Zhang *et al.*
Cell Communication and Signaling (2024) 22:460
https://doi.org/10.1186/s12964-024-01837-x

Cell Communication and Signaling

REVIEW

Open Access

Molecular genetics, therapeutics and RET inhibitor resistance for medullary thyroid carcinoma and future perspectives

Ying Zhang[1,2†], Wei-Hui Zheng[1,3†], Shi-Hong Zhou[4†], Jia-Lei Gu[1,5], Qing Yu[1,3], Yi-Zhou Zhu[1,6], Yu-Jie Yan[2], Zhi Zhu[7*†] and Jin-Biao Shang[1,3,5*†]

Abstract

Medullary thyroid carcinoma (MTC) is a rare type of thyroid malignancy that accounts for approximately 1–2% of all thyroid cancers (TCs). MTC include hereditary and sporadic cases, the former derived from a germline mutation of rearrangement during transfection (RET) proto-oncogene, whereas somatic RET mutations are frequently present in the latter. Surgery is the standard treatment for early stage MTC, and the 10-year survival rate of early MTC is over 80%. While for metastatic MTC, chemotherapy showing low response rate, and there was a lack of effective systemic therapies in the past. Due to the high risk (ca. 15–20%) of distant metastasis and limited systemic therapies, the 10-year survival rate of patients with advanced MTC was only 10–40% from the time of first metastasis. Over the past decade, targeted therapy for RET has developed rapidly, bringing hopes to patients with advanced and progressive MTC. Two multi-kinase inhibitors (MKIs) including Cabozantinib and Vandetanib have been shown to increase progression-free survival (PFS) for patients with metastatic MTC and have been approved as choices of first-line treatment. However, these MKIs have not prolonged overall survival (OS) and their utility is limited due to high rates of off-target toxicities. Recently, new generation TKIs, including Selpercatinib and Pralsetinib, have demonstrated highly selective efficacy against RET and more favorable side effect profiles, and gained approval as second-line treatment options. Despite the ongoing development of RET inhibitors, the management of advanced and progressive MTC remains challenging, drug resistance remains the main reason for treatment failure, and the mechanisms are still unclear. Besides, new promising therapeutic approaches, such as novel drug combinations and next generation RET inhibitors are under development. Herein, we overview the pathogenesis, molecular genetics and current management approaches of MTC, and focus on the recent advances of RET inhibitors, summarize the current situation and unmet needs of these RET inhibitors in MTC, and provide an overview of novel strategies for optimizing therapeutic effects.

†Zhi Zhu and Jin-Biao Shang contributed equally to this work and shared the corresponding authorship.

†Ying Zhang, Wei-Hui Zheng and Shi-Hong Zhou contributed equally to this work and shared the first authorship.

*Correspondence:
Zhi Zhu
zhuzhi@xmu.edu.cn
Jin-Biao Shang
shangjb@zjcc.org.cn
Full list of author information is available at the end of the article

Keywords Medullary thyroid carcinoma (MTC), Rearrangement during transfection (RET), Tyrosine kinase inhibitors (TKIs), Targeted therapy

Background

Over the past 50 years, the incidence of thyroid cancer (TC) has rapidly increased worldwide. In 2023, TC constituted about 3% of newly diagnosed cancer cases, with an estimated incidence of 44,020 new cases, and ranked the ninth most common cancer in the United States [1]. Medullary thyroid carcinoma (MTC) is a relatively rare malignant thyroid neoplasm accounting for only 1–2% of all TC cases worldwide [2, 3]. Whereas, MTC is associated with high mortality, comprising disproportionately 8.6% of TC-related deaths, and the 10-year survival rate is approximately 50–60% [4–6]. As a neuroendocrine tumor (NET), MTC derives from the thyroid C-cells or parafollicular cells, and is able to secrete calcitonin (Ctn) and carcinoembryonic antigen (CEA) [7]. MTC may be inherited or sporadic, the familial cases are secondary to a germline mutation in the rearranged during transfection (RET) proto-oncogene, and more than half of sporadic cases harbor a somatic RET mutation [8, 9].

Compared to differentiated thyroid cancer (DTC), which is the most common type of TC, MTC is more aggressive, about 50% of MTC patients were observed to have lymph node invasion, and 15% have distant metastases at the first diagnosis. What's more, about 20–40% of patients will experience distant metastases during the disease, leading to a lower 10-year survival rate of 40%, compared to 96% for patients with MTC confined to the thyroid [4].

The low incidence and limited number of large-scale studies of MTC have led to a lack of high-quality data needed to establish a consensus on diagnosis and treatment. For patients with early stage MTC, surgical resection is the mainstay treatment and the only curative treatment for MTC, the main surgical strategy is total thyroidectomy with dissection of cervical lymph node compartments, and depending on the serum Ctn levels and preoperative cervical US imaging, a more extensive surgery with lateral neck dissection should be considered [5]. Whereas, in the presence of widespread regional or distant disease, more extensive surgery is not related to a higher cure rate or survival benefit and should be considered primarily for local symptom control [5, 10]. While for patients with locally advanced MTC that is inoperable or those with distant metastasis, there is no effective therapeutic and curative option. Local treatments like external beam radiotherapy (EBRT), have only limited and short-term benefits [5], systemic therapies like radioactive iodine treatment is ineffective in MTC due to the inability of MTC cells to uptake iodine-131, even though is the main adjuvant therapy for DTC [11]. The conventional cytotoxic chemotherapy has shown poor results in advanced or metastatic MTC, with a response rate of approximately 20% [12–14]. However, with the deepening understanding of the molecular mechanism of MTC and intracellular signaling pathway that involved in MTC pathogenesis, targeted treatments have gradually developed, which represented by tyrosine kinase inhibitors (TKIs) that target RET proto-oncogene, have demonstrated considerable promise in treatment for advanced and recurrent MTC and have positioned targeted therapy as the current standard of treatment [15].

Current TKIs for RET include multi-tyrosine kinase inhibitors (MKIs) and RET-selective TKIs, the former incompletely inhibit multiple kinases including RET and often impair multiple signaling pathways, while the latter is highly selective for RET [16, 17]. During recent decades, two MKIs, Cabozantinib and Vandetanib, have been successfully introduced for treatment of patients with advanced MTC, based on the beneficial results of randomized phase III trials [18, 19]. Nevertheless, the degree of overall clinical benefit achieved with these MKIs has been relatively low, which mainly due to partial inhibition of RET, suboptimal pharmacokinetic properties, and toxicities resulting from more effective inhibition of non-RET kinases, including VEGFR2/KDR, EGFR, KIT, BRAF and MET, i.e., off-target effects [18]. Consequently, a majority of patients undergoing therapy with MKIs encounter significant toxicities that need the interruption, reduction, or discontinuation of the prescribed dosage. Moreover, some pathogenic RET mutations fail to respond to MKI treatment, ie, are non-responsive to nonspecific RET inhibitors. Although these MKIs have been shown to increase progression-free survival (PFS) for patients with advanced and progressive MTC, while have not prolonged overall survival (OS) and their utility is limited due to high rates of unacceptable off-target toxicities [20].Therefore, in order to overcome the weaknesses of MKIs and enhance both their efficacy and safety, highly selective RET inhibitors have emerged. Currently approved small-molecule RET-selective inhibitors include Selpercatinib (LOXO-292) [21–24] and Pralsetinib (BLU-667) [25–27]. Both drugs were approved as second-line treatment options by the US Food and Drug Administration (FDA) in 2020 [28, 29], and the European

Medicines Agency (EMA) approved Selpercatinib in 2020 and Pralsetinib in 2021 [30, 31]. The timeline of the landmark discoveries and clinical trials of RET gene and MTC are shown in Fig. 1.

