients receiving vandetanib. Five patients on vandetanib had AEs leading to death (aspiration pneumonia, respiratory arrest, respiratory failure, staphylococcal sepsis, and arrhythmia with cardiac failure).[5] A Risk Evaluation and Mitigation Strategy (REMS) is required to prescribe vandetanib due to its black box warning indicating the possibility of QT prolongation, torsade de pointes or sudden death. Cabozantinib had dose reduction, interruption, and discontinuation rates of 79%, 65%, and 16% of patients, respectively. Cabozantinib led to rare but serious AEs associated with its VEGF pathway inhibition (perforation, fistula, hemorrhage, thrombosis, impaired wound healing, osteonecrosis, and HFSR).[6] Cabozantinib has a black box warning listing gastrointestinal perforations, fistulas, and hemorrhage (including hemoptysis and gastrointestinal hemorrhage); therefore, it should be used with caution or avoided in patients with a history of radiation to the neck or mediastinum and injury to the respiratory or gastrointestinal mucosa (i.e. diverticulitis, inflammatory bowel disease, active peptic ulcer disease). In addition, tumor invasion of the trachea, bronchi, and esophagus increases the risk of fistula formation. Tumors that encase major blood vessels or invade the GI mucosa are associated with a high risk of bleeding; whereas conditions known to cause acute surgical abdomen such as cholecystitis and appendicitis are at increased risk of organ perforation.[69,70] Clinically relevant QTc prolongation >500 ms was not appreciated in the cabozantinib phase III trial, as it was with vandetanib.[6,71]

## Selective RET inhibitors

These are small molecule, ATP-competitive, potent, and highly selective RET inhibitors designed to overcome gatekeeper *RET* mutations and associated with less toxicity, dose reductions, and treatment discontinuations (Table 3).[72]

### *Selpercatinib*

The safety and efficacy of selpercatinib (formerly known as LOXO-292) was evaluated in an international, multicenter, open-label, phase I/II trial known as LIBRETTO-001 (NTC03157128).[65] A total of 531 patients (⩾12 years old) with any locally advanced or metastatic solid tumor type harboring an activating *REt al*teration were enrolled in the study, out of which 162 patients had a *RET*-altered thyroid cancer. In the thyroid cancer population, 55 patients had *RET*-mutant MTC previously treated with vandetanib and cabozantinib, 88 patients had *RET*-mutant MTC not previously treated with vandetanib or cabozantinib, and 19 patients had *RET* fusion-positive previously treated non-MTC (13 PTC, 3 PDTC, 2 ATC, 1 Hurthle cell). *RET* M918T mutation and *CCDC6-RET* fusion were the most common *REt al*terations; in addition, patients with the acquired gatekeeper-resistance mutation *RET* V804 were included in the study.

**Table 3.** Efficacy of selective RET inhibitors phase I/II clinical trials.

| Agents | References | Trial design (name) | Subjects (*n*)/ cancer type | ORR[a] *n* (%) | CR *n* (%) | PR *n* (%) | SD *n* (%) | PFS (at 1 year) | OS (at 1 year) |
|---|---|---|---|---|---|---|---|---|---|
| Selpercatinib | Wirth *et al.* | Phase I/II trial (LIBRETO-001) | 55 RET + MTC previously treated[b] | 38 (69%) | 5 (9%) | 33 (60%) | 14 (25%) | 82% | NR |
| | | | 88 RET + MTC treatment naive | 64 (72%) | 10 (11%) | 54 (61%) | 20 (23%) | 92% | NR |
| | | | 19 RET fusion + thyroid cancer[c] | 15 (79%) | 1 (5%) | 14 (74%) | 4 (21%) | 64% | NR |
| Pralsetinib | Subbiah/Hu *et al.* | Phase I/II trial (ARROW) | 55 RET + MTC previously treated[b] | 33 (60%) | 1 (2%) | 32 (58%) | 18 (33%) | 75% | 89% |
| | | | 21 RET + MTC treatment naïve | 15 (71%) | 1 (5%) | 14 (67%) | 6 (29%) | 91% | 91% |
| | | | 9 RET fusion + thyroid cancer[d] | 8 (89%) | 0 | 8 (89%) | 0 | 81% | 91% |

CR, complete response; MTC, medullary thyroid cancer; NR, not reported; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; PR partial response; SD, stable disease.
[a]Primary outcome.
[b]With vandetanib and cabozantinib.
[c]Thirteen papillary thyroid cancer (PTC), three poorly differentiated thyroid carcinoma (PDTC), two anaplastic thyroid carcinoma, one Hurthle cell carcinoma.
[d]Ten PTC, one PDTC (9/11 response-evaluable patients).

