The importance of the *RET* gene in thyroid cancer and therapeutic implications

Domenico Salvatore[1], Massimo Santoro[2] & Martin Schlumberger[3†]

[1]Department of Public Health, University of Naples “Federico II”, Naples, Italy.

[2]Department of Molecular Medicine and Medical Biotechnology, University of Naples "Federico II", Naples, Italy.

[3]Département de Médecine Nucléaire et Cancérologie Endocrinienne, Gustave Roussy, Université Paris-Saclay, Villejuif, France.

[†]Email: Martin.SCHLUMBERGER@gustaveroussy.fr

Abstract

Since the discovery of the RET receptor tyrosine kinase in 1985, alterations of this protein have been found in diverse thyroid cancer subtypes. *RET* gene rearrangements are observed in papillary thyroid carcinoma, which result in RET fusion products. By contrast, single amino acid substitutions and small insertions and/or deletions are typical of hereditary and sporadic medullary thyroid carcinoma. *RET* rearrangements and mutations of extracellular cysteines facilitate dimerization and kinase activation, whereas mutations in the *RET* kinase coding domain drive dimerization-independent kinase activation. Thus, RET kinase inhibition is an attractive therapeutic target in patients with *RET* alterations. This approach was initially achieved using multikinase inhibitors, which affect multiple deregulated pathways that include RET kinase. In clinical practice, use of multikinase inhibitors in patients with advanced thyroid cancer resulted in therapeutic efficacy, which

was associated with frequent and sometimes severe adverse effects. However, remarkable progress has been achieved with the identification of novel potent and selective RET kinase inhibitors for patients with advanced thyroid cancer. Although expanded clinical validation in future trials is needed, the sustained anti-tumoural activity and the improved safety profile of these novel compounds is opening a new exciting era in precision oncology for *RET*-driven cancers.

[H1] Introduction

Since the discovery of the *RET* gene in 1985, considerable progress has been made in the understanding of its physiology and role in diseases, particularly thyroid cancer. *RET* encodes a receptor tyrosine-protein kinase (RET kinase) that is involved in numerous cellular mechanisms. In 2008, we reviewed the role of *RET* in thyroid cancer for *Nature Clinical Practice Endocrinology & Metabolism* (later renamed *Nature Reviews Endocrinology*)[1]. Since that time, major advances have been made, including the approval of tyrosine kinase inhibitors (TKIs) that inhibit vascular endothelial growth factor receptor type 2 (VEGFR2) and RET kinase. Furthermore, potent and selective RET kinase inhibitors have been identified. The 2020 approval of selpercatinib, one of these new RET TKIs, by the US Food and Drug Administration (FDA) prompted us to produce this updated Review[2, 3].

[H1] The role of *RET* in human malignancies

In 1985, Takahashi and colleagues cloned a novel oncogene from a human T lymphoma; this oncogene was able to transform fibroblasts after transfection in culture. The transforming oncogene was named *RET* (REarranged during Transfection) because it was generated by recombination between two unlinked DNA fragments, which probably occurred during the transfection procedure. The researchers showed that fusion occurred

between the 3′-terminal sequence of wild-type *RET* and the 5′-terminal sequence of *RFP* (encoding RET Finger Protein)[4]. In 1989, *RET* was mapped to the long arm of chromosome 10 (10q11.2)[5], a milestone that indicated *RET* as a candidate gene for multiple endocrine neoplasia type 2 (MEN2) syndrome (**Box 1**), which had also been mapped to that chromosomal region[6]. Also in 1989, *RET* was demonstrated to encode a transmembrane receptor with tyrosine kinase (RTK) activity[7]. Following this discovery, in 1990, *RET* fusions were shown to be a common oncogenic driver event of papillary thyroid carcinoma (PTC, the fusions were called *RET*–PTC)[8]. Furthermore, in 1993, single amino-acid substitutions and small insertions and/or deletions were identified in *RET* in hereditary and sporadic forms of medullary thyroid carcinoma (MTC)[9]. Finally, in 2012, *RET* fusions have been identified in non-small cell lung carcinoma (NSCLC)[10-12], and subsequently were identified in colon, breast and other cancer types[13-15] (**Figure 1**).

[H1] RET kinase structure and physiology

[H2] RET kinase structure

RET encodes a transmembrane RTK (RET) (**Figure 2**). The extracellular domain features four repeats of ~110 amino acids with similarities to cadherins (that is, cadherin-like domains CLD1–4), one Ca^{2+} binding site between CLD2 and CLD3, and one cysteine-rich domain (CRD). The plasma membrane is traversed by the transmembrane segment and the intracellular domain comprises the intra-cellular juxtamembrane segment and the tyrosine kinase domain (TKD). This TKD folds in a typical twin-lobed catalytic core, with a N-terminal lobe that contains the glycine-rich nucleotide-binding loop involved in ATP binding, and a C-terminal lobe that contains a kinase insert of 14 amino acids. The two lobes are separated by a hinge[16]. The TKD C-terminal lobe contains the activation segment, which is a major regulator of the catalytic activity. The conformation of this activation segment is misshapen by the M918T mutation in MTC (see later), with this

change leading to increased and faster kinetics of autophosphorylation than in the wild-type protein[14, 17, 18]. The TKD is followed by one of two alternatively spliced carboxyl-terminal tails, which diverge, starting from residue 1063, for their last 9 (forming the RET-9 isoform) or 51 (forming the RET-51 isoform) amino acids[13, 14, 19].

[H2] RET kinase physiology

[H3] Cellular and tissue expression. RET kinase has important roles in several tissues and cell types, which suggests that other pathologies, besides cancer, could benefit from the use of drugs that act on RET kinase. Furthermore, knowledge of RET kinase physiology could also help with anticipating the potential on-target toxic effects of pharmacological RET kinase inhibition. The function of RET kinase is important in neuronal populations of the central nervous system (CNS), including spinal cord motoneurons, ventral midbrain dopaminergic neurons (these neurons are involved in Parkinson disease) and appetite centres. RET kinase function is also essential for the correct development of parasympathetic enteric neurons of the myenteric plexus and sub-mucosal plexus, which are involved in bowel peristalsis. RET kinase is also expressed in parafollicular thyroid C-cells, haematopoietic stem cells, ureteric bud tip cells (during kidney organogenesis), spermatogonia, growth hormone-secreting pituitary somatotrophs and gut haematopoietic cells (which form Peyer's patches)[19-25].

[H3] RET kinase signalling. RET kinase is stimulated by ligands of the glial cell line-derived neurotrophic growth factor (GDNF) family, named GDNF-family ligands, that belong to the TGFβ superfamily. They include GDNF, neurturin, persephin and artemin, and bind to glycosylphosphatidylinositol (GPI)-linked cell surface co-receptors, named GDNF family receptors (GFRα1–4). Thus, a tripartite complex forms between the GDNF family ligand–GFRα complex and RET kinase, thereby stimulating RET kinase[14, 26]. For

example, in the GDNF–GFRα1 complex, GFRα1 contacts the RET CLD2–3, and both GDNF and GFRα1 contact RET CRD, which leads to homodimerization of the membrane-proximal RET portion and kinase activation (**Figure 2**)[18, 26]. This mechanism is hijacked by mutations in the CRD of *RET* that occur in MTC (see later text).

Growth differentiation factor 15 (GDF15, also known as MIC-1) is an alternative functional RET ligand[27, 28]. GDF15 acts on GDNF receptor alpha-like (GFRAL)[29], a distant relative of the GFRα family. GDF15–GFRAL binds to and stimulates RET kinase, thereby regulating the appetite centres of the CNS. In patients with cancer as well as patients with a chronic injury or inflammation, rising serum levels of GDF15 induce a RET kinase-mediated anorexia or cachexia syndrome. Thus, the GDF15–GFRAL–RET system seems to be an interesting target for therapies aimed at controlling appetite, obesity, anorexia or cachexia[27, 28].

In addition to acting through ligand stimulation, RET kinase functions as a 'dependence receptor', able to actively transmit constitutive pro-apoptotic intracellular signals in the absence of ligand stimulation [22, 30].

Upon homodimerisation of RET kinase and kinase activation, the RET kinase intracellular domain undergoes phosphorylation at several tyrosine residues, which are in turn involved in signal transduction. These include Y687 (in the juxtamembrane segment), Y900 and Y905 (in the activation segment), Y981 (in the TKD), and Y1015, Y1029, Y1062 and Y1096 (in the C-terminal tail; Y1096 is specific for the RET-51 isoform). Y1062 is the first tyrosine to be phosphorylated, followed by Y900, Y905 and Y981 at later time points[17]. Of note, Y1062 is crucial for intracellular signalling, in particular to the MAPK and PI3K pathways. Upon kinase activation**,** RET kinase also auto-phosphorylates on S909, thus amplifying RET kinase catalytic function [31].

[H3] RET kinase as a driver of disease. Mutations of the GDNF family ligand–GFRα–RET system impair development of parasympathetic enteric neurons, which causes intestinal aganglionosis with intestinal obstruction and congenital megacolon (known as Hirschsprung disease)[32, 33]. Hirschsprung-associated *RET* mutations are found in 30–70% of patients with Hirschsprung disease. They typically reduce RET kinase protein folding and trafficking to the cell surface of parasympathetic enteric neurons, or decrease its intrinsic catalytic function, or reduce its binding to intracellular signalling effectors[19, 34].

Increased expression or activity of wild type RET kinase in response to GDNF family ligands present in the tumour microenvironment is involved in several cancer types, including breast, prostate, pancreatic and myeloid malignancies [35]. Moreover, as discussed later, small *RET* mutations at the germline or somatic level, as well as somatic *RET* rearrangements are *bona fide* genetic drivers of thyroid cancer and other malignancies, a notion that has launched the search for effective RET TKIs to treat these malignancies.