Despite the tremendous development of RET inhibitors, the management of advanced and progressive MTC remains challenging, drug resistance remains the main reason for treatment failure, and the mechanisms are still unclear. Besides, new promising therapeutic approaches, such as novel drug combinations and next- generation targeted therapies such as immunotherapy and peptide receptor radionuclide therapy (PRRT) are under development.

Herein, we review the pathogenesis, molecular genetics, and current management approaches of MTC, and focus on the recent advances in RET inhibitors, summarize the current situation and unmet needs of these RET inhibitors in MTC, and provide an overview of novel strategies for optimizing therapeutic effects.

Pathogenesis and Molecular Genetics of MTC

During the last decades, the molecular biology of MTC has been elucidated. MTC can occur either in hereditary or sporadic forms, and the majority (nearly 75%) of MTC cases develop sporadically, only nearly 25% of cases are familial [32]. Hereditary MTCs are secondary to germline mutations in RET proto-oncogene, which is inherited as an autosomal dominant trait [32, 33]. Furthermore, inherited MTC appears as a predominant part of multiple endocrine neoplasia type 2 (MEN2) syndrome [34, 35], which can be divided into two clinically distinct subtypes: MEN2A and MEN2B, with the former being the most common, almost 95% [36]. Most (95–98%) hereditary MTC patients harbor germline-activating RET mutations. Although somatic RET mutations are present in about half (40–50%) of sporadic MTC (sMTC) patients [21, 36–42], these were discovered to increase to a striking 91.4% in patients with advanced and progressive MTC [43].

Pathogenesis of MTC

The RET gene was identified in 1985 as a novel transforming gene during transfection of NIH/3T3 cells with human T lymphoma DNA [44]. RET is a proto-oncogene, which is a 21-exon gene located on the long arm of chromosome 10 (10q11.2). As a tyrosine kinase receptor gene, it encodes for a transmembrane receptor with tyrosine kinase (RTK), called RET kinase, which is primarily expressed in organs that derive from the embryologic neural crest, including parafollicular C cells in the thyroid gland. Under physiological conditions, RET kinase plays an important role in numerous cellular mechanisms (including cell growth, proliferation, migration, differentiation and survival) [45]. RET is vital 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 [46–48]. RET signaling also plays a role in the regulation of hematopoietic cells and spermatogenesis [49, 50]. During adulthood, RET is mainly present in organs derived from neural crest cells [51]. 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 [46, 52].

RET protein is a transmembrane glycoprotein RTK that consists of three domains: an N-terminal extracellular domain, which features four cadherin-like regions (CLD1-4), a calcium binding site and one cysteine-rich

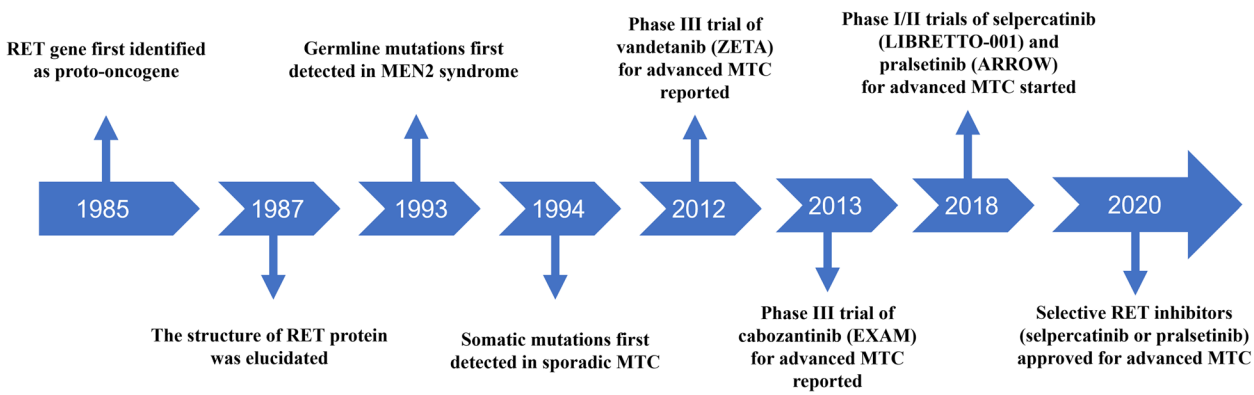


Fig. 1 Timeline of the landmark discoveries and clinical trials of RET gene and MTC. RET, rearranged during transfection; MTC, Medullary thyroid cancer

region (CRD); a hydrophobic transmembrane region that transverses the plasma membrane; and an intracellular cytoplasmic domain including a juxta-membrane segment, tyrosine kinase activity domains (TKD) and an isoform specific C-terminal [53–58].

RET kinase is stimulated by forming a tripartite complex with glial cell line-derived neurotrophic growth factor (GDNF) family ligands (GFLs) binding to glycosylphosphatidylinositol (GPI)-linked cell surface co-receptor called GDNF-family receptor-α(GFRα) (Fig. 2) [58]. These ligands include GDNF, neurturin (NRTN), persephin (PSPN), and artemin (ARTN) [45]. GFRαis comprised of four different subtypes (GFRα1-4) that are able to bind preferentially to GDNF, NRTN, ARTN, and PSPN, respectively [59]. The main intracellular signaling pathways involved in RET activation are the mitogen-activated protein kinase (MAPK) and the phosphatidylinositol-3 kinase (PI3K) pathways. Stimulation of RET kinase results in downstream signaling of mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinases (PI3K), and protein kinase B, as well as focal adhesion kinase, signal transducer and activator of