Eligible patients had advanced disease that have progressed following prior treatment or had no acceptable alternative treatment options. In addition, eligible *RET* fusion-positive thyroid cancer patients were defined as having RAIR disease (except for ATC in which radioiodine is not used) and had received at least one previous systemic therapy other than radioiodine. It is important to note that radiographic tumor progression was not a definite inclusion criterion, and patients with progressive disease on study drug could continue selpercatinib if they were receiving clinical benefit overall.

Phase I was the dose-escalation portion of the study and determined the recommended phase II dose of 160 mg twice daily by mouth. *RET*-mutant MTCs previously treated with vandetanib and cabozantinib had an ORR (primary endpoint) of 69% [CR 9% ($n=5$), PR 60% ($n=33$), SD 25% ($n=14$)]; and a 1-year PFS of 82%. *RET*-mutant MTCs not previously treated with vandetanib or cabozantinib had an ORR of 73% [(CR 11% ($n=10$), PR 61% ($n=54$), SD 23% ($n=20$)]; and a 1-year PFS of 92% (95% CI, 82–97). *RET* fusion-positive non-MTC patients had an ORR of 79% with activity seen across all histologic types [CR 5% ($n=1$), PR 74% ($n=14$)] and a 1-year PFS of 64%. Patients with *RET*-mutant previously treated MTC had a biochemical response of 91% (54 patients evaluated) regarding calcitonin (median time to biochemical response = 0.5 months) and 66% (53 patients evaluated) regarding CEA (median time to biochemical response = 1.8 months). The efficacy of selpercatinib treatment was observed regardless of the number of previous MKI therapies received, radioiodine treatments, or type of *RET* mutation/fusion. Notably, responses were observed in 3 of the 8 MTC patients with *RET* V804 gatekeeper mutations.

The most common treatment-related adverse events (TRAEs) were dry mouth, hypertension, fatigue, peripheral edema, diarrhea, constipation, nausea, headache, QTc prolongation, rash, vomiting, abdominal distention, dizziness, arthralgia, increased weight, abdominal pain, cough, and back pain. Laboratory abnormalities consisted of elevated LFTs, increased serum creatinine, elevated TSH, and electrolyte abnormalities (hypocalcemia, hyponatremia). Most TRAEs were grade 1 or 2; however, the most common grade 3 or 4 AE was hypertension (12%) followed by increased LFTs, diarrhea, and prolonged QTc. The investigators deemed all grade 5 AEs (hemoptysis,

postprocedure hemorrhage, sepsis, cardiac failure, and cardiac arrest) to be unrelated to selpercatinib. One patient with MTC developed grade 3 tumor lysis syndrome. Of the total 531 cohort of patients who received selpercatinib, 160 (30%) had a dose reduction and 12 (2%) had drug discontinued due to TRAEs.

Based on results from the phase I/II LIBRETTO-001 trial, selpercatinib was approved by the FDA in May 2020 and by the EMA in February 2021 for the treatment of advanced or metastatic *RET* fusion-positive non-small cell lung cancer (NSCLC), *RET* fusion-positive RAIR-thyroid cancer, and *RET*-mutant MTC in adults and children ⩾ 12 years old. The treatment dose is 160 mg oral twice daily or a reduced dose of 120 mg twice daily in patients who weigh <50 kg.[73] A large, international phase III trial comparing selpercatinib with standard of care (based on investigator's choice of cabozantinib or vandetanib) is actively enrolling *RET*-mutated, treatment naïve MTC patients with the clinically meaningful primary outcome of treatment failure-free survival (NCT04211337).