[H1] *RET* point mutations in MTC

[H2] Germline RET mutations in MEN2

In ~25% of patients, MTC occurs as a hereditary monogenic autosomal dominant disorder in MEN2 syndrome (**Box 1**)[36]. Diffuse C-cell hyperplasia can be detected as the initial histological manifestation of the hereditary MTC, which is frequently multifocal in MEN2 syndrome[36,37]. A germline *RET* mutation is present in almost all patients with MEN2 syndrome. Germline *RET* mutations have an estimated age-standardized global incidence of 0.06 per 100,000 people per year and a prevalence of 1.3 per 100,000 people[38, 39]. Genetic screening is recommended in families with two or more members with MTC and also in patients with apparently sporadic MTC (sMTC), because *RET* germline mutations can be found in patients with sMTC (see later text) (**Box 2**). The identified *RET*-mutation

positive relatives must eventually undergo a search for MTC and for phaeochromocytoma and/or hyperparathyroidism[40, 41, 42].

[H3] MEN2A syndrome. This syndrome accounts for 80% of hereditary MTC, although this proportion might increase when including familial MTC (FMTC, **Box 1**). MEN2A syndrome with a *RET* mutation at codon 634, the most frequent *RET* mutation in MEN2A, is characterized by the presence of MTC in 100% of patients; 50% of patients have phaeochromocytoma (which is bilateral in half of these patients) and 15% a hyperparathyroidism, known as the classic presentation[43]. Around 30% of patients with a *RET* mutation at codon 634 have another subtype characterized by cutaneous lichen amyloidosis (CLA), known as MEN2A CLA (**Box 1**). Phaeochromocytoma is less frequent and occurs later and hyperparathyroidism is absent in patients with MEN2A due to another *RET* mutation. Phaeochromocytoma can be associated with any *RET* mutation and should be screened for in all patients with *RET* mutations[44] . Hirschsprung disease can occur in patients with a *RET* mutation at exon 10.

In ~95% of patients with MEN2A syndrome, germline *RET* mutations cluster in cysteine C609, C611, C618, C620 (in exon 10), or C630 and C634 (in exon 11) within the extracellular CRD, with mutation of C634 being the most frequent[44, 45, 36, 38]. Many other single amino acid changes and small insertions and/or deletions have been reported (**Figure 2, Table 1**)[44, 45, 36, 38]. These mutations lead to constitutive activation of the RET kinase. In the wild type receptor, the affected cysteines are engaged in intra-molecular disulfide bonds. By contrast, in mutated RET kinase, their replacement with other amino acids leads to the formation of aberrant inter-molecular disulfide bonds, which causes ligand-independent RET kinase dimerization and kinase activation [46].

Isolated FMTC accounts for about ~15% of patients with hereditary MTC. The most common *RET* mutations in FMTC affect extracellular cysteines other than C634, or

intracellular codons (768, 790, or 804) at exons 13 or 14, with a mutation at codon 804 being the most frequent (**Table 1**) [44, 45, 36, 38]. Currently, FMTC is regarded as a variant of MEN2A that is only diagnosed if MTC is present in another family member. Thus, including FMTC, up to 95% of patients with hereditary MTC can be classified as having MEN2A[47]. Of note, the genetic screening for MTC has led to the discovery of an unsuspected germline *RET* mutation in up to 6.5% of patients with apparent sMTC. Furthermore, these patients showed a statistically significant higher representation of FMTC (87%) with respect to classic MEN2A syndrome (13%), thus leading to an overall increased prevalence of the FMTC phenotype in individuals with hereditary MTC [48]

[H3] MEN2B syndrome. This syndrome is rare (accounting for ~5% of hereditary MTC) and is characterized by MTC, phaeochromocytoma, generalized ganglioneuromatosis, ocular abnormalities and a phenotype given by typical facies, skeletal malformations and marfanoid habitus [49, 50]. MEN2B is almost exclusively associated with a mutation in *RET* exon 16, which causes a methionine to threonine (M918T) substitution within the activation segment of RET kinase (**Figure 2 and Table 1**) [16, 51]. This substitution increases ATP-binding and autophosphorylation activity, thereby mediating a dimerization-independent activation of RET kinase [17]. In addition, mutant RET M918T can be further activated by endogenous ligands [51]. Moreover, this mutation has been reported to change the substrate specificity of RET kinase. In less than 10% of patients, MEN2B is associated with an A883F mutation or to double mutations, which include V804M and other mutations [36, 38, 52-55]. Phaeochromocytoma occurs in ~50% of patients with MEN2B syndrome; the occurrence of phaeochromocytoma is earliest and most frequent in patients with MEN2B and in patients with MEN2A with C634R mutation than in other patients with MEN2.

[H2] Somatic RET point mutations in sMTC

Somatic *RET* mutations have been found in up to 55% of patients with sMTC [56]. This prevalence is higher in patients with large tumours[41], and up to 85% of patients with distant metastases have somatic RET mutations[57]. The most common somatic mutation is M918T, which is present in up to 40% of patients with sMTC and is associated with disease aggressiveness. Other single amino acid changes might occur at residues C611, C618, C620, C630, C634, E768, A883 and S891; small *RET* deletions and/or insertions (indels) have also been detected[58, 56]. One instance of a *RET* gene fusion has been reported in a patient with MTC[59]. Point mutations in *RAS* (mainly *H-RAS* and *K-RAS*) are commonly present in patients with *RET* mutation-negative MTC[56, 60-62].

[H1] *RET* gene fusions in cancer

[H2] Thyroid cancer

Somatic gene rearrangements are a common mechanism of *RET* oncogenic conversion in cancer[63]. For example, RET kinase fusion proteins involve a NH_2-terminal partner fused in frame to the RET TKD and COOH-tail. RET kinase fusions occur in <10–20% of patients with papillary thyroid carcinoma (PTC), which is the most common differentiated thyroid cancer (DTC) subtype[64] (**Table 2**). *RET* fusions are most common in PTC occurring after radiation exposure during childhood, and in PTC in children[65-67]. *RET* fusions can be present in poorly-differentiated thyroid carcinoma and in rare patients with anaplastic thyroid carcinoma (ATC)[68, 69].

[H2] Non-thyroid cancer

RET fusions occur in 1–2% of NSCLC, predominantly in the lung adenocarcinoma (LADC) subtype, where they correlate with young age, female sex, Asian ethnicity and minimal tobacco exposure[10, 15, 70]. Other cancer types can also rarely harbour *RET*

rearrangements[63]. These include breast carcinoma (about 1% of patients)[71], colorectal carcinoma (<1% of patients)[72], Spitz tumour (3% of patients)[73] and other rare neoplasms such as paediatric spindle mesenchymal tumours[74], salivary gland carcinomas[75, 76] and chronic myeloproliferative neoplasms[77]. Overall, a total of 78 *RET* fusions from 46,697 cancer samples (0.17%) of various histotypes are recorded in the cBioPortal for Cancer Genomics [https://www.cbioportal.org][10, 15, 63, 70].

[H2] Mechanisms of gene fusion

In *RET* gene fusions, the most common breakpoint is in *RET* intron 11 (**Figure 2**). The most frequent *RET* fusions in PTC are coiled-coil domain containing 6 (*CCDC6)–RET* (also named RET–PTC1) and nuclear receptor coactivator 4 (*NCOA4)–RET* (also named RET–PTC3). Of note, kinesin family member 5B (*KIF5B*)–*RET* is the most common fusion in LADC (**Table 2**).

A chromosome 10q paracentric (that is, not including the centromere) inversion is the mechanism for *CCDC6–RET* and *NCOA4–RET* fusions, whereas a chromosome 10 pericentric (that is, including the centromere) inversion is the mechanism of *KIF5B–RET* fusion[63]. Illegitimate repair of a DNA double-strand break underlies these recombination events[78]. Accordingly, *RET* fusions in PTC have been mechanistically linked to exposure to ionizing radiation[79] or to reactive oxygen species[80], both agents known to cause double-strand break formation.

Gene fusions mediate constitutive activation of the RET kinase, which can drive oncogenesis. This process is primarily caused by the aberrant RET TKD expression, due to the switch of the *RET* transcriptional promoter with that of the fusion partner, and by ligand-independent RET TKD activation, due to dimerization mediated by the fusion partners (**Figure 2**)[15, 63, 70]. In PTC and LADC, RET kinase fusions are mutually exclusive with other driver mutations, such as *BRAF* and *RAS* mutations and other RTK fusions.

This suggests that the *RET* fusion is a key driver event in these cancers, a notion that has fostered attempts to target RET kinase therapeutically and launched the search of RET kinase inhibitors.

[H1] RET kinase as a target for TKIs

With the identification of key driver oncogenes as targetable activated kinases, a new era of treatment options emerged in patients with advanced thyroid cancer, particularly MTC. In these patients, RET kinase was the first mutated kinase that was selected for targeted inhibition[1]. Based on similarities between kinase domains of RET and vascular endothelial growth factor receptor type 2 (VEGFR2), the first anti-RET TKIs that reached clinical development also had considerable activity against VEGFR2 and other VEGFR family members. The use and approval of such TKIs for treatment of advanced MTC or DTC represented a major breakthrough for the systemic treatment of these thyroid cancers (discussed later) and prompted the search for the next generation of potent or selective RET TKIs[81 82]. **Table 3** summarizes the biochemical activity of TKIs that are approved for MTC and DTC, and of novel RET TKIs that have already reached the clinical trial phase.