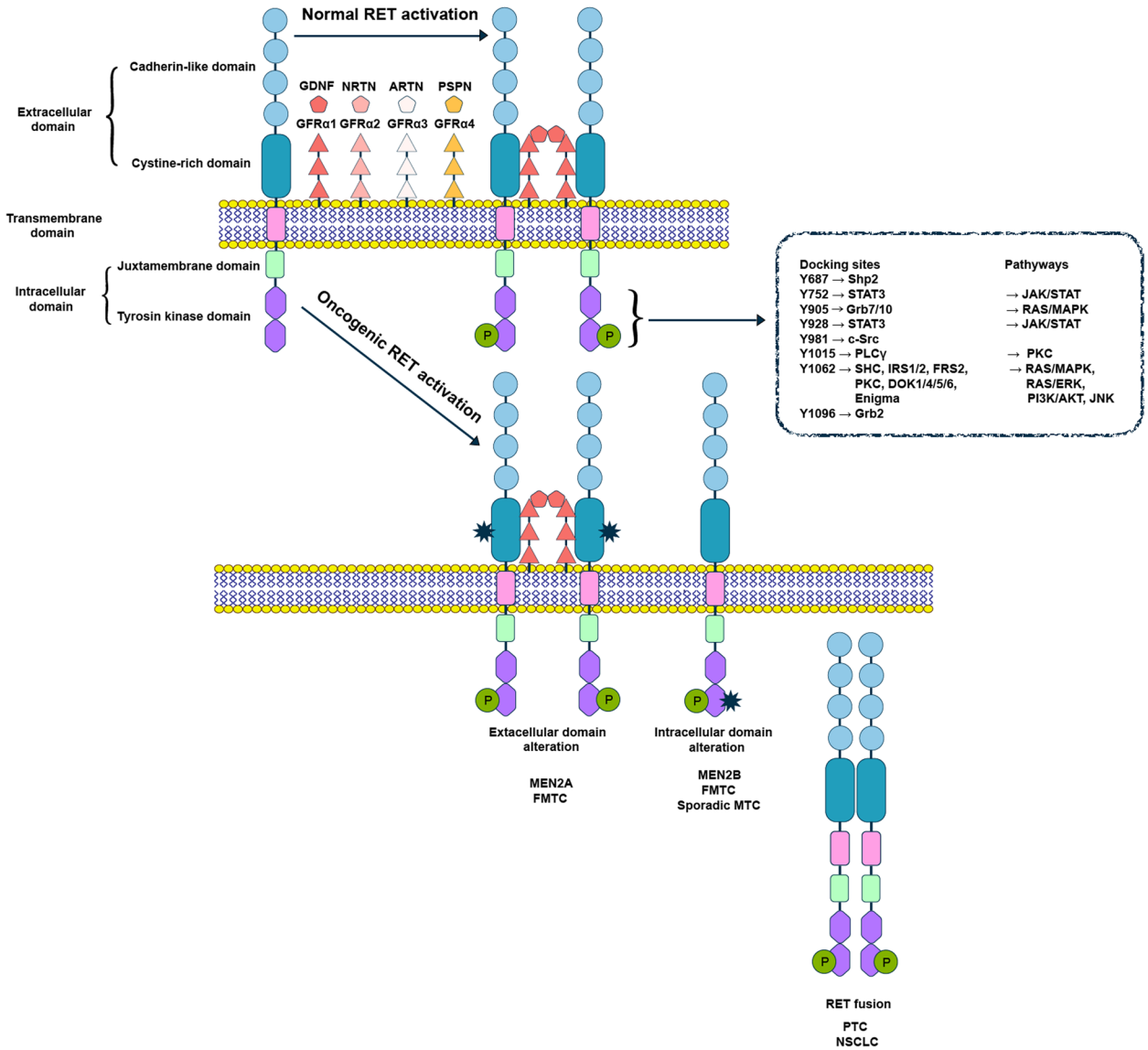


Fig. 2 Schematic structure and oncogenic mechanism of RET in a cancer cell. It shows the extracellular, transmembrane and intracellular domains of RET, as well as the normal activation and oncogenic activation of RET. The main docking sites and related pathways are also shown. GDNF, glial cell line-derived neurotrophic growth factor; GFR, GDNF-family receptor; NRTN, neurturin; ARTN, artemin; PSPN, persephin; MEN2A, multiple endocrine neoplasia type; FMTC, familial medullary thyroid cancer; PTC, papillary thyroid cancers; NSCLC, non-small cell lung cancer

transcription (STAT), and steroid receptor coactivator-1 (Src1) pathways [45].

Disruption of the integrity of this tripartite complex, as well as the structure and function of the RET receptor, can drive the development of disease. However, the mechanism of action differs depending on the mutation, and different RET mutations have diverse transforming activities. Mutations in RET can contribute to loss or gain of function mutations in the RET kinase signaling pathway. For instance, loss of function mutations in RET is associated with Hirschsprung disease, which is due to the injury of parasympathetic enteric neuron development [60, 61]. Additionally, gain of function mutations in RET are linked with several malignancies, such as breast, prostate, pancreatic, myeloid, and TCs including MTC [58].

Oncogenic RET is activated by two major mechanisms, including chromosomal rearrangements and point mutations, and the mechanism of RET activation is different and specific to the type of tumor. RET chromosomal rearrangements can produce hybrid proteins, leading to gene fusions that harbor the RET kinase domain with a partner protein, the latter contains a dimerization domain, which generates chimeric RET homodimers, which are persistently active, therefore promoting the proliferation of cancer cells [62]. RET fusions are most commonly observed in papillary thyroid cancers (PTC) and non-small cell lung cancers (NSCLC), approximately 2.5–73% and 1–3%, respectively [63–65]. While point mutations in the kinase domain of RET at the somatic or germline level can directly or indirectly cause abnormal activation of RET kinase, which is common in inherited or sporadic MTC [66, 67]. These mutations substitute cysteine with another amino acid and lead to the formation of disulfide-bonded RET homodimers with subsequent ligand-independent constitutive activation of RET kinase region [46]. The schematic structure and oncogenic mechanism of RET in a cancer cell was shown in Fig. 2.

Molecular genetics of hereditary MTC

Most (95–98%) hereditary MTC patients harbor germline-activating RET mutations. Germline activating RET mutations cause the autosomal dominant inheritance of MEN2 syndromes, which is a group of hereditary, autosomal dominant syndromes characterized by the occurrence of various endocrine tumors (including MTC, in combination or not with pheochromocytoma (PHEO), hyperparathyroidism (HPTH), mucosal neuroma and extra-endocrine features) [34, 35], and MTC is the most common cause of death in patients with MEN2 [68]. According to genotype–phenotype correlation, MEN2 can be classified as two clinically distinct subtypes: MEN2A and MEN2B [34]. MEN2A is the most common type, accounting for almost 95% [46], and familial MTC (FMTC) is viewed as a phenotypic variant of MEN2A [69]. MEN2A is characterized by MTC, pheochromocytoma (PHEO) and parathyroid hyperplasia or adenoma. MEN2A is most frequently connected with activation of point mutations in the RET cysteine codon 634 (in exon 11), as well as other sites of point mutations, including cysteine codons 609, 611, 618, and 620 (in exon 10) and 630 (in exon 11) [5]. MEN2A patients carry cysteine-specific mutations in the extracellular domain of RET, that are involved in conformational stability and kinase activity via intramolecular disulfide bridges. These mutations cause covalent dimerization of RET leading to ligand-independent kinase activation [70–72]. MEN2B only corresponds to 5% of MEN2 cases, it is the most aggressive type with early onset of MTC and occurs earlier in life. Patients do not have parathyroid involvement, but often show a number of unique physical features, such as ganglioneuromas, musculoskeletal manifestation (including Marfanoid habitus and other skeletal malformations like hip epiphysiolysis, scoliosis and feet abnormalities), mucosal neuromas of the lips and the tongue and gastrointestinal manifestations [34, 73, 74]. Unfortunately, knowledge of MEN2B syndrome is still insufficient and several cases are still unrecognized nowadays, despite this syndrome has been discovered several years ago and has typical physical malformation [75]. MEN2B is associated with Met918Thr in exon 16 of RET (~95%), Met918 is located near the kinase activation loop: mutation to threonine causes the opening of the activation loop and leading to constitutive activation via promoting a high level of autophosphorylation of RET receptors [5, 64, 76]. FMTC patients only develop MTC, lacking an association with other endocrine tumors or nonendocrine manifestations; thus, some authors view FMTC as a phenotypic variant of MEN2A without adrenal gland involvement [69]. In FMTC, germline mutations are distributed throughout the RET gene, with an accumulation in exon 13 (codons 768, 790, and 791) and exon 14 (codons 804, and 844); some of these mutations have also been identified in families with MEN 2A [77]. There is geographic variability between the RET variants that are linked with the development of MEN2A [78]. Specific germline RET variants are correlated with age of presentation and aggressiveness of MTC, as well as the occurrence of other manifestations, and this genotype–phenotype association has been used to determine the optimal age for the initiation of screening and surveillance, as well as recommending early total thyroidectomy [79–81]. It is of great significance for patients with germline RET mutations to screen and monitor for MTC, to help detect MTC early and improve prognosis [79, 80, 82–84]. In addition, more