### *Pralsetinib*

Subbiah *et al.*[74] reported that the antitumor activity of pralsetinib (formerly known as BLU-667) was ⩾10-fold than vandetanib and cabozantinib in preclinical models harboring different *RET* oncogenic alterations (*KIF5B-RET*, *CCDC6-RET*, *RET* C634W, *RET* M918T, and gatekeeper mutations *RET* V804L/M/E) with comparatively less activity against VEGFR-2.

ARROW is an ongoing, international, multicenter, open-label phase I/II clinical trial (NCT03037385) evaluating pralsetinib in patients with unresectable, locally advanced, or metastatic RET-driven solid cancers. Patients ⩾ 18 years old with both kinase inhibitor-naive and kinase inhibitor-refractory disease, as well as any number of prior therapies, were eligible. The phase I dose-escalation study recommended the phase II dose of 400 mg oral once daily. The phase II portion enrolled the following thyroid cancer cohorts: (1) *RET*-mutant MTC and (2) *RET*-fusion-positive thyroid cancer. For inclusion in the MTC group, patients were required to have disease progression within 14 months before enrollment. All subjects required measurable disease at the time of enrollment and a confirmed pathogenic *RET* mutation. The primary outcomes from phase II were ORR and safety. Most secondary outcomes at the time of data cut-off were not reached and included median duration of response (DoR), clinical benefit rate, disease control rate (DCR), PFS, and OS.[66]

The *RET*-mutant MTC cohort consisted of a total of 84 patients with mostly sporadic disease and harboring different activating mutations: 58% M918T, 31% cysteine- rich domains, 7% V804L/M (including 3 of whom also had a coincident M918T mutation), and 7% other mutations. Of these, 55 response-evaluable patients (REP) were previously treated with cabozantinib and vandetanib and 21 REP were treatment-naïve.

In *RET*-mutant MTC patients previously treated with cabozantinib and vandetanib, the ORR was 60% ($n = 33/55$; 95% CI = 46–73) with 2% CR ($n = 1/55$). Median time to first response was 3.7 months. The median DoR was not reached with median follow-up of 11.2 months. The estimated ongoing response at 6 months was 96% and at 12 months was 92%. The estimated 1-year PFS was 75% after a median follow-up of 14.9 months. The estimated 1-year OS was 89% after median follow-up of 16.5 months.[66]

Among treatment naïve *RET*-mutant MTC patients, the ORR was 71% ($n = 15/21$; 95% CI = 48–89) with 5% CR ($n = 1/21$). Median time to first response was 5.6 months. The median DoR was not reached with median follow-up of 10.8 months. The estimated ongoing response at 6 months was 93% and at 12 months was 84%. The estimated 1-year PFS was 81% after a median follow-up of 15.1 months. The estimated 1-year OS was 91% after median follow-up of 18.5 months.[66]

Responses were observed regardless of the *RET* mutation in both MTC cohorts, including gatekeeper mutations V804L/M. Disease-related diarrhea resolved in 14/15 patients by the end of the second cycle. Biochemical response rates of calcitonin and CEA were 87% ($n = 72/83$) and 66% ($n = 52/79$), respectively.[66]

The *RET*-fusion positive thyroid cancer cohort included 10 PTC and 1 PDTC. *RET*-fusion partners included *CCDC6* (6, 55%), *NCOA4* (2, 18%), and other (3, 27%). Patients with RAIR disease and any prior systemic therapy were allowed in the study. Of the 9 REP, the ORR was

89% ($n$ = 8/9; 95% CI = 52–100) with all PR. Median time to first response was 1.9 months. The median DOR was not reached with a median follow-up of 9.5 months. The estimated ongoing response at 6 months was 100% and at 12 months was 86%. The estimated 1-year PFS was 81% after a median follow-up of 12.9 months. The estimated 1-year OS was 91% after median follow-up of 15.8 months. Responses were observed across fusion genotypes.[66]