It is important to note that RET kinase inhibition could be a viable approach for *RET*-driven cancers of non-thyroid origin. A detailed review of the clinical results of the treatment of *RET*-rearranged lung carcinomas with vandetanib, cabozantinib and other multikinase inhibitors that target RET kinase has been is reported elsewhere[70]. Of the novel RET TKIs (see later), BLU-667 (pralsetinib) showed an objective response rate (ORR) of 58% in patients with *RET* fusion-positive NSCLC (ARROW trial, NCT03037385). Furthermore, in the LIBRETTO-001 trial (NCT03157128), LOXO-292 (selpercatinib) showed an ORR of 68% in patients with *RET* fusion-positive NSCLC [2, 3, 82-86].

[H1] Mechanistic evidence

Initial studies to identify RET kinase inhibitors revealed that the orally available anilinoquinazoline, vandetanib (ZD6474), which is a TKI with activity against VEGFR2 and EGFR, had substantial RET kinase inhibitory activity[87] (**Table 3**). Vandetanib belongs to the 'type I' class of TKIs, defined as an inhibitor that binds the ATP-binding pocket with the activation segment positioned in the active conformation (referred to as DFG-in, due to the position of the DFG motif)[88]. In cell-based studies, vandetanib inhibited the activity of *RET* fusion products as well as *RET* point mutants. Furthermore, mouse-based experiments showed that vandetanib inhibits the growth of xenografts of NIH3T3 fibroblasts that express the *RET*–PTC3 fusion product[87]. Cabozantinib, another TKI with inhibitory activity for VEGFR2 and hepatocyte growth factor receptor (MET), showed activity against RET kinase (**Table 3**). In contrast with vandetanib, cabozantinib is a 'type II' inhibitor, defined as an inhibitor that binds the catalytically inactive conformation of the kinase, in which the DFG motif is flipped out (DFG-out)[88]. Of note, several other TKIs have shown anti-RET kinase activity, including sorafenib, sunitinib, alectinib, ponatinib and lenvatinib[89 70]

[H2] TKIs with variable VEGFR selectivity

The simultaneous block of RET kinase and VEGFR2 could provide an advantage in controlling tumour growth; although such dual activity can also increase the toxicity of TKIs and therefore hamper the possibility of achieving safe doses that are sufficient to inhibit RET kinase. Thus, medicinal chemistry and structure–activity relationship research efforts have led to the identification of novel RET TKIs with variable VEGFR inhibitory activity[89]. Among them, Pz1 is a novel TKI that has been shown in preclinical studies to act at a sub-nanomolar concentration on RET kinase and several RET kinase variations[90]. Of note, Pz1 shows an approximately equal inhibitory activity against both RET kinase and VEGFR2 and a good global kinome selectivity[90]. Importantly, it is active against RET

kinase with a mutation at the V804 residue, which is resistant to other TKIs (see later text)[90]

At least four additional molecules designed as VEGFR-sparing RET TKIs have been reported: BLU-667 (pralsetinib), LOXO-292 (selpercatinib), BOS172738 and TPX-0046. For example, pralsetinib was identified through the screening of >10,000 compounds for selective activity against RET kinase compared with other kinases[91]. Biochemical assays showed that pralsetinib inhibited wild-type RET kinase as well as oncogenic *RET* mutants at sub-nanomolar concentrations, without concomitant VEGFR2 inhibition, and the drug showed only minor activity against JAK1. Furthermore, pralsetinib abrogated RET signalling and proliferation in *RET*-driven cancer cell lines, demonstrated antitumour activity in *in vivo* models of *RET*-driven cancers and was active against RET kinase with a mutation at the V804 residue[91].

Selpercatinib was specifically designed to target diverse *RET*-activating mutations. *In vitro* studies demonstrated that at a concentration of 0.1 μM, the drug was at least 250-fold more selective for RET than for 98% of the other kinases tested [86]. In addition, selpercatinib exerted substantial activity against diverse RET kinase-activating mutations, including MTC-associated mutations as well as V804 mutations (see later text). Importantly, in diverse RET kinase-dependent mouse tumour models, selpercatinib showed substantial inhibition of tumor growth[82, 86].

BOS172738 is another potent RET TKI with a high selectivity over VEGFR2 (>300-fold) that demonstrated potency against several *RET* mutants, including V804L and V804M (**Table 3**). In preclinical studies, the drug demonstrated potent activity in *RET*-driven tumours and BOS172738 is currently being evaluated in a phase I clinical trial (NCT03780517)[92]. Finally, TPX-0046 is another new VEGFR2-sparing TKI with dual RET kinase–SRC inhibitory activity at low nanomolar concentrations that demonstrated anti-

tumour efficacy in cell lines and patient-derived xenografts, and is currently being evaluated in a phase I/II clinical trial (NCT04161391) [93].

[H2] Mechanisms of resistance to RET TKIs

Either primary or acquired resistance to RET TKIs can occur in patients with *RET*-driven cancer or in cell-based models. Acquired resistance primarily involves a modification of the target molecule (that is, RET kinase) or a mutation that enables bypass signalling[15]. Several single amino acid changes within the RET kinase domain are able to provide target-mediated resistance to various TKIs (**Figure 2**). These include mutations of RET V804, Y806 and G810 in the hinge segment of the RET kinase, and of S904 in the activation segment of the RET kinase. Importantly, RET kinase V804 occupies the so-called 'gatekeeper' position, controlling access to the hydrophobic ATP-binding and drug-binding pocket and potentially controlling the binding affinity of the RET kinase for ATP. V804M, V804L and V804E substitutions mediate resistance to vandetanib and cabozantinib[94, 95 91, 94]. In addition, the change of the adjacent amino acid (Y806C) also mediates resistance to vandetanib[96 97].

V804 (V804L and V804M) and Y806 (Y806C) RET kinase substitutions are pathogenetic lesions identified in patients with MTC who are naive for previous TKI treatment (**Table 2**). Of note, V804M has been also identified in patients with MTC and patients with NSCLC who relapsed upon treatment with vandetanib, cabozantinib or both, suggesting that the V804M mutation could also be under selective pressure to mediate secondary resistance[98 99]. This finding supports the speculation that RET kinase inhibition by these specific TKIs is an important component of their clinical activity. However, a clinical response to vandetanib has been reported in 3 of 4 patients with MTC who have a RET kinase V804 mutation, which suggest that non-RET kinase activity might be important as well[100]. Notably, new inhibitors, such as Pz1, selpercatinib, pralsetinib and BOS172738,

maintained their activity against RET kinase V804 mutants, findings that were replicated *in vivo* in patients[90, 10186, 91].

Mutations at glycine 810 (G810A or G810S) in the hinge of the RET kinase were initially demonstrated to mediate resistance to vandetanib in preclinical models[102 103] (**Figure 2**). Importantly, RET kinase G810R, G810S or G810C mutations were also identified as acquired resistance mechanisms in patients with *KIF5B–RET* or *CCDC6–RET* positive NSCLC and in patients with RET mutant-positive MTC, who progressed with vandetanib, lenvatinib and with the new generation TKIs selpercatinib and pralsetinib[101]. Structural modelling showed that replacement of G810 with bulky, charged or polar residues could directly interfere with selpercatinib binding to the kinase with just minor effects on ATP affinity[101]. Of note, TPX-0046 maintained activity against the RET kinase G810R mutation and nintedanib against G810A[97].

Finally, S904 in RET kinase maps between two autophosphorylation tyrosine residues (Y900 and Y905) within the activation segment of RET kinase (**Figure 2**). S904F mutation increases the ATP affinity and autophosphorylation activity of RET kinase, and this change was identified as a mechanism of on-target resistance to vandetanib in a patient with *CCDC6–RET* positive NSCLC[104]. However, the new generation inhibitors selpercatinib and pralsetinib were active *in vitro* also against RET kinase with the S904F mutation [101].

Monitoring circulating tumour DNA or cell-free DNA in patients before and during treatment with TKIs (**BOX 2**) could provide new insights into these mechanisms of resistance in individual patients[105].

[H1] Clinical data

Since the early 2000s, multi kinase inhibitors have been used to treat patients with advanced thyroid cancer. A decade later, inhibitors with selective activity for specific

oncogenic kinases became available and the use of these selective inhibitors has therefore been restricted to tumours with mutations of the target kinase. Mutational screening is mandatory in all patients with extended thyroid cancer, ideally of their metastatic tissue, which enables patients to be treated with selective TKIs.

[H2] Progressive refractory advanced DTC

Several TKIs have entered into clinical trials in patients with progressive radioiodine-refractory advanced DTC. For example, two drugs, sorafenib that targets VEGFR and RET kinase, and lenvatinib that targets VEGFR, RET kinase and FGFR, proceeded through phase III trials. In the DECISION trial (sorafenib versus placebo) in 417 patients with DTC, the median progression free survival (PFS) was improved from 5.8 to 10.8 months (HR: 0.59, *P*<0.0001) and the objective response rate (ORR) was 12% (REF. [106]). In the SELECT trial (lenvatinib versus placebo) in 392 patients with DTC, the median PFS was improved from 3.6 to 18.3 months (HR: 0.21, *P* <0.0001) and the ORR was 65% (REF. [107]). The significant improvement of median PFS over placebo led to the US FDA and European Medicine Agency (EMA) approvals for both drugs for patients with progressive refractory advanced DTC (in 2013 for sorafenib and 2015 for lenvatinib). Other TKIs have been effective in phase II trials for progressive refractory advanced DTC, such as cabozantinib[108], pazopanib[109], sunitinib[110] and axitinib[111]. In the DECISION (sorafenib versus placebo) and SELECT (lenvatinib versus placebo) trials, no association could be identified between specific oncogenic mutations and change in PFS or response rate to the drug.