than 90% of cases with MEN2B and approximately 2% of cases with MEN2A have de novo germline mutation (without any family history) [75, 85]. Thus, in all cases of histologically detected MTC, or in cases where a family history of MTC is detected before surgery, RET germline mutation must be evaluated since about 6% of the cases without a familial history of MTC carried germline mutations [86, 87].

Specific germline pathogenic variants involving RET gene result in different cancer risks and aggressiveness. According to current American Thyroid Association (ATA) guidelines, patients with germline RET variants are stratified into "highest risk" (HST), "high risk" (H) and "moderate risk" (MOD) levels of aggressive MTC. The HST category includes patients with MEN2B and RET p.M918T (Fig. 3). The H category includes patients with the RET variants involving codon 634F/G/R/S/W/Y and RET p.A883F. Patients with hereditary MTC and other germline pathogenic RET variants belong to the MOD category. The risk stratification of germline RET variants is showed in Fig. 3. The ATA also recommends the appropriate timing of prophylactic thyroidectomy for children based on the concrete RET variants and serum Ctn levels [5]. For cases of positive germline mutation in children, particularly younger children, Ctn evaluation over time is a safe method that able to detect the disease before it became clinically evident, and when young patients are followed on this way, there is a high possibility to reduce the post-operative complication without any influence on the course of the disease [88].

Molecular genetics of somatic MTC

Approximately 75% of MTC occurred sporadically, and somatic pathogenic RET mutations exist in more than 60% cases of sMTC. Although somatic-activating RET mutations are present in 40–50% of sMTC patients [21, 37, 38], in advanced and progressive MTC patients with distant metastases [58, 89] and cervical lymph node metastases [90], this number increase to a striking 91.4% [43]. The most frequent somatic mutation related to sMTC is Met918Thr, accounting for up to 80% of these pathogenic RET variants (Fig. 3) [2, 37]. Other somatic mutations have been identified involving codons 634, 804 and many others, and codon 634 is the second most common site of mutations associated with sMTC, accounting for about 15% prevalence [2, 37, 91].. Besides, somatic pathogenic variants involving the RAS genes, a downstream signaling molecule of RET, occur in about 30% of sMTC (Fig. 3) [37, 92, 93]. Somatic RAS variants (HRAS, KRAS, NRAS) are mutually exclusive of RET variants, accounting for about 70% of RET mutation-negative sMTC [37, 89, 92, 94–96]. HRAS alterations account for about 70% of all MTCs with RAS variants, and NRAS variants are less common [37]. Besides point mutations, deletions and insertions have also been described on next-generation sequencing (NGS) tumor testing [97, 98], and there is an even smaller proportion of sMTC having unknown genetic causes. Beyond RET and RAS, mutations of NF-1 gene have also been very recently reported in sporadic MTC [99].

Although sMTC is the most common means of presentation, 1–7% of apparently sMTC cases are due to de novo germline mutations and have no suggestive family history [5]. Since there may be an underlying hereditary disease, every patient should undergo a DNA analysis to detect RET germline mutations [100, 101].

One previous study found the correlation of somatic mutations with the pathological characteristics of the tumors and with both the clinical features and outcome of patients affected with sMTC by NGS targeted sequencing [37], it was observed that the presence of RET mutations and in particular, the M918T was confirmed to be significantly associated with a worse outcome, a higher tumoral staging, a higher T category and the presence of lymph-node and distant metastases. While RAS-positive cases were significantly associated with a better outcome, a lower tumor staging and a lower rate of T categories than RET-positive cases, independent from the presence of RET mutation. Thus, it was confirmed that sMTCs patients with somatic RET p.M918T variant have the worst prognosis, whereas those with somatic RAS mutations have a less aggressive phenotype and the best prognosis.

Current approaches in the management of MTC

For many reasons, there is lack of consensus in current guidelines for appropriate diagnosis, treatment, and follow-up of MTC. First, MTC can take a variety of clinical forms; for some patients, especially sMTC patients, tumors can remain stable and even unchanged for a long time, thus indicating a higher survival rate. In contrast, in another group of patients, MTC may be extremely aggressive and may show early distant metastasis and high mortality [102, 103]. Second, compared with common types of TC, MTCs exhibit a lower incidence. Thus, there is a limited number of large-scale studies about MTC, causing a lack of effective evidence to reach a consensus. Owing to the reasons above, there are obvious differences in clinical practices. According to recent advances of MTC diagnosis and treatment, we summarized the current treatment recommendations of MTC based on the clinical stage, which is shown in Fig. 4.

Surgical management of MTC

For patients with MTC, surgical resection is the mainstay of treatment and the only curative treatment for