The ARROW study reported safety for the total 142 patients with *RET*-altered thyroid cancer who initiated pralsetinib at the recommended phase II dose of 400 mg PO daily. The most common TRAEs were anemia, musculoskeletal pain, constipation, elevated AST, and hypertension. Common grade 3 and above AEs were hypertension and blood cell alterations (neutropenia, lymphopenia, and anemia). The most frequent serious TRAE was pneumonitis in 4% ($n$ = 5). Dose reduction owing to TRAEs occurred in 46% ($n$ = 66), dose interruptions in 54% ($n$ = 76), and treatment discontinuation in 4% ($n$ = 5 due to anemia in 2 patients, pneumonia in 1, elevated CPK in 1, and ARDS and pneumonitis in 1). One patient died after developing interstitial pneumonitis considered a TRAE who succumbed due to *Pneumocystis jirovecii* pneumonia. QTc prolongation was seen in 7 patients, mostly grade 1–2 with one being grade 3.[66]

Based on the data from the phase I/II ARROW study, the FDA granted breakthrough therapy designation to pralsetinib in September 2020 for the treatment of adults with metastatic *RET* fusion-positive NSCLC. In December 2020, the FDA approved pralsetinib for the treatment of adults and pediatric patients ⩾12 years old with advanced or metastatic *RET*-mutant MTC who require systemic therapy, or with advanced or metastatic *RET* fusion-positive nonmedullary RAIR thyroid cancer who require systemic therapy. The initial treatment dose for both adults and children ⩾12 years old is 400 mg oral once daily. The ARROW study is ongoing and continues to enroll patients in the non-MTC cohort and other solid tumors (excluding NSCLC) with *RET*-alterations with an estimated primary completion date of December 2021.[66]

Both selpercatinib and pralsetinib seem equally effective for these patient populations. The only clearly distinguishable features are the difference in dosing interval (selpercatinib given twice a day; pralsetinib given once a day) and that selpercatinib can be dissolved per specified instructions.

### *TPX-0046*

TPX-0046 is a novel, potent, and selective inhibitor of both RET and SRC with a rigid macrocyclic structure unlike other RET inhibitors which makes it active against various mutations, especially the emergent SFM *RET* G810 that conveys resistance to selpercatinib and pralsetinib (discussed later in this article). Its potency was evidenced *in vitro* with cell culture proliferation assays where at low nanomolar levels, TPX-0046 inhibited wild-type RET, many mutated RET receptors, SRC, but spared VEGFR2. TPX-0046 was also able to overcome the SFM G810R at a mean IC50 of 17 nM compared with selpercatinib and pralsetinib which have IC50s >500 nM. In addition, TPX-0046 showed robust anti-tumor efficacy with *in vivo* cell-derived and patient-derived xenograft *RET*-driven tumor models.[75] However, TPX-0046 does not target the gatekeeper V804, which limits its effectiveness in some patients with MTC especially if they harbor both gatekeeper and SFMs. It is currently undergoing a phase I/II clinical trial (NCT04161391) in adult subjects (⩾18 years old) with advanced, progressive, or metastatic solid tumors harboring *RET* fusions or mutations.

### *BOS172738*

BOS172738 is a small molecule RET inhibitor that has demonstrated robust low nanomolar potency (kd ⩽1 nM) against wild-type RET and fusion and mutated protein receptors including M918T, V804L, and V804M, while keeping approximately 300-fold selectivity against VEGFR2. BOS172738 produced durable tumor regression and tumor growth inhibition at similar or lower $IC_{50}$ concentrations compared with ponatinib in preclinical studies.[76,77] It is currently undergoing a phase I (NCT03780517), open label, multicenter, dose escalation study to evaluate safety, tolerability, pharmacokinetics, ad pharmacodynamics in adult patients (⩾18 yo) with advanced solid tumors with *REt al*terations.