[H2] Advanced MTC

For advanced MTC, vandetanib (which targets VEGFR2, RET and EGFR kinases) and cabozantinib (which targets VEGFR2, RET and MET kinases) have entered phase III trials

(**Table 3**). In the ZETA trial (vandetanib versus placebo) in 331 patients with metastatic (but not necessarily progressive) MTC, median PFS was improved from 19.3 months to an estimated 30.5 months (HR: 0.46, *P*<0.001) and the ORR was 44% (REF. [81]). In the EXAM trial (cabozantinib versus placebo) in 330 patients with progressive metastatic MTC, median PFS improved from 4.0 months to 11.2 months (HR: 0.28, *P*<0.001) and the ORR was 28% (REF. [112]). The significant improvement of median PFS over placebo led to FDA and EMA approvals for both drugs for patients with progressive advanced MTC (in 2011 for vandetanib and in 2012 for cabozantinib). Of note, between the two trials, the markedly different outcomes in the placebo treated arms as well as the active drug arms are probably related to differences in eligibility criteria. However, a non-prespecified sub-analysis of the ZETA trial could demonstrate similar benefits of vandetanib in patients with documented progression at baseline[113]. Long term treatment responses lasting 10 years or even more were observed in ~10% of patients with MTC treated with vandetanib[114 115], suggesting that in some tumours no resistance mechanisms appear during the long-term treatment. Other TKIs have been effective in phase II trials in patients with MTC, such as lenvatinib[116].

In the vandetanib and cabozantinib trials in MTC, the presence of a *RET* mutation in the tumour tissue was associated with a better outcome. For example, in the cabozantinib study, patients whose tumours contained the somatic *RET* M918T mutation showed a greater improvement in PFS than the overall study cohort (13.9 months versus 4.0 months; HR: 0.15, *P*< 0.0001) and a significant overall survival improvement (median survival: 44.3 months versus 18.9 months, HR: 0.6, *P*=0.03) (REF. [117]). In the vandetanib trial, patients with the somatic *RET* M918T mutation were more likely than patients with other mutations to experience an improved PFS[81]. It is worth noting that these drugs are potent VEGFR2 inhibitors and might exert their effects on MTC by directly targeting either RET or VEGFR2 or both[118]. In MTC, *RET* activating mutations, besides promoting C-cell

proliferation, stimulate the production of calcitonin. In some patients, treatment with vandetanib or cabozantinib induces a rapid decrease in circulating levels of calcitonin, even before and, in some cases independently from, any decrease in tumour burden. In these patients, it is plausible that the rapid reduction of calcitonin production is the effect of a direct inhibition of RET kinase[119].

[H2] Limitations of currently approved drugs

Despite the impressive changes in PFS, none of these four drugs approved for treating advanced thyroid carcinoma (sorafenib and lenvatinib in DTC, vandetanib and cabozantinib in MTC) were shown to improve overall survival. Study design could have affected this outcome in three of the studies, as only the cabozantinib trial did not permit cross-over from placebo upon progression. Furthermore, only one pre-specified analysis of the lenvatinib trial showed that patients older than 65 years lived longer if randomized to lenvatinib rather than placebo (HR: 0.53, $P = 0.02$) (REF. [120]).

Unfortunately, the use of these agents might decrease quality of life and is associated with adverse effects that could be fatal in some patients. Adverse effects include diarrhoea, nausea, fatigue, rash and skin lesions, as well as hypertension and cardiovascular complications including QT prolongation attributable to VEGFR2, EGFR and hERG inhibition. These effects might limit the treatment dose of these drugs[70]. Moreover, some patients might simply be not eligible for these therapies on the basis of contraindication due to systemic disability or to an unacceptable risk of bleeding or fistula. In patients with DTC, adverse effects that necessitated dose reduction occurred in 64% of patients on sorafenib and 68% of patients on lenvatinib, and permanent discontinuation of therapy in 19% and 14% of patients, respectively. In addition, in patients with MTC, adverse effects necessitated dose reduction in 35% of patients on vandetanib and 79% of

patients on cabozantinib, and permanent discontinuation of therapy in 12% and 16% of patients, respectively.

[H2] Selective TKIs

Preliminary data on the use of selective RET TKIs in patients with thyroid cancer with *RET*-activating mutations suggest that they might be more effective and less toxic than those drugs already in practice. For example, the safety and efficacy of selpercatinib in advanced *RET*-mutant MTC and in *RET* fusion-positive thyroid cancer were evaluated in LIBRETTO-001 (NCT03157128), a phase I/II clinical trial in adolescent and adult patients. In 88 patients with *RET*-mutated MTC, the ORR was 73% for patients who had not received vandetanib and cabozantinib, whereas the 1-year PFS rate was 92%; for the 55 patients who had been pre-treated with vandetanib or cabozantinib, the ORR was 69%, the 1-year PFS rate was 82% and the calcitonin response rate was 91%. In addition, in this trial, 19 patients had previously treated *RET*-fusion gene positive DTC; for these patients the ORR was 79% and the 1-year PFS rate was 64% (REF. [83]).

The safety and efficacy of pralsetinib were evaluated in ARROW, a phase I/II trial (NCT03037385). Preliminary results are available in 32 patients with *RET*-mutant MTC, including 16 patients previously treated with cabozantinib or vandetanib; for all these patients the ORR was 63% and the disease control rate was 94%. In 12 patients with *RET*–fusion gene positive DTC, the ORR was 75% and the median duration of response was 14.5 months[91, 121]. These specific RET TKIs appeared to be well tolerated, with the most common drug-related adverse effects including dry mouth, increased serum levels of markers of liver function (AST and ALT), hypertension, diarrhoea and fatigue. However, toxicities typically associated with other TKIs, such as hypertension and diarrhoea, were less frequent and of lower grades. Dose reductions were fairly uncommon, and treatment discontinuation due to treatment-related adverse effects was necessary in only 3% of

patients. In May 2020, the FDA granted approval for selpercatinib in patients with lung and thyroid cancer carrying alterations in the *RET* gene[2, 3].

[H1] Conclusions

In conclusion, the use of these novel RET kinase inhibitors are promising in terms of both efficacy and tolerance. Ongoing phase III trials comparing the efficacy and tolerance of one of the novel selective RET kinase inhibitors to the TKIs already in practice (that is, vandetanib or cabozantinib) will determine which agent can be recommended as the first line treatment in patients with a *RET* mutation. Trials will also determine at which stage of the disease initiation of treatment is recommended, and whether the use of these novel RET kinase inhibitors should be used at an earlier stage than that recommended for TKIs already in clinical practice. The emergence of target-dependent resistance mechanisms, such as RET kinase G810 mutations, might also be an issue with these new drugs and necessitates a search for second line compounds to overcome resistance. In the absence of a *RET* mutation, other treatment modalities, such as the other TKIs already in clinical practice, can be used for patients with advanced thyroid cancer, either alone or in combination with other treatment modalities, such as immunotherapy.

REFERENCES

1. Schlumberger, M., Carlomagno, F., Baudin, E., Bidart, J.M. & Santoro, M. New therapeutic approaches to treat medullary thyroid carcinoma. *Nat Clin Pract Endocrinol Metab* **4**, 22-32 (2008).
2. Markham, A. Selpercatinib: First Approval. *Drugs* **80**, 1119-24 (2020).
3. FDA Approves Selpercatinib; Pralsetinib May Soon Follow. *Cancer Discov* **10**, OF1 (2020).
4. Takahashi, M., Ritz, J. & Cooper, G.M. Activation of a novel human transforming gene, ret, by DNA rearrangement. *Cell* **42**, 581-8 (1985).

This article describes the first isolation of *RET* as well as the first demonstration of its oncogenic activity

5. Ishizaka, Y. et al. Human ret proto-oncogene mapped to chromosome 10q11.2. *Oncogene* **4**, 1519-21 (1989).

6. Simpson, N.E. et al. Assignment of multiple endocrine neoplasia type 2A to chromosome 10 by linkage. *Nature* **328**, 528-30 (1987).
7. Takahashi, M., Buma, Y. & Hiai, H. Isolation of ret proto-oncogene cDNA with an amino-terminal signal sequence. *Oncogene* **4**, 805-6 (1989).
8. Grieco, M. et al. PTC is a novel rearranged form of the ret proto-oncogene and is frequently detected in vivo in human thyroid papillary carcinomas. *Cell* **60**, 557-63 (1990).
9. Mulligan, L.M. et al. Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. *Nature* **363**, 458-60 (1993).
10. Kohno, T. et al. KIF5B-RET fusions in lung adenocarcinoma. *Nat Med* **18**, 375-7 (2012).
11. Lipson, D. et al. Identification of new ALK and RET gene fusions from colorectal and lung cancer biopsies. *Nat Med* **18**, 382-4 (2012).
12. Takeuchi, K. et al. RET, ROS1 and ALK fusions in lung cancer. *Nat Med* **18**, 378-81 (2012).
13. Mulligan, L.M. 65 YEARS OF THE DOUBLE HELIX: Exploiting insights on the RET receptor for personalized cancer medicine. *Endocr Relat Cancer* **25**, T189-T200 (2018).
14. Plaza-Menacho, I. Structure and function of RET in multiple endocrine neoplasia type 2. *Endocr Relat Cancer* **25**, T79-T90 (2018).
15. Subbiah, V. & Cote, G.J. Advances in Targeting RET-Dependent Cancers. *Cancer Discov* **10**, 498-505 (2020).
16. Knowles, P.P. et al. Structure and chemical inhibition of the RET tyrosine kinase domain. *J Biol Chem* **281**, 33577-87 (2006).