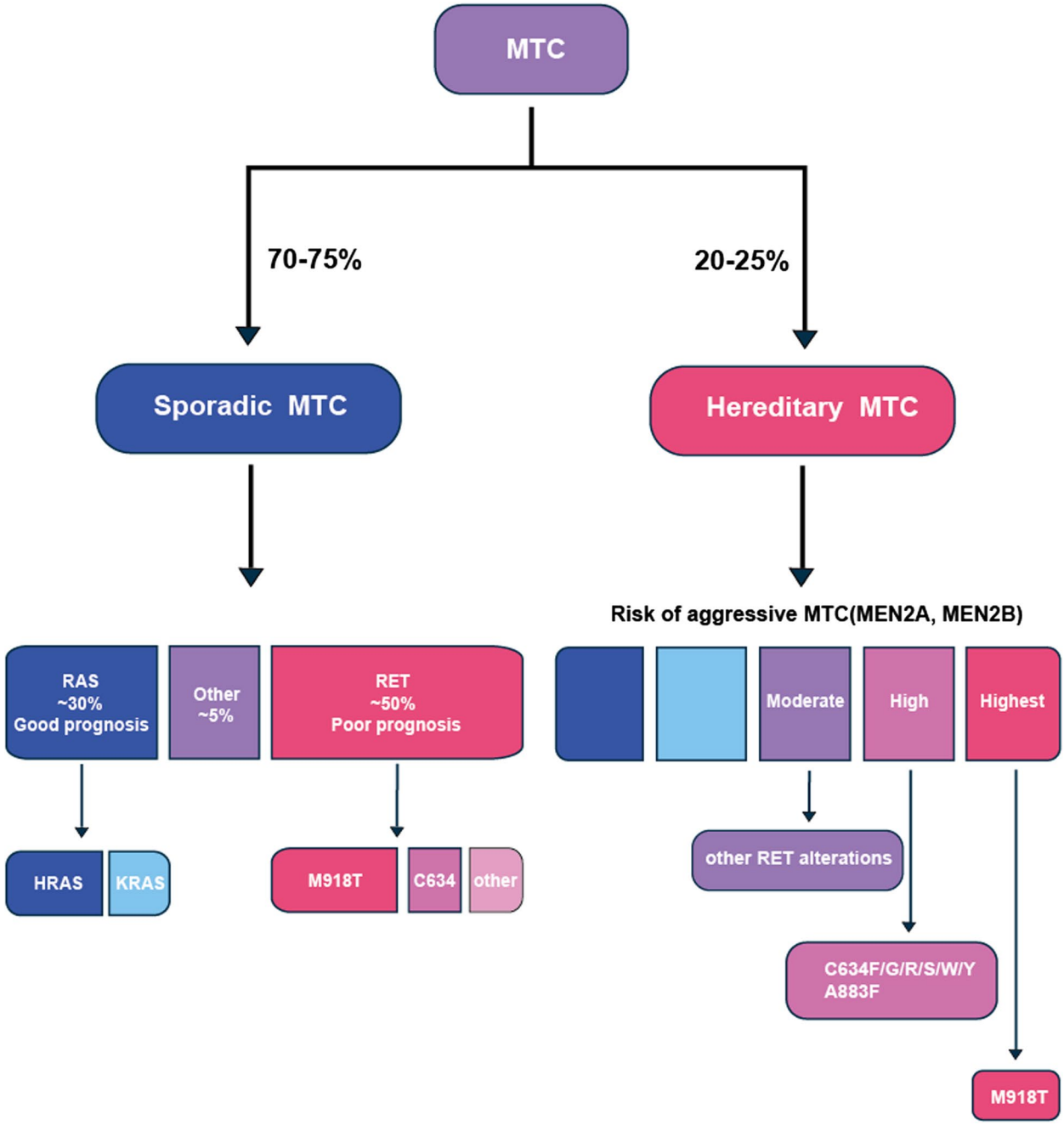


Fig. 3 Risk stratification of hereditary and sporadic medullary thyroid carcinoma (MTC) according to molecular profiles. Current American Thyroid Association guidelines classify patients with pathogenic germline RET variants into 3 groups based on the aggressiveness of the MTC: highest risk includes patients with RET p.M918T variant, high risk includes patients with RET p.C634F/G/R/S/W/Y variants or p.A883F alteration, moderate risk includes patients with other pathogenic RET variants. Germline RET p.M918T and p.A883F variants mainly occur in multiple endocrine neoplasia type 2B (MEN2B), whereas germline RET alterations at codon C634 occur in MEN2A. In sporadic MTCs, patients with somatic RET p.M918T variant have the worst prognosis, whereas those with somatic RAS mutations have the best prognosis

MTC, the main operation is total thyroidectomy with dissection of cervical lymph node compartments, and depending on the serum tumor markers levels and preoperative imaging, a more extensive surgery with lateral neck dissection may be considered [5], and currently the scientific community is moving towards a less aggressive surgery for MTC in selected cases [104]. Preoperative imaging includes high-resolution neck ultrasonography

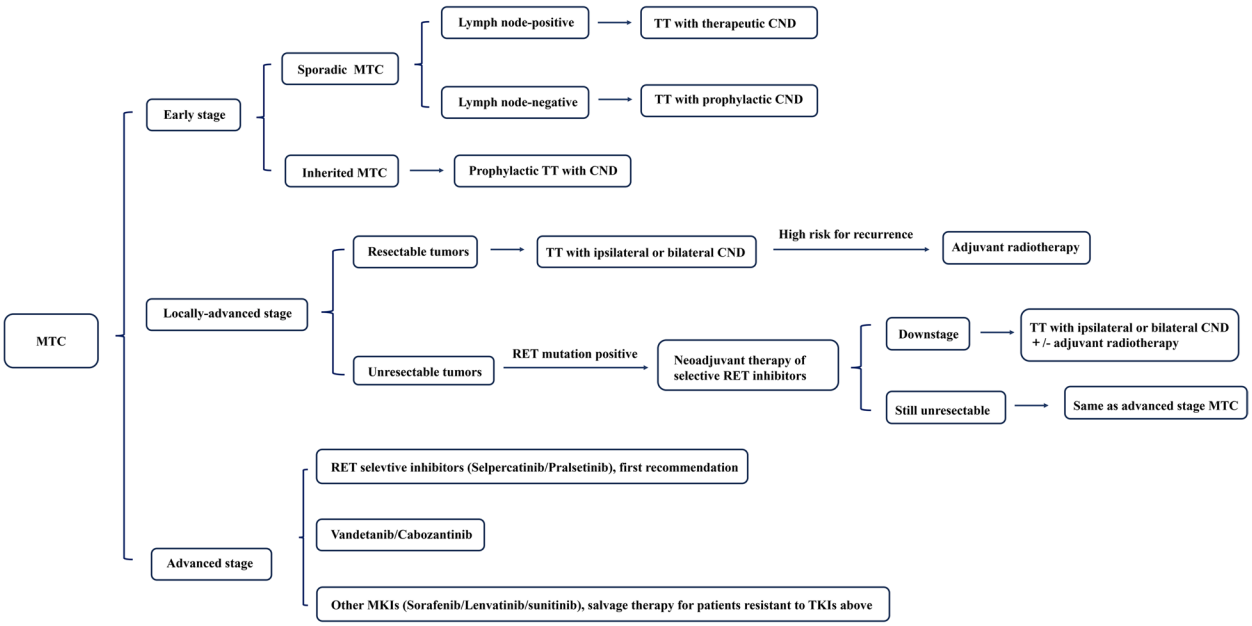


Fig. 4 Classification of the management of MTC based on clinical stage. MTC, medullary thyroid carcinoma; TT, total thyroidectomy; CND, central neck dissection. MKI, multi-kinase inhibitor

(US), which is the first-line imaging choice [105, 106], computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET)/CT (^{18}F-DOPA and ^{68}Gallium-DOTATATE are the preferred radiotracers). Serum tumor markers include Ctn and CEA, which are widely used biomarkers for the diagnosis, prognosis, and follow-up of MTC patients [7].