### *TAS0953/HM06*

TAS0953/HM06 is a selective RET inhibitor undergoing a phase I/II clinical trial (MARGARET study) (NCT04683250) in adult patients (⩾18

**Table 4.** Mechanisms of resistance and IC50 (μM) for each drug.

| Mutation status | | $IC_{50}$ (μM) | | | | |
|---|---|---|---|---|---|---|
| | | Lenvatinib | Vandetanib | Cabozantinib | Selpercatinib | Pralsetinib |
| | RET wild type | **0.19** | **0.1** | **0.0098** | **0.0004** | **0.0004** |
| | M918T | 1.42 | 1.83 | 1.57 | **0.009** | **0.001** |
| Gatekeeper | V804L | 10.60 | 6.10 | 3.22 | **0.0172** | **0.0018** |
| | V804M | 5.42 | 5.83 | 4.26 | **0.0559** | **0.0168** |
| Solvent front | G810A | **0.11** | 2.76 | **0.22** | – | – |
| | G810R | – | – | – | 2.744 | 2.650 |
| | G810S | 0.67 | 5.47 | 1.05 | 0.8802 | 0.3906 |
| | G810C | – | – | – | 1.227 | 0.6417 |
| Fusion | CCDC6-RET | – | **0.02** | **0.034** | **0.01** | **0.00045** |
| | VEGFR2 | **0.004** | **0.04** | **0.000035** | **0.1** | **0.035** |

$IC_{50}$ concentration causes 50% inhibition of growth.
The values are mean.
Gray: resistant; black: nonresistant.

years old) with advanced, progressive, or metastatic RET-altered solid tumors with or without prior MKI therapy. Phase I aims to recommend the maximum tolerated dose and dose expansion whereas phase II will determine ORR as primary outcome.

## Mechanisms of resistance to RET inhibitors

Resistance mechanisms are usually first identified in preclinical studies followed by patient studies, and later overcome with the discovery of new drugs. However, with the accessibility and wider use of NGS of progressing tumors and liquid biopsy, real-time identification of emergent mutations in patients that progress on treatments can offer insight into possible resistance mechanisms optimizing further treatment planning. Acquired resistance to RET inhibitors takes place *via* two different mechanisms: (1) on-target mutations (target modification) that prevent drug binding and (2) activated alternative mechanisms which bypass the targeted kinase (bypass signaling).

### *Target modification*

The presence of the gatekeeper *RET* V804M/L mutations will convey resistance to MKIs with low selectivity for RET (Table 4).[27,63,78–81] This takes place because MKIs bind to the front and back clefts of the RET kinase catalytic pocket by inserting through the V804 gate residue that separates the two clefts. V804 mutants with bulkier leucine or methionine side chains prevent insertion of the MKIs between the front and back clefts (Figure 3(a)).[18,63] *RET* gatekeeper mutations V804M/L have been reported in NSCLC and MTC.[78,80,82,83] Another point mutation of *RET* S904F in the activation loop reduces vandetanib drug binding.[84]

The selective RET inhibitors were designed to overcome gatekeeper mutations. Both selpercatinib and pralsetinib bind to the front and back clefts of the RET kinase catalytic pocket without going through the gate between V804 and K758. Instead, they pass around the gate wall K758 residue to access the back pocket (Figure 3(b)). In this way, gatekeeper mutations do not disrupt their binding mode, but they remain vulnerable to several identified nongatekeeper mutations.[63,72,74]

In addition, several emergent nongatekeeper mutations have been identified recently. The RET G810 residue is located at the C-lobe solvent front side. Mutations at this site are known as SFMs, where glycine (which has a smaller side chain) is replaced with aminoacids like alanine,