This article describes the crystal structure of the RET kinase domain as well as of it in complex with ATP-competitive kinase inhibitors

17. Plaza-Menacho, I. et al. Oncogenic RET kinase domain mutations perturb the autophosphorylation trajectory by enhancing substrate presentation in trans. *Mol Cell* **53**, 738-51 (2014).
18. Redaelli, S., Plaza-Menacho, I. & Mologni, L. Novel targeted therapeutics for MEN2. *Endocr Relat Cancer* **25**, T53-T68 (2018).
19. Ibanez, C.F. Structure and physiology of the RET receptor tyrosine kinase. *Cold Spring Harb Perspect Biol* **5,** a009134 (2013).
20. Meng, X. et al. Regulation of cell fate decision of undifferentiated spermatogonia by GDNF. *Science* **287**, 1489-93 (2000).
21. Lindfors, P.H., Lindahl, M., Rossi, J., Saarma, M. & Airaksinen, M.S. Ablation of persephin receptor glial cell line-derived neurotrophic factor family receptor alpha4 impairs thyroid calcitonin production in young mice. *Endocrinology* **147**, 2237-44 (2006).
22. Canibano, C. et al. The dependence receptor Ret induces apoptosis in somatotrophs through a Pit-1/p53 pathway, preventing tumor growth. *EMBO J* **26**, 2015-28 (2007).
23. Veiga-Fernandes, H. et al. Tyrosine kinase receptor RET is a key regulator of Peyer's patch organogenesis. *Nature* **446**, 547-51 (2007).
24. Ohgami, N. et al. c-Ret-mediated hearing loss in mice with Hirschsprung disease. *Proc Natl Acad Sci U S A* **107**, 13051-6 (2010).
25. Fonseca-Pereira, D. et al. The neurotrophic factor receptor RET drives haematopoietic stem cell survival and function. *Nature* **514**, 98-101 (2014).
26. Goodman, K.M. et al. RET recognition of GDNF-GFRalpha1 ligand by a composite binding site promotes membrane-proximal self-association. *Cell Rep* **8**, 1894-904 (2014).
27. Breit, S.N., Tsai, V.W. & Brown, D.A. Targeting Obesity and Cachexia: Identification of the GFRAL Receptor-MIC-1/GDF15 Pathway. *Trends Mol Med* **23**, 1065-7 (2017).

28. Tsai, V.W.W., Husaini, Y., Sainsbury, A., Brown, D.A. & Breit, S.N. The MIC-1/GDF15-GFRAL Pathway in Energy Homeostasis: Implications for Obesity, Cachexia, and Other Associated Diseases. *Cell Metab* **28**, 353-68 (2018).
29. Yang, L. et al. GFRAL is the receptor for GDF15 and is required for the anti-obesity effects of the ligand. *Nat Med* **23**, 1158-66 (2017).
30. Bordeaux, M.C. et al. The RET proto-oncogene induces apoptosis: a novel mechanism for Hirschsprung disease. *EMBO J* **19**, 4056-63 (2000).
31. Plaza-Menacho, I. et al. RET Functions as a Dual-Specificity Kinase that Requires Allosteric Inputs from Juxtamembrane Elements. *Cell Rep* **17**, 3319-32 (2016).
32. Heanue, T.A. & Pachnis, V. Enteric nervous system development and Hirschsprung's disease: advances in genetic and stem cell studies. *Nat Rev Neurosci* **8**, 466-79 (2007).
33. Amiel, J. et al. Hirschsprung disease, associated syndromes and genetics: a review. *J Med Genet* **45**, 1-14 (2008).
34. Carlomagno, F. et al. Molecular heterogeneity of RET loss of function in Hirschsprung's disease. *EMBO J* **15**, 2717-25 (1996).
35. Mulligan, L.M. GDNF and the RET Receptor in Cancer: New Insights and Therapeutic Potential. *Front Physiol* **9**, 1873 (2018).
36. Wells, S.A., Jr., Pacini, F., Robinson, B.G. & Santoro, M. Multiple endocrine neoplasia type 2 and familial medullary thyroid carcinoma: an update. *J Clin Endocrinol Metab* **98**, 3149-64 (2013).
37. Landsvater, R.M. et al. The clinical implications of a positive calcitonin test for C-cell hyperplasia in genetically unaffected members of an MEN2A kindred. *Am J Hum Genet* **52**, 335-42 (1993).
38. Machens, A. & Dralle, H. Long-term outcome after DNA-based prophylactic neck surgery in children at risk of hereditary medullary thyroid cancer. *Best Pract Res Clin Endocrinol Metab* **33**, 101274 (2019).
39. Mathiesen, J.S. et al. Incidence and prevalence of sporadic and hereditary MTC in Denmark 1960-2014: a nationwide study. *Endocr Connect* **7**, 829-39 (2018).
40. Eng, C. et al. Low frequency of germline mutations in the RET proto-oncogene in patients with apparently sporadic medullary thyroid carcinoma. *Clin Endocrinol (Oxf)* **43**, 123-7 (1995).
41. Romei, C. et al. Low prevalence of the somatic M918T RET mutation in micro-medullary thyroid cancer. *Thyroid* **22**, 476-81 (2012).
42. Elisei, R. et al. Twenty-Five Years Experience on RET Genetic Screening on Hereditary MTC: An Update on The Prevalence of Germline RET Mutations. *Genes (Basel)* **10,** 698 (2019).
43. Wells, S.A., Jr. Advances in the management of MEN2: from improved surgical and medical treatment to novel kinase inhibitors. *Endocr Relat Cancer* **25**, T1-T13 (2018).
44. Eng, C. et al. The relationship between specific RET proto-oncogene mutations and disease phenotype in multiple endocrine neoplasia type 2. International RET mutation consortium analysis. *JAMA* **276**, 1575-9 (1996).
45. Mulligan, L.M. et al. Specific mutations of the RET proto-oncogene are related to disease phenotype in MEN 2A and FMTC. *Nat Genet* **6**, 70-4 (1994).
46. Santoro, M. et al. Activation of RET as a dominant transforming gene by germline mutations of MEN2A and MEN2B. *Science* **267**, 381-3 (1995).
47. Kloos, R.T. et al. Medullary thyroid cancer: management guidelines of the American Thyroid Association. *Thyroid* **19**, 565-612 (2009).

48. Romei, C. et al. RET genetic screening of sporadic medullary thyroid cancer (MTC) allows the preclinical diagnosis of unsuspected gene carriers and the identification of a relevant percentage of hidden familial MTC (FMTC). *Clin Endocrinol (Oxf)* **74**, 241-7 (2011).
49. Castinetti, F., Moley, J., Mulligan, L. & Waguespack, S.G. A comprehensive review on MEN2B. *Endocr Relat Cancer* **25**, T29-T39 (2018).
50. Castinetti, F. et al. Natural history, treatment, and long-term follow up of patients with multiple endocrine neoplasia type 2B: an international, multicentre, retrospective study. *Lancet Diabetes Endocrinol* **7**, 213-20 (2019).
51. Gujral, T.S., Singh, V.K., Jia, Z. & Mulligan, L.M. Molecular mechanisms of RET receptor-mediated oncogenesis in multiple endocrine neoplasia 2B. *Cancer Res* **66**, 10741-9 (2006).
52. Miyauchi, A. et al. Two germline missense mutations at codons 804 and 806 of the RET proto-oncogene in the same allele in a patient with multiple endocrine neoplasia type 2B without codon 918 mutation. *Jpn J Cancer Res* **90**, 1-5 (1999).
53. Menko, F.H. et al. Atypical MEN type 2B associated with two germline RET mutations on the same allele not involving codon 918. *J Clin Endocrinol Metab* **87**, 393-7 (2002).
54. Cranston, A.N. et al. RET is constitutively activated by novel tandem mutations that alter the active site resulting in multiple endocrine neoplasia type 2B. *Cancer Res* **66**, 10179-87 (2006).
55. Nakao, K.T. et al. Novel tandem germline RET proto-oncogene mutations in a patient with multiple endocrine neoplasia type 2B: report of a case and a literature review of tandem RET mutations with in silico analysis. *Head Neck* **35**, E363-8 (2013).
56. Ciampi, R. et al. Genetic Landscape of Somatic Mutations in a Large Cohort of Sporadic Medullary Thyroid Carcinomas Studied by Next-Generation Targeted Sequencing. *iScience* **20**, 324-36 (2019).
57. Heilmann, A.M. et al. Comprehensive Genomic Profiling of Clinically Advanced Medullary Thyroid Carcinoma. *Oncology* **90**, 339-46 (2016).
58. Elisei, R. et al. Prognostic significance of somatic RET oncogene mutations in sporadic medullary thyroid cancer: a 10-year follow-up study. *J Clin Endocrinol Metab* **93**, 682-7 (2008).
59. Grubbs, E.G. et al. RET fusion as a novel driver of medullary thyroid carcinoma. *J Clin Endocrinol Metab* **100**, 788-93 (2015).
60. Moura, M.M., Cavaco, B.M. & Leite, V. RAS proto-oncogene in medullary thyroid carcinoma. *Endocr Relat Cancer* **22**, R235-52 (2015).
61. Agrawal, N. et al. Exomic sequencing of medullary thyroid cancer reveals dominant and mutually exclusive oncogenic mutations in RET and RAS. *J Clin Endocrinol Metab* **98**, E364-9 (2013).
62. Boichard, A. et al. Somatic RAS mutations occur in a large proportion of sporadic RET-negative medullary thyroid carcinomas and extend to a previously unidentified exon. *J Clin Endocrinol Metab* **97**, E2031-5 (2012).
63. Santoro, M., Moccia, M., Federico, G. & Carlomagno, F. RET Gene Fusions in Malignancies of the Thyroid and Other Tissues. *Genes (Basel)* **11**, 424 (2020).
64. Cancer Genome Atlas Research Network. Integrated genomic characterization of papillary thyroid carcinoma. *Cell* **159**, 676-90 (2014).