MTC diagnosed presurgically

The initial surgical management of biopsy-proven MTC mainly depends on the presence of lateral neck lymph node metastases and serum Ctn/CEA levels or the detection of a germline mutation in the RET proto-oncogene [107]. Nowadays, basal and stimulated Ctn level remains the cornerstone of the diagnosis of MTC [108, 109]. Neck US and fine-needle aspiration cytology (FNAC) (mainly if serum Ctn testing is performed) are useful tools but should be integrated into the work-up of patients with thyroid nodule suspicious for MTC, as the sensitivity is around 50% if considered alone [106, 110, 111]. Moreover, there are other potential markers in diagnosing MTC, including procalcitonin, CA19-9 and chromogranin A [112]. Owing to the occurrence of multifocal disease in 15% and bilateral disease in 5%, total thyroidectomy is commonly the preferred treatment option [107]. And due to the high risk of occult central compartment lymph node metastases, total thyroidectomy with prophylactic bilateral central neck lymph node dissection (CND) is recommended as the treatment for node-negative MTC in adults.

MTC diagnosed following lobectomy

As previously reported, RET germline mutation analysis should be performed in all cases of MTC at histology [87]. Thus, for patients proven with MTC after thyroid lobectomy, further examinations must be undertaken to identify hereditary MTC including RET proto-gene mutational analysis. And total thyroidectomy must be performed to prevent contralateral MTC for diagnosed cases [113, 114]. Examination should comprise basal serum Ctn levels, neck US, identifying RET mutations and a detailed family history [114].

MTC with diameters < 1 cm are defined as microMTC [115], and there is a controversy about the necessity of implementing aggressive therapy in these patients. Nevertheless, previous evidence has shown that, even in patients with < 5 mm microMTC, there is a 23% risk of lymph node metastasis [116]. Hence, patients with microMTC are recommended to receive thyroidectomy with CND in most guidelines [115].

There is no consensus on the role of prophylactic lateral neck dissections (LND) based on current published guidelines [5, 116, 117]. Performance of prophylactic ipsilateral or bilateral neck dissections (without clinical or radiologic evidence of metastatic disease) in patients with high serum levels of tumor markers is supported by observational data. The studies showed that the risks

of ipsilateral central and lateral and contralateral central and lateral lymph node metastases increase incrementally with increasing serum Ctn and CEA levels among a cohort of patients with both sporadic and familial MTC [118]. In addition, because increasing primary tumor size is associated with an increased risk of lymph node metastases [119], there should be some consideration for ipsilateral neck dissection in patients with larger primary tumors. However, the risks of LND, including chyle leak and spinal accessory nerve injury, should be carefully weighed when considering an individual patient's risk of occult metastases and incorporated into shared decision-making with individual patients when making decisions about prophylactic lateral neck dissection [120]. Besides, there is also an alternative approach using the postoperative serum Ctn and CEA levels to stage the lateral neck compartment after initial total thyroidectomy and bilateral CND.

For patients with locally-advanced or metastatic MTC, more extensive surgery is not related to a higher cure rate or survival benefit and should be considered primarily for local symptom control [5, 10]. Therefore, with a palliative intent, total thyroidectomy with resection of the involved lymph node compartments is recommended for most patients. During surgery, for the primary tumor and lymph node dissection in central and lateral neck compartments, a less aggressive function-preserving approach is preferred to preserve speech, swallowing, parathyroid function, and mobility of the shoulder [5]. The approach should be individualized, taking the patient's wishes, other comorbidities, and life expectancy into account.

Prophylactic thyroidectomy in hereditary MTC

Because the penetrance for MTC is nearly 100% in patients with germline RET mutations, prophylactic total thyroidectomy is indicated to prevent or lead to a definitive cure of MTC by intervening before the development of a primary tumor or lymph node metastases [68, 84]. For those with germline RET mutations without clinical evidence of MTC, prophylactic thyroidectomy is recommended to reduce MTC-related long-term morbidity and mortality [5]. Current ATA Guidelines suggest a classification to define categories of RET mutations connected with stepwise increasing aggressiveness (from HST, H, to MOD) [5], as showed in Fig. 3.Due to the highly aggressive nature of MTC in patients in the HST group, total thyroidectomy with CND in the first year of life is recommended, preferably in the first month after birth [5]. For those with MEN 2A syndrome in the ATA-H category, close monitoring, including periodical biochemical screening for urine catecholamine and epinephrine, as well as annual physical examination, cervical US and measurement of serum calcium, Ctn and parathyroid hormone levels from 3 years of age is recommended [5], and total thyroidectomy and CND by 5 years of age is also suggested [5]. In case of a subject with clinically and genetically confirmed MEN2B, as in all other cases of RET positive germline mutation, the genetic screening for RET germline mutation should be extended to all first-degree relatives. Although very rare in clinical practice, since MEN 2B is mostly a de novo syndrome, total thyroidectomy (either prophylactic or therapeutic) with central compartment and oriented lateral cervical compartment lymph node dissection should be performed if some relatives had the same RET germline mutation. It is optimal to delay prophylactic thyroidectomy in patients with mutations known to lead to MTC at older ages, because of the increased risks and potential adverse effects (AEs) of hypothyroidism (due to insufficient thyroid hormone replacement during key times for growth and development), hypoparathyroidism, and recurrent laryngeal nerve injury in children [5].

Since early surgery with complete resection of tumor largely determines the likelihood of being cure for MTC, the widespread use of RET genetic screening has dramatically changed the prognostic of gene carriers in hereditary MTC [7].

Follow-up of patients with MTC after surgery

According to ATA guidelines [5], clinicians should consider TNM classification, the number of lymph node metastases, and postoperative serum Ctn levels in predicting outcome and planning long-term follow-up of patients treated by thyroidectomy for MTC. Serum levels of Ctn and CEA, should be measured 3 months postoperatively, and if undetectable or within the normal range, they should be measured every six months for 1 year, and then yearly thereafter. Patients with elevated postoperative serum Ctn levels less than 150 pg/mL should have a physical examination and US of the neck. If these studies are negative the patients should be followed with physical examinations, measurement of serum levels of Ctn and CEA, and US every 6 months. If the postoperative serum Ctn level exceeds 150 pg/mL patients should be evaluated by imaging procedures, including: neck US, chest CT, contrast-enhanced MRI or three-phase contrast-enhanced CT of the liver, and bone scintigraphy and MRI of the pelvis and axial skeleton.

During follow-up, patients that are cured after surgery (undetectable Ctn, CEA values, and negative imaging) have an almost negligible possibility to have recurrence [121]. Conversely, in patients with detectable calcitonin but without structural disease, the possibility of developing metastatic disease in a medium-long term follow up

is present [122, 123]. Moreover, metastatic cases should be strictly followed over time [124].

Adjuvant and neoadjuvant therapy for MTC

The adjuvant and neoadjuvant therapies including radiotherapy, systemic medical therapy, and other nonsurgical therapies should be considered to achieve local tumor control for patients with the presence of extensive regional or metastatic disease [5]. Local treatments like external beam radiotherapy (EBRT), have only limited and short-term benefits, systemic therapy like ^{131}I treatment is the main postoperative comprehensive treatment for DTC, but it is ineffective in MTC because MTC cells do not absorb iodine-131 [11]. The conventional cytotoxic chemotherapy has shown poor results in advanced or metastatic MTC, with a response rate of approximately 20% [12, 13].