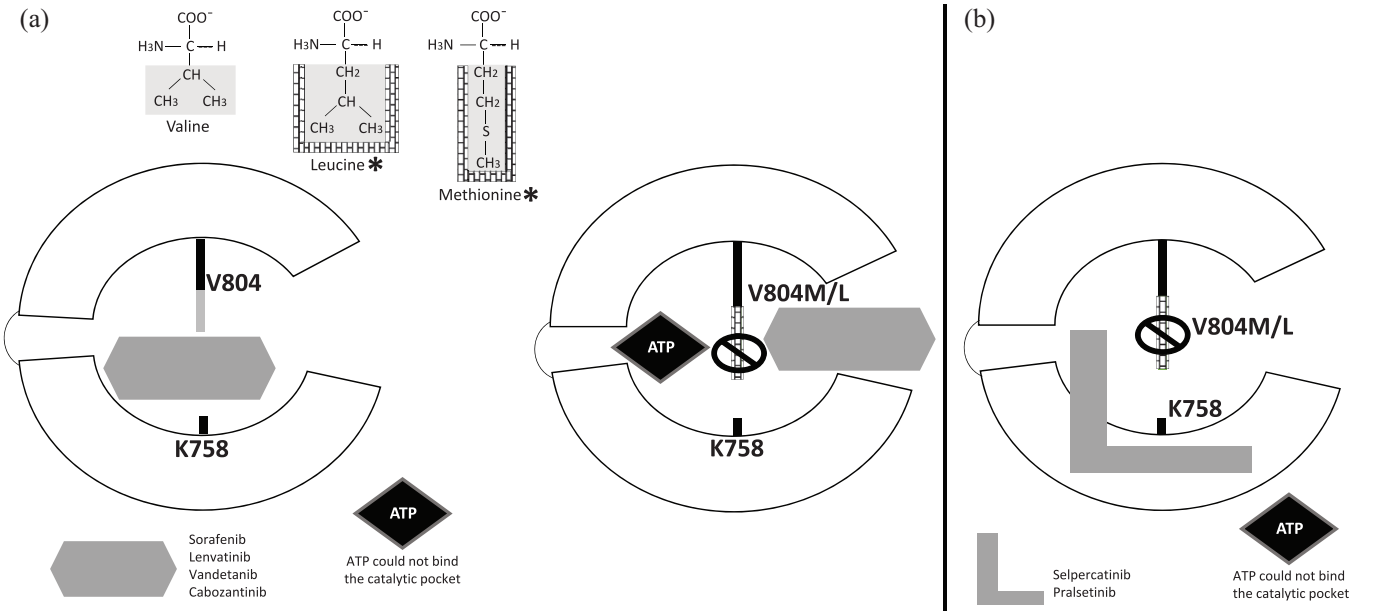


**Figure 3.** Mechanism of resistance to RET inhibition: Target modification V804M/L gatekeeper mutation. RET tyrosine kinase domain catalytic cleft as shown in Figure 2. (a) Asterisks indicate structural differences of leucine and methionine with bulkier side chains compared with nonmutant valine residue. The bulkier side chains (in brick wall) prevent multitargeted tyrosine kinase inhibitors (sorafenib, lenvatinib, vandetanib, cabozantinib) to communicate between the front and back clefts of the RET receptor kinase domain catalytic cleft. (b) Selpercatinib and pralsetinib can overcome gatekeeper mutations as they bind to the front and back clefts of the RET kinase domain catalytic pocket without going through the gate between V804 and K758. Instead, they pass around the gate wall K758 residue to access the back pocket.

cysteine, serine, arginine, or valine (G810A/C/S/R/V, which have larger side chains that prevent drug binding).[63,85] A resistant SFM was first described in a *KIF5B-RET*-fusion NSCLC patient who initially responded to selpercatinib on study but then progressed correlating with detectable circulating tumor DNA for G810R/S/C.[86] In addition, a MTC patient with dual mutations of *RET* M918T and V804M had initial durable response to selpercatinib but then progressed with demonstration of rising levels of cell-free DNA for M918T, V804M as well as G810C/S mutations.[63] Acquired SFMs G810C/S were also reported in *CCDC6-RET* fusion NSCLC patients at the time of progression while on pralsetinib.[87]

In addition to *RET* gatekeeper and SFMs, Subbiah *et al.*[63] identified additional mutations affecting other portions of the RET receptor which contributed to diminished efficacy of selective RET-inhibitors. The RET Y806 residue is located at the hinge site of the kinase domain where the hydrophobic side chain of tyrosine allows van der Waals interactions with selpercatinib and pralsetinib rings. These interactions would be interrupted if cysteine or asparagine are substituted in for tyrosine (Y806C/N) as they have nonhydrophobic, shorter side chains. In addition, selpercatinib and pralsetinib interact with the side chain V738 located on the ß2 strand of N-lobe in the front pocket. This interaction would be lost if V738 was substituted with the shorter side chain of alanine (V738A). The IC50s of both selpercatinib and pralsetinib against *RET* Y806 hinge and V738A mutations were approximately over 150 nM.[63]