This article describes the comprehensive characterization of the genetic lesions associated with human papillary thyroid carcinoma and the identification of molecularly defined papillary thyroid carcinoma subgroups

65. Caudill, C.M., Zhu, Z., Ciampi, R., Stringer, J.R. & Nikiforov, Y.E. Dose-dependent generation of RET/PTC in human thyroid cells after in vitro exposure to gamma-radiation: a model of carcinogenic chromosomal rearrangement induced by ionizing radiation. *J Clin Endocrinol Metab* **90**, 2364-9 (2005).
66. Alzahrani, A.S. et al. Genetic Alterations in Pediatric Thyroid Cancer Using a Comprehensive Childhood Cancer Gene Panel. *J Clin Endocrinol Metab* **105**, 3324-34 (2020).
67. Pekova, B. et al. RET, NTRK, ALK, BRAF, and MET Fusions in a Large Cohort of Pediatric Papillary Thyroid Carcinomas. *Thyroid* **30,** 1771-80 (2020).
68. Dias-Santagata, D. et al. Response to RET-Specific Therapy in RET Fusion-Positive Anaplastic Thyroid Carcinoma. *Thyroid* **30**, 1384-9 (2020).
69. Landa, I. et al. Genomic and transcriptomic hallmarks of poorly differentiated and anaplastic thyroid cancers. *J Clin Invest* **126**, 1052-66 (2016).
70. Drilon, A., Hu, Z.I., Lai, G.G.Y. & Tan, D.S.W. Targeting RET-driven cancers: lessons from evolving preclinical and clinical landscapes. *Nat Rev Clin Oncol* **15**, 151-67 (2018).
71. Paratala, B.S. et al. RET rearrangements are actionable alterations in breast cancer. *Nat Commun* **9**, 4821 (2018).
72. Pietrantonio, F. et al. RET fusions in a small subset of advanced colorectal cancers at risk of being neglected. *Ann Oncol* **29**, 1394-401 (2018).
73. Wiesner, T. et al. Kinase fusions are frequent in Spitz tumours and spitzoid melanomas. *Nat Commun* **5**, 3116 (2014).
74. Antonescu, C.R. et al. Spindle Cell Tumors With RET Gene Fusions Exhibit a Morphologic Spectrum Akin to Tumors With NTRK Gene Fusions. *Am J Surg Pathol* **43**, 1384-91 (2019).
75. Guilmette, J., Dias-Santagata, D., Nose, V., Lennerz, J.K. & Sadow, P.M. Novel gene fusions in secretory carcinoma of the salivary glands: enlarging the ETV6 family. *Hum Pathol* **83**, 50-8 (2019).
76. Skalova, A. et al. NCOA4-RET and TRIM27-RET Are Characteristic Gene Fusions in Salivary Intraductal Carcinoma, Including Invasive and Metastatic Tumors: Is "Intraductal" Correct? *Am J Surg Pathol* **43**, 1303-13 (2019).
77. Ballerini, P. et al. RET fusion genes are associated with chronic myelomonocytic leukemia and enhance monocytic differentiation. *Leukemia* **26**, 2384-9 (2012).
78. Mizukami, T. et al. Molecular mechanisms underlying oncogenic RET fusion in lung adenocarcinoma. *J Thorac Oncol* **9**, 622-30 (2014).
79. Ricarte-Filho, J.C. et al. Identification of kinase fusion oncogenes in post-Chernobyl radiation-induced thyroid cancers. *J Clin Invest* **123**, 4935-44 (2013).

This article describes the systematic characterization of gene fusion events driving the formation of post-Chernobyl thyroid papillary carcinoma

80. Ameziane-El-Hassani, R. et al. Role of H2O2 in RET/PTC1 chromosomal rearrangement produced by ionizing radiation in human thyroid cells. *Cancer Res* **70**, 4123-32 (2010).
81. Wells, S.A., Jr. et al. Vandetanib in patients with locally advanced or metastatic medullary thyroid cancer: a randomized, double-blind phase III trial. *J Clin Oncol* **30**, 134-41 (2012).

This article describes the clinical study that led to the registration of the first targeted therapy for advanced medullary thyroid carcinoma

82. Subbiah, V., Yang, D., Velcheti, V., Drilon, A. & Meric-Bernstam, F. State-of-the-Art Strategies for Targeting RET-Dependent Cancers. *J Clin Oncol* **38**, 1209-21 (2020).
83. Wirth, L.J. et al. Efficacy of Selpercatinib in RET-Altered Thyroid Cancers. *N Engl J Med* **383**, 825-35 (2020).

This article describes the prospective trial that led to the first registration of a RET specific TKI in patients with advanced thyroid carcinoma who have a *RET* mutation

84. Drilon, A. et al. Efficacy of Selpercatinib in RET Fusion-Positive Non-Small-Cell Lung Cancer. *N Engl J Med* **383**, 813-24 (2020).
85. Kurzrock, R. Selpercatinib Aimed at RET-Altered Cancers. *N Engl J Med* **383**, 868-9 (2020).
86. Subbiah, V. et al. Selective RET kinase inhibition for patients with RET-altered cancers. *Ann Oncol* **29**, 1869-76 (2018).
87. Carlomagno, F. et al. ZD6474, an orally available inhibitor of KDR tyrosine kinase activity, efficiently blocks oncogenic RET kinases. *Cancer Res* **62**, 7284-90 (2002).
88. Zhao, Z. et al. Exploration of type II binding mode: A privileged approach for kinase inhibitor focused drug discovery? *ACS Chem Biol* **9**, 1230-41 (2014).
89. De Falco, V., Carlomagno, F., Li, H.Y. & Santoro, M. The molecular basis for RET tyrosine-kinase inhibitors in thyroid cancer. *Best Pract Res Clin Endocrinol Metab* **31**, 307-18 (2017).
90. Frett, B. et al. Fragment-Based Discovery of a Dual pan-RET/VEGFR2 Kinase Inhibitor Optimized for Single-Agent Polypharmacology. *Angew Chem Int Ed Engl* **54**, 8717-21 (2015).
91. Subbiah, V. et al. Precision Targeted Therapy with BLU-667 for RET-Driven Cancers. *Cancer Discov* **8**, 836-49 (2018).
92. Schoffski, P. et al. A phase I study of BOS172738 in patients with advanced solid tumors with RET gene alterations including non-small cell lung cancer and medullary thyroid cancer. *J Clin Oncol* https://doi.org/10.1200/JCO.2019.37.15_suppl.TPS3162 (2019).
93. Drilon, A. et al. TPX-0046 is a novel and potent RET/SRC inhibitor for RET-driven cancers. *Ann Oncol* https://doi.org/10.1093/annonc/mdz244.068 (2019).
94. Carlomagno, F. et al. Disease associated mutations at valine 804 in the RET receptor tyrosine kinase confer resistance to selective kinase inhibitors. *Oncogene* **23**, 6056-63 (2004).
95. Mologni, L., Redaelli, S., Morandi, A., Plaza-Menacho, I. & Gambacorti-Passerini, C. Ponatinib is a potent inhibitor of wild-type and drug-resistant gatekeeper mutant RET kinase. *Mol Cell Endocrinol* **377**, 1-6 (2013).
96. Carlomagno, F. et al. Identification of tyrosine 806 as a molecular determinant of RET kinase sensitivity to ZD6474. *Endocr Relat Cancer* **16**, 233-41 (2009).
97. Liu, X., Shen, T., Mooers, B.H.M., Hilberg, F. & Wu, J. Drug resistance profiles of mutations in the RET kinase domain. *Br J Pharmacol* **175**, 3504-15 (2018).
98. Busaidy, N.L. et al. Emergence of V804M resistance gatekeeper mutation in sporadic medullary thyroid carcinoma patients treated with TKI tyrosine kinase inhibitors. *Thyroid* **27**, A166-88 (2017).
99. Wirth, L.J. et al. Emergence and Targeting of Acquired and Hereditary Resistance to Multikinase RET Inhibition in Patients With RET-Altered Cancer. *JCO Precis Oncol* **3**, PO.19.00189 (2019).
100. Valerio L. et al. V804M RET mutation and vandetanib response in metastatic medullary thyroid cancer. *Eur Thyr J* 6 (suppl 1), 23-118 (2017).
101. Solomon, B.J. et al. RET Solvent Front Mutations Mediate Acquired Resistance to Selective RET Inhibition in RET-Driven Malignancies. *J Thorac Oncol* **15**, 541-49 (2020).
102. Huang, Q. et al. Preclinical Modeling of KIF5B-RET Fusion Lung Adenocarcinoma. *Mol Cancer Ther* **15**, 2521-9 (2016).
103. Terzyan, S.S. et al. Structural basis of resistance of mutant RET protein-tyrosine kinase to its inhibitors nintedanib and vandetanib. *J Biol Chem* **294**, 10428-37 (2019).
104. Nakaoku, T. et al. A secondary RET mutation in the activation loop conferring resistance to vandetanib. *Nat Commun* **9**, 625 (2018).

105. Rich, T.A. et al. Analysis of Cell-Free DNA from 32,989 Advanced Cancers Reveals Novel Co-occurring Activating RET Alterations and Oncogenic Signaling Pathway Aberrations. *Clin Cancer Res* **25**, 5832-42 (2019).

106. Brose, M.S. et al. Sorafenib in radioactive iodine-refractory, locally advanced or metastatic differentiated thyroid cancer: a randomised, double-blind, phase 3 trial. *Lancet* **384**, 319-28 (2014).
This article describes the clinical study that led to the registration of the first targeted therapy for advanced differentiated thyroid carcinoma

107. Schlumberger, M. et al. Lenvatinib versus placebo in radioiodine-refractory thyroid cancer. *N Engl J Med* **372**, 621-30 (2015).
This article describes the clinical study that led to the registration of lenvatinib therapy for advanced differentiated thyroid carcinoma

108. Cabanillas, M.E. et al. Cabozantinib As Salvage Therapy for Patients With Tyrosine Kinase Inhibitor-Refractory Differentiated Thyroid Cancer: Results of a Multicenter Phase II International Thyroid Oncology Group Trial. *J Clin Oncol* **35**, 3315-21 (2017).