The goal of adjuvant radiation therapy in MTC is to prevent or provide local control of disease in patients at high risk of locoregional recurrence. There are data to support external beam radiation therapy (EBRT) to prevent recurrent local disease, but definitive data supporting a benefit to OS are lacking [125–128]. A recent systematic review and meta-analysis, including data from 27 nonrandomized studies, found that EBRT for MTC with lymph node metastases, microscopic residual disease, or extrathyroidal extension was associated with a 38% reduction in locoregional recurrence but had no association with OS, with data from multiple studies favoring doses of greater than 60 Gy [129]. However, the interpretation of these data is limited based on nonrandom treatment assignment, with patients having higher risk disease being more likely to receive radiation therapy. It was recently been suggested that postoperative adjuvant radiotherapy is an important means of increasing local tumor control for MTC patients who still have nodal involvement, extrathyroidal extension or residual microscopic or macroscopic tumors after surgery [129]. However, there is no evidence of benefit from adjuvant radiotherapy for patients undergoing R0 or R1 resection. Generally, EBRT has been reserved for selected patients with a high likelihood of tumor recurrence after thyroidectomy, and merits consideration in cases of advanced disease at high risk for locoregional recurrence [5].

Chemotherapy regimens for patients with MTC included therapy with dacarbazine in combination with other agents, including vincristine, cyclophosphamide, streptozotocin, and doxorubicin. As described above, MTC is not sensitive to radiotherapy and chemotherapy, and the efficacy of neoadjuvant radiotherapy and chemotherapy in locally advanced MTC has not yet been confirmed [130]. And with the development of targeted therapy, there have been no subsequent reports on the application of neoadjuvant radiotherapy and chemotherapy in MTC.

At present, there are also reports of locally advanced MTC treated with neoadjuvant therapy of RET inhibitors. For example, one patient has undergone radical resection following Selpercatinib neoadjuvant therapy, and there has been no recurrence on biochemical and imaging examinations [131]. A recent Chinese expert consensus recommended that patients with locally advanced unresectable MTC with RET mutations should consider neoadjuvant treatment with Selpercatinib or Pralsetinib (Evidence level: C; Recommendation level: B) [132]. What's more, there is an ongoing phase II clinical trial (NCT04759911) that designed to define the effect of Selpercatinib given before surgery in treating patients with RET-altered TC [133]. However, neoadjuvant therapy with Selpercatinib is not fully risk-free, one peculiar case has been reported of developing tumor lysis syndrome (TLS) secondary to Selpercatinib neoadjuvant therapy [134].Therefore, for the treatment of locally advanced MTC, it is necessary to evaluate and design individualized treatment for TC multi-disciplinary treatment (MDT) with rich clinical experience. After evaluation, if the patient is expected to achieve R0 or R1 resection, surgical treatment is preferred. When it is difficult to achieve R0 or R1 resection, or when it is difficult to preserve adjacent structures or organ functions due to the large surgical scope, neoadjuvant therapy may be considered.

There is currently no evidence-based medical data regarding the need for targeted or immune maintenance therapy for patients undergoing surgical treatment after neoadjuvant therapy. In cases of RET-mutated-MTC treated with Selpercatinib neoadjuvant therapy, due to the patients' concurrent lung, bone, and liver metastases, Selpercatinib maintenance therapy was continued after surgical removal of local lesions [131]. However, the indications and duration of maintenance therapy are currently unclear, and more clinical studies are needed to confirm the survival benefits. In MTC patients, it is recommended to monitor the dynamic changes of biochemical indicators like serum Ctn and carcinoembryonic antigen as auxiliary means for efficacy evaluation and selection of adjuvant therapy.

Targeted therapies for patients with advanced and recurrent MTC

It is worth noting that 15–20% of MTC patients exhibited distant metastasis at the initial diagnosis, and a frightening 10-year survival rate of 10–40% from the time of first metastasis was reported in previous retrospective studies [4, 135].In the past, cytotoxic chemotherapy has shown poor results in patients with persistent or

recurrent MTC, only a fraction of patients responded to it, and durable control of disease was uncommon [12, 136]. While with the deepening understanding of the molecular mechanism of MTC and intracellular signaling pathway that involved in MTC pathogenesis, targeted treatments have gradually developed, which represented by RET-targeted TKIs, have demonstrated considerable promise in treatment for advanced and recurrent MTC and have positioned targeted therapy as the current standard of treatment [12, 14]. Current TKIs for RET include multi-tyrosine kinase inhibitors (MKIs) and RET-selective TKIs, the former incompletely inhibit multiple kinases including RET and often impair multiple signaling pathways, while the latter is highly selective for RET [16, 17].

Multi-kinase Inhibitors

MKI, i.e., nonselective RET inhibitors, were the first targeted systemic therapies approved by the US-FDA for the treatment of patients with progressive or symptomatic, advanced, or metastatic MTC. During past years, several small-molecule MKI compounds have been tested in clinical trials for the treatment of MTC, but only Vandetanib and Cabozantinib were approved, in 2011 and 2012, respectively. The small molecule Vandetanib targets the RET tyrosine-kinase receptor (TKR), in addition to other TKRs including vascular endothelial growth factor (VEGFR)2, VEGFR3, and EGFR, which contribute to tumor angiogenesis, cell proliferation, and cell migration and are commonly overexpressed in advanced MTC [137, 138]. Cabozantinib, another MKI, targets RET, VEGFR2, and hepatocyte growth factor receptor (MET) TKRs.

In a multicenter phase III randomized, placebo-controlled trial (ZETA) involving 331 patients with advanced MTC, Vandetanib showed a statistically significant improvement in median progression-free survival (PFS) of 30.5 months for patients treated with Vandetanib compared to 19.3 months for placebo [19]. It is worth noting that the approval did not require the presence of a RET activating mutation. In 298 patients with sporadic MTC enrolled in this study, 155 patients were with a RET mutation, 8 patients were without RET mutation and RET mutation status was unknown in 135 patients. According to the post-hoc analysis, EMA allowed the use of Vandetanib in case of somatic/germline positive RET mutation in Europe. Therefore, having the data about germline/somatic RET mutation become fundamental, not only for prescribing highly selective RET inhibitors but also for prescribing Vandetanib. Germline RET mutation is tested at diagnosis, and somatic RET testing can be performed with the use of an algorithm to optimize the cost/benefit ratio [121].

In phase-III randomized, placebo-controlled EXAM trial for Cabozantinib involving 330 patients with unresectable, locally advanced, or metastatic MTC, the estimated median PFS was 4.0 months for the placebo versus 11.2 months for Cabozantinib [18]. In final analysis of the EXAM results after a long-term follow-up, Cabozantinib did not lead to statistically significant improvement in OS when compared with the placebo (median OS 26.6 vs 21.1 months, HR = 0.85), but subgroup analyses suggested an increased benefit in patients with a somatic RET M918T mutation(OS: 44.3 months for Cabozantinib compared to 18.9 months for placebo) [139].

Sorafenib, Lenvatinib, Anlotinib and Sunitinib are other MKIs that have been studied in MTC with less impressive results and notable toxicities, but they may have possible applications as salvage therapy for patients with progressive disease and resistance to other TKIs [140–145].