Second-generation selective RET inhibitors are being developed with the aim to overcome evolving mutations. Although TPX-0046 has the benefit of overcoming SFMs, it is not effective against the gatekeeper V804 mutations.[75]

### *Bypass signaling*

*RET*-altered tumors can develop escape mechanisms to drug receptor inhibition by activating oncogenic alternative or downstream pathways independent of RET activation. Preclinical and clinical data on *RET*-fusion NSCLC have shown that RET-inhibition can be overcome through oncogenic activation of *MET*, *EGF*, and *RAS*.[88–91] Coincident activating *BRAF*, *KRAS*, and *NRAS* mutations have been demonstrated in *RET*-altered

PTCs, although it is hard to assess whether they are present in the same cell or represent different clones of cells within the same tumor.[92,93] Hu *et al.*[94] reported the emergence of *KRAS* pan G12/G13 bypass mutation alone or combined with *RET* gatekeeper mutation V804M in sporadic MTC patients with progressive disease after a minimum of 6-month treatment with cabozantinib and vandetanib. A targeted drug combination therapy such as a selective kinase inhibitor with a MEK-inhibitor (for patients with *RAS* bypass mutations) or an m-TOR inhibitor (for inhibition of the PI3K/AKT pathway) could potentially be used to overcome bypass signaling.[95–97]

## Uncertainty of RET inhibition on nontumor cells

The effects of long-term RET inhibition on normal tissues are not completely understood but remain as potential risks that should be carefully considered. For instance, potential linear growth and development retardation in children with open epiphyses remain uncertain as well as the effects on fertility. There is animal toxicity data showing abnormal bone growth and tooth dysplasia and discoloration in rats and minipigs, hence the suggestion to monitor open growth plates in adolescent patients.[98] The selective RET-inhibitors, pralsetinib, and selpercatinib, are known to have good penetration into the central nervous system (CNS) which is clinically beneficial for patients with CNS metastases; however, there is concern of neuron disruption, particularly dopaminergic neurons where GFL-RET signals are important for survival. The inhibition of RET signaling could also potentially affect hematopoietic cells with a negative impact on immune responses as seen in animal models.[99] Since these medications are relatively new for use in *RET*-altered cancer patients, long-term monitoring will help clarify potential repercussions on normal physiology from decreased RET activity.

## Conclusion and further directions

Precision oncology in the treatment of *RET*-dependent cancers is an evolving field with promising outcomes with the development of highly selective and potent RET inhibitors. The approval of selpercatinib and pralsetinib in 2020 based on phase I/II trials demonstrating high response rates in both *RET*-mutated MTC (previously treated and kinase inhibitor-naïve) and *RET*-fusion non-MTC balanced with fewer off-target side effects gives rise to an optimistic new phase in the management of these rare cancers. However, the development of on-target or bypass resistance mechanisms likely will become more common with wider use of RET inhibitors. Further research and development of potent RET inhibitors with broader coverage of known (gatekeeper and SFMs) and potential resistance mechanisms are much needed. In addition, exploration of combination therapies should be undertaken to optimally target-activated intracellular pathways. Advanced thyroid cancers with *RAS* or other nontargetable mutations can be treated with the approved nonselective MKIs, but as discussed in this review, dose-limiting toxicities often limit their effectiveness. More effective treatments are much needed for such patients with targetable mutations. The potential long-term repercussions of potently inhibiting normal RET physiology of other cells remain incompletely understood and should not be dismissed. It is tempting to consider implementing a highly effective and well-tolerated drug earlier on in a patient's care if there is biochemical progression without significantly burdensome or progressive structural disease. However, unless clinical trials demonstrate oncologic benefit of this treatment paradigm and long-term risk of RET inhibition is found to be minimal, use of RET inhibitors for advanced *RET*-altered thyroid cancer should remain reserved for patients with structurally progressive disease not amenable to other treatments.

## Author contribution(s)

**Danica M. Vodopivec:** Conceptualization; Data curation; Investigation; Methodology; Resources; Visualization; Writing – original draft.

**Mimi I. Hu:** Conceptualization; Data curation; Investigation; Methodology; Resources; Supervision; Validation; Visualization; Writing – review & editing.

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