109. Bible, K.C. et al. A multicenter phase 2 trial of pazopanib in metastatic and progressive medullary thyroid carcinoma: MC057H. *J Clin Endocrinol Metab* **99**, 1687-93 (2014).

110. Ravaud, A. et al. A multicenter phase II study of sunitinib in patients with locally advanced or metastatic differentiated, anaplastic or medullary thyroid carcinomas: mature data from the THYSU study. *Eur J Cancer*. **76**, 110-7 (2017)

111. Locati, L.D. et al. Treatment of advanced thyroid cancer with axitinib: Phase 2 study with pharmacokinetic/pharmacodynamic and quality-of-life assessments. *Cancer* **120**, 2694-703 (2014).

112. Elisei, R. et al. Cabozantinib in progressive medullary thyroid cancer. *J Clin Oncol* **31**, 3639-46 (2013).
This article describes the clinical study that led to the registration of cabozantinib therapy for advanced medullary thyroid carcinoma

113. Kreissl, M.C. et al. Efficacy and safety of vandetanib in progressive and symptomatic medullary thyroid cancer – post hoc analysis from the ZETA trial. *J Clin Oncol* **38,** 2773-81 (2020).

114. Valerio, L. et al. Medullary thyroid cancer treated with vandetanib: predictors of a longer and durable response. *Endocr Relat Cancer* **27**, 97-110 (2020).

115. Chougnet, C.N. et al. Vandetanib for the treatment of advanced medullary thyroid cancer outside a clinical trial: results from a French cohort. *Thyroid* **25**, 386-91 (2015).

116. Schlumberger, M. et al. A Phase II Trial of the Multitargeted Tyrosine Kinase Inhibitor Lenvatinib (E7080) in Advanced Medullary Thyroid Cancer. *Clin Cancer Res* **22**, 44-53 (2016).

117. Schlumberger, M. et al. Overall survival analysis of EXAM, a phase III trial of cabozantinib in patients with radiographically progressive medullary thyroid carcinoma. *Ann Oncol* **28**, 2813-9 (2017).

118. Verrienti, A. et al. RET mutation and increased angiogenesis in medullary thyroid carcinomas. *Endocr Relat Cancer* **23**, 665-76 (2016).

119. Akeno-Stuart, N. et al. The RET kinase inhibitor NVP-AST487 blocks growth and calcitonin gene expression through distinct mechanisms in medullary thyroid cancer cells. *Cancer Res* **67**, 6956-64 (2007).

120. Brose, M.S., Worden, F.P., Newbold, K.L., Guo, M. & Hurria, A. Effect of Age on the Efficacy and Safety of Lenvatinib in Radioiodine-Refractory Differentiated Thyroid Cancer in the Phase III SELECT Trial. *J Clin Oncol* **35**, 2692-9 (2017).
121. Subbiah, V. et al. Clinical activity of the RET inhibitor pralsetinib (BLU-667) in patients with RET fusion+ solid tumors. *J Clin Oncol* **38**, 109 (2020).
122. Herbst, R.S., Heymach, J.V., O'Reilly, M.S., Onn, A. & Ryan, A.J. Vandetanib (ZD6474): an orally available receptor tyrosine kinase inhibitor that selectively targets pathways critical for tumor growth and angiogenesis. *Expert Opin Investig Drugs* **16**, 239-49 (2007).
123. Yakes, F.M. et al. Cabozantinib (XL184), a novel MET and VEGFR2 inhibitor, simultaneously suppresses metastasis, angiogenesis, and tumor growth. *Mol Cancer Ther* **10**, 2298-308 (2011).
124. Wilhelm, S.M. et al. Preclinical overview of sorafenib, a multikinase inhibitor that targets both Raf and VEGF and PDGF receptor tyrosine kinase signaling. *Mol Cancer Ther* **7**, 3129-40 (2008).
125. Wilhelm, S. et al. Discovery and development of sorafenib: a multikinase inhibitor for treating cancer. *Nat Rev Drug Discov* **5**, 835-44 (2006).
126. Plaza-Menacho, I. et al. Sorafenib functions to potently suppress RET tyrosine kinase activity by direct enzymatic inhibition and promoting RET lysosomal degradation independent of proteasomal targeting. *J Biol Chem* **282**, 29230-40 (2007).
127. Matsui, J. et al. Multi-kinase inhibitor E7080 suppresses lymph node and lung metastases of human mammary breast tumor MDA-MB-231 via inhibition of vascular endothelial growth factor-receptor (VEGF-R) 2 and VEGF-R3 kinase. *Clin Cancer Res* **14**, 5459-65 (2008).
128. Matsui, J. et al. E7080, a novel inhibitor that targets multiple kinases, has potent antitumor activities against stem cell factor producing human small cell lung cancer H146, based on angiogenesis inhibition. *Int J Cancer* **122**, 664-71 (2008).
129. Okamoto, K. et al. Antitumor activities of the targeted multi-tyrosine kinase inhibitor lenvatinib (E7080) against RET gene fusion-driven tumor models. *Cancer Lett* **340**, 97-103 (2013).
130. Keegan, M., Wilcoxen, K. & Ho, P.T. BOS172738: A novel highly potent and selective RET kinase inhibitor in Phase 1 clinical development. *Cancer Res* **79**, Abstract nr. 2199 (2019).
131. El-Gebali, S. et al. The Pfam protein families database in 2019. *Nucleic Acids Res* **47**, D427-D432 (2019).
132. Wells, S.A., Jr. et al. Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. *Thyroid* **25**, 567-610 (2015).

Comprehensive guidelines for all aspects of the diagnosis, classification and management of medullary thyroid carcinoma

133. Li, A.Y. et al. RET fusions in solid tumors. *Cancer Treat Rev* **81**, 101911 (2019).
134. Cote, G.J. et al. Prognostic Significance of Circulating RET M918T Mutated Tumor DNA in Patients With Advanced Medullary Thyroid Carcinoma. *J Clin Endocrinol Metab* **102**, 3591-9 (2017).

Acknowledgements

The authors acknowledge the support of a grant under the European Union's Horizon 2020 Programme – EU FP7 contract Thyrage (grant number 666869) awarded to D.S. and

the POR Campania FESR 2014-2020 “SATIN” grant awarded to M. Santoro. The authors gratefully acknowledge F. Carlomagno for critically reading the manuscript.

Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

M. Santoro is inventor of patent WO/2015/187818. The other authors declare no competing interests.

Peer review information

Nature Reviews Endocrinology thanks L. Mulligan, C. Romei and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Related links

The cBioPortal for Cancer Genomics [https://www.cbioportal.org]

Multiple endocrine neoplasia type 2A Online Mendelian Inheritance in Man [https://www.omim.org/entry/171400]

Multiple endocrine neoplasia type 2B Online Mendelian Inheritance in Man [https://www.omim.org/entry/162300]

Familial medullary thyroid carcinoma Online Mendelian Inheritance in Man [https://www.omim.org/entry/155240]

The PubChem database [https://pubchem.ncbi.nlm.nih.gov/]

National Cancer Institute Drug Dictionary [https://www.cancer.gov/publications/dictionaries/cancer-drug]

Pfam [https://pfam.xfam.org/]

Key points

- Alterations in *RET* are frequent key events in thyroid tumorigenesis.
- Single amino acid mutations and small deletions and/or insertions, or gene fusions involving *RET* represent oncogenic driving forces in medullary thyroid carcinoma and papillary thyroid carcinoma, respectively.
- Multi-targeted tyrosine kinase inhibitors (TKIs) currently represent the mainstay of treatment for advanced radioiodine-refractory thyroid cancer and advanced medullary thyroid carcinoma.
- Novel RET TKIs that target RET kinase potently with increased selectivity might be effective and better tolerated than the drugs already in clinical practice for treatment of advanced thyroid cancer.

Table 1: *RET* single amino acid substitutions in MEN2 syndrome and sMTC

***RET* exon**	**Codon**	**Mutation in MEN2[a]**	**MEN2 subtype**	**Risk level**	**Mutation in sMTC**
10	C609	C609R, C609G, C609F, C609S, C609W, C609Y	MEN2A	Moderate	Rare in sMTC
10	C611	C611R, C611G, C611F, C611S, C611W, C611Y	MEN2A	Moderate	Rare in sMTC
10	C618	C618R, C618G, C618F, C618S, C618W, C618Y	MEN2A	Moderate	C618R, C618G
10	C620	C620R, C620G, C620F, C620S, C620W, C620Y	MEN2A	Moderate	C620R
11	C630	C630R, C630Y, C630F, C630S	MEN2A	Moderate	Rare in sMTC
11	C634	C634R, C634G,	MEN2A	High	C634R, C634F,

		C634F, C634S, C634W, C634Y, C634K			C634S, C634W, C634Y
13	E768	E768D	MEN2A	Moderate	Rare in sMTC
13	L790	L790F	MEN2A	Moderate	Rare in sMTC
14	V804	V804L, V804M	MEN2A	Moderate	Rare in sMTC
15	A883	A883F	MEN2B	High or Highest	A883F, A883S
15	S891	S891A	MEN2A	Moderate	S891A
15	S904	S904F	MEN2A	Moderate	Rare in sMTC
16	M918	M918T	MEN2B	Highest	M918T

MEN2, multiple endocrine neoplasia type 2; sMTC**,** sporadic medullary thyroid carcinoma. [a]Only some of the most common mutations of RET kinase hot spots are listed. For a complete list, the reader is referred to comprehensive reviews[36, 38,56].

Table 2: *RET* fusions in thyroid carcinoma and in other malignancies

RET Fusion[a]	Thyroid carcinoma	Other malignancies
ACBD5–RET	PTC	NA
AFAP1L2–RET	PTC	NA
AKAP13–RET	PTC	NA
ANKRD26–RET	PTC	NA
CCDC6–RET (known as *RET*–PTC1)	PTC, PDTC	LADC, BRCA, SCT, CRC, STAD
DLG5–RET	PTC	NA
ERC1–RET	PTC,	BRCA
FKBP15–RET	PTC	NA
GOLGA5–RET (known as *RET*–PTC5)	PTC,	SN
HOOK3–RET	PTC	NA
KIAA1468–RET	PTC	IMA
KTN1–RET (known as *RET*–PTC8)	PTC	NA
MYH13–RET	MTC	NA
NCOA4–RET (known as *RET*–PTC3)	PTC, PDTC	LADC, BRCA, IC, SCT, CRC
PCM1–RET	PTC	NA
PDCD10–RET	PDTC	NA
PPFIBP2–RET	PTC	NA
PRKAR1A–RET (known as *RET*–PTC2)	PTC	NA
RUFY2–RET	PTC	LADC,
SPECC1L–RET	PTC	LPF, LPF-NT
SQSTM1–RET	PTC	NA

TBL1XR1–RET	PTC	NA
TFG–RET	PDTC	SCT, LPF
TRIM24–RET (known as *RET*–PTC6)	PTC	LADC, CRC
TRIM27–RET	PTC	IC
TRIM33–RET (known as *RET*–PTC7)	PTC	LADC
UEVLD–RET	PTC	NA

BRCA, breast invasive carcinoma; CRC, colorectal carcinoma; IC, intraductal carcinoma of the salivary gland; IMA, invasive mucinous lung adenocarcinoma; LADC, lung adenocarcinoma; LPF, lipofibromatosis; LPF-NT, lipofibromatosis-like neuronal tumours; MTC, medullary thyroid carcinoma; NA, not identified in other malignancies; PDTC, poorly differentiated thyroid carcinoma; PTC, papillary thyroid carcinoma; SCT, spindle cell tumour of soft tissues; SN, spitzoid neoplasms; STAD, stomach adenocarcinoma. [a]Data originally presented in Ref[63].

Table 3: Activity of clinical small molecule tyrosine kinase inhibitors targeting RET

Compound[a]	Other names	Targets (IC_{50} nM)[b]			Reference
		RET kinase	VEGFR2	Others (IC_{50} nM)[c]	
Vandetanib	ZD6474, Zactima, Caprelsa	130 (4)	40 (4)	VEGFR3 (110), EGFR (500)	[122], [86]
Cabozantinib	BMS-907351, XL-184, Cometriq	5.2 (11)	0.035 (2)	MET (1.3), KIT (4.6), AXL (7), FLT3 (11.3), TIE2 (14.3)	[123], [86]
Sorafenib	BAY 43-9006, Nexavar	5.9	15	RAF1 (6), mVEGFR3 (20), BRAF (22), mPDGFRb (57), FLT3 (58)	[124-126]
Lenvatinib	Lenvima, E7080	1.5	4	VEGFR3 (5.2)	[127-129]
Pralsetinib	BLU-667	0.4	35	NR	[86]
Selpercatinib	LOXO-292	0.4	100	NR	[82]
BOS172738	DS-5010	1.0[d]	>300 fold vs RET	NR	[130]

NR: not reported. [a]From https://pubchem.ncbi.nlm.nih.gov/ and https://www.cancer.gov/publications/dictionaries/cancer-drug. [b]Data from *in vitro* kinase assays (standard deviations are omitted) from the original publications. Based on the different experimental setting relative potency for the different targets within the same study might be appreciated, while direct comparisons between absolute values from different studies should be considered very cautiously. Between dashes are the values from reference[86]. [c]Targets with IC_{50} values in a 10-fold range with respect to RET kinase

are reported. BLU-667 inhibits JAK1 but at a potency about 20-fold lower than RET. [d]In this case, biochemical Kd values are reported.

Figure 1: Timeline of key discoveries for *RET* as a driver oncogene and its therapeutic targeting. Schematic representation of the major discoveries about RET kinase structure and function, and the role of RET kinase in the pathogenesis of human cancer and other diseases. The approval of drugs acting on RET kinase is also shown. GDNF, glial cell line-derived neurotrophic growth factor; LADC: lung adenocarcinoma; MEN2: multiple endocrine neoplasia type 2; MTC: medullary thyroid carcinoma; PTC: papillary thyroid carcinoma; TKD, tyrosine kinase domain.

Figure 2: Structure of RET protein and of major RET fusion partners. a | Schematic representation of RET protein. C634 is commonly mutated in multiple endocrine neoplasia type 2A (MEN2A) syndrome. b | Schematic representation of CCDC6, NCOA4 and KIF5B proteins. For each protein, the structural domains are indicated. DUF2046 (domain of unknown function 2046) of CCDC6 contains several potential segments able to fold into coiled-coil structures (amino acids 56–104, 120–140, 142–212, 214–234 and 262–325). The first ARA70 domain in NCOA4 contains a potential segment (amino acids 12–128) able to fold into a coiled-coil structure. Protein domain information was obtained from Pfam (https://pfam.xfam.org/)[131]. Arrows indicate the most frequent breakpoint sites in RET kinase and partner proteins. c | Schematic representation of the RET TKD. V804, Y806, G810 and S904 are shown; mutation of these residues might cause resistance to some kinase inhibitors. M918, whose mutation is associated with sporadic MTC and patients with MEN2B syndrome, is also shown. AS, activation segment; CC, coiled-coil; CLD 1 to 4, Cadherin-like domains; CRD, Cysteine-rich domain; EC, extracellular region; G, Glycine-

rich loop mediating binding to the nucleotide; H, hinge; I, kinase insert; TM, transmembrane segment; JM, juxtamembrane segment; TKD, tyrosine kinase domain.

Box 1: Clinical characteristics of multiple endocrine neoplasia type 2 syndromes

There are two clinically distinct types of multiple endocrine neoplasia type 2 (MEN2) syndrome, termed [MEN2A https://www.omim.org/entry/171400] and [MEN2B https://www.omim.org/entry/162300]. Within MEN2A, there are four variants. First, classic MEN2A, represented by the uniform presence of medullary thyroid carcinoma (MTC) and the less frequent occurrence of phaeochromocytoma, or primary hyperparathyroidism, or both. Second, MEN2A with cutaneous lichen amyloidosis. Third, MEN2A with Hirschsprung disease. Fourth, familial MTC [(FMTC) https://www.omim.org/entry/155240], which is defined as individuals with MTC only and without any other inherited pathology in their family members. MEN2B is associated with MTC, phaeochromocytoma, typical facies, skeletal abnormalities and marfanoid habitus, generalized ganglioneuromatosis and ocular abnormalities[132 36].

A germline *RET* mutation is present in almost all patients with MEN2. *RET* mutations are stratified into three risk levels, that is, highest, high and moderate risk, based on the penetrance, aggressiveness and latency of the MTC[132]. This risk level will indicate the age at which thyroidectomy should be performed in mutation carriers. Children with MEN2B (mutation at codon 918) are in the highest risk category and should undergo thyroidectomy in their first year of life or perhaps even in their first months of life. In children in the high-risk category (mutation at codon 634), thyroidectomy should typically be performed at the age of 5 or at an earlier age if serum levels of calcitonin become elevated. In children in the moderate risk group (with any of the other *RET* mutations), disease expression varies considerably. As such, the decision regarding the age of prophylactic thyroidectomy is no longer based upon genotype alone but is currently driven by the increasing trend of serum levels of calcitonin, surgery being recommended for elevated levels but below 30 pg/ml, because the occurrence of lymph node metastases becomes more frequent for higher levels. Personalized management also includes decisions about the best age to begin biochemical screening for phaeochromocytoma and primary hyperparathyroidism [132].

Box 2: *RET* testing in patients with thyroid carcinoma

[bH1] Testing for germline mutations

The identification of *RET* point mutations is fairly simple when a specific germline mutation is already known in the family. Such knowledge allows clinicians to detect the mutated *RET* allele in at-risk family members. The strategy is either to initially sequence the most commonly mutated *RET* cysteine codons located in exons 10 and 11 as well as hot spots located in exons 13 to 16, or sequencing all *RET* exons. The second approach is more expensive, but feasible and allows detection of 'rare' *RET* double or multiple mutations [133].

[bH1] Testing for somatic alterations

[bH2] Detection of point mutations. Uses the above methods.

[bH2] Detection of gene fusions. Several different genes can contribute to the 5′ sequences of chimeric *RET* products, therefore the use of DNA based methods is limited. RT-PCR assays can detect *RET* and fusions partners, but detect only a few genes at once and cannot detect novel fusion partners. Fluorescence in situ hybridization or immunohistochemistry are rarely used to detect RET fusion proteins due to the weak reactivity of available antibodies. Next generation sequencing offers the possibility to assess multiple *RET* mutations, gene fusions and variant allele frequencies with high sensitivity. RNA sequencing approaches enable a more comprehensive detection of gene rearrangements and are considered as a complementary approach when DNA-based approaches fail to detect fusion products.

[bH2] Liquid biopsy

Liquid biopsy has finally emerged as a novel method that is particularly useful in the clinical and therapeutic monitoring of advanced thyroid cancers with a specific *RET* alteration. The technique aims to identify and quantify *RET* alterations in plasma DNA, although specificity and sensitivity of the assay should be addressed [134]. In a 2019 study, *RET* point mutations and fusions were investigated in plasma DNA of nearly 33,000 patients affected by various cancer types; somatic activating *RET* alterations were found in ~1 in 200 patients in a wide range of solid tumour types[105] .

Alterations of RET kinase have been found in diverse thyroid cancer subtypes. This Review describes the *RET* mutations and gene fusions that can occur in thyroid cancer and highlights specific RET kinase inhibitors that are in clinical and preclinical use.