Despite the ability in slowing or stabilizing advanced MTC, the use of MKIs in clinical practice has been limited by the intolerable adverse reactions, which frequently contribute to dose reductions (ranging from 35 to 79%) and drug discontinuation in clinical trials [146, 147].

The most common side effects seen with Vandetanib include nausea, fatigue, and rash, in addition to hypertension and QT prolongation. In patients who received Cabozantinib, which inhibits MET and VEGFR2 in addition to RET often experienced diarrhea, palmar-plantar erythrodysesthesia, decreased weight and appetite, nausea, and fatigue. In addition, the utility of these systemic therapies is time-limited due to eventual tumor resistance, and neither Vandetanib or Cabozantenib have been shown to lead to complete responses and improve OS in patients with MTC [148]. Furthermore, off-target toxicity was observed related to more potent inhibition of non-RET kinases, especially VEGFR2. All this suggests a need for more effective and more selective RET-targeting therapies.

RET selective Inhibitors

Recently, selective RET inhibitors have been developed to achieve higher potency anti-tumor effects with less toxicity. Two small-molecule inhibitors, named Selpercatinib and Pralsetinib, are US-FDA-approved for the management of RET-mutated unresectable MTC. Both drugs have demonstrated more efficient and wide-spectrum inhibition of RET mutations (including RET V804L and V804M mutations), as well as fewer adverse reactions as compared to MKIs in clinical trials, which may be attributed to more specific RET-targeting activity and decreased activity against VEGFR2 [149].

The results of clinical trials of MKIs and RET-selective inhibitors were summarized in Table 1. In a phase I/II open-label LIBRETTO 001 trial of Selpercatinib in patients with progressive RET-mutant MTC with and without prior treatment with MKIs, an objective response rate (ORR) was observed in 69% of patients with prior Vandetanib or Cabozantinib treatment (with 86% of responses ongoing at 1 year) and 73% of patients who had not received prior TKI therapy (with 91% of responses ongoing at 1 year), which is called as TKI-naïve patients [21]. Of note, Selpercatinib was well tolerated with grade 3 AEs including hypertension (21%) and diarrhea (6%), only 12 of 531 (2%) patients in this trial discontinued therapy due to drug-related AEs. In a phase III multicenter, open-label, randomized LIBRETTO-531 trial (NCT04211337) comparing Selpercatinib as first-line therapy with Cabozantinib or Vandetanib (control group) in patients with in advanced RET-mutant MTC, Selpercatinib showed a significantly better result than MKI both in improving efficacy and reducing toxicities [150, 151]. At a median follow-up of 12 months, median PFS was not reached in the Selpercatinib group and was 16.8 months in MKI group ($P < 0.001$). PFS rate at 12 months was 86.8% vs. 65.7% in the Selpercatinib group and in MKI group, respectively. Median treatment failure–free survival (FFS) was not reached in the Selpercatinib group and was 13.9 months in the control group ($P < 0.001$). Treatment failure–free survival at 12 months was 86.2% for Selpercatinib versus 62.1% for MKI. The overall response was 69.4% for Selpercatinib versus 38.8% for MKI. AEs led to a dose reduction in 38.9% of the patients with Selpercatinib, as compared with 77.3% with MKI, and to treatment discontinuation in 4.7% and 26.8%, respectively.

And the newly released expert consensus in China especially emphasizes that selective RET inhibitors such as Selpercatinib are strongly recommended for treatment of advanced NSCLC patients with RET-fusion [152]. A recent case report indicated that Selpercatinib is also effective and safe in RET-altered pediatric tumors with limited treatment options, including MTC and soft-tissue sarcomas [153].

In updated results of the phase I/II ARROW study released in 2020, Pralsetinib showed an ORR of 71% in TKI-naïve RET-mutated MTC, with a 60% objective response in patients who had previously received MKI therapy [154]. Less than 3% of patients discontinued Pralsetinib treatment due to treatment-related AEs, which included hypertension, fatigue, and diarrhea [149]. Based on these data showing remarkable efficacy and better-tolerated side effect profiles, Selpercatinib or Pralsetinib is recommended for patients with symptomatic or progressive metastatic MTC. In addition, according to results of ARROW trial, Pralsetinib has shown an excellent efficacy in RET-fusion NSCLC [155]. However, Pralsetinib has recently been declared no longer available for advanced or metastatic RET-positive MTC in the United States, as the manufacturers involved in its development have chosen to withdraw the indication [156]. The decision was made not due to any new safety or efficacy data, while because the phase III AcceleRET-MTC trial (NCT04760288) needed to convert the agent's accelerated approval into a full approval is no longer feasible.

There are other promising selective RET inhibitors currently under investigation in the management of MTC, including BOS172738 [157] and TPX-0046 [158], which may lead to additional treatment options and second-line therapies targeting mutations that lead to resistance to other TKIs.

Overlapping areas of concern for patients taking Selpercatinib or Pralsetinib are hepatotoxicity, hypertension, and hematological concerns, since either drug can inhibit the vascular endothelial growth factor (VEGF) pathway. In particular, patients on Selpercatinib have been additionally monitored to minimize the risk of QTc interval prolongation by performing electrocardiograms (ECGs) and correction of abnormal electrolytes at baseline; Before initiation of Selpercatinib, a QTcF interval of ≤ 470 ms is recommended [159]. Any present hypokalemia, hypomagnesemia, and hypocalcemia should all be corrected prior to initiating Selpercatinib [160]. Periodic testing of serum electrolytes should also be performed to ensure continuous monitoring of patients taking Selpercatinib [160]. Additionally, patients on Selpercatinib should be aware of the potential for hypersensitivity reaction, which is not a concern for those on Pralsetinib [160, 161]. A Pralsetinib-specific monitoring parameter is used to routinely assess patients for signs of interstitial lung disease (ILD) or pneumonitis at each follow-up [162, 163].The toxicity of these RET-targeted TKIs was summarized in Table 2. Besides of the common AEs reported by these above trials, several peculiar AEs of Selpercatinib have also been reported in literatures, including gastrointestinal toxicities (gastric and small-bowel edema, mucosal edema), lung toxicities (Langerhans cell histiocytosis and obliterative bronchiolitis), chylous effusions (chylothorax and chylous ascites) and erectile dysfunction [164–169].

Genetic testing of MTC and other novel therapeutic methods of MTC

It is recommended in the National Comprehensive Cancer Network (NCCN) guidelines that all patients diagnosed with MTC receive germline RET screening to determine if MTC is hereditary or sporadic, and all MTC patients who are RET germline unknown or

Table 1 Efficacy data of clinical trials evaluating Vandetanib, Cabozantinib, Selpercatinib and Pralsetinib treatments in MTC patients

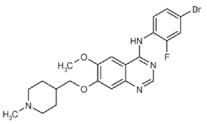
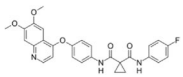
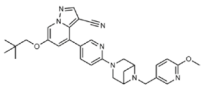
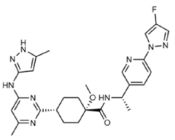
	Multikinase Inhibitors, MKI		**RET selective inhibitors**	
	Vandetanib	**Cabozantinib**	**Selpercatinib**	**Pralsetinib**
Drug target	RET, EGFR, VEGFR-2	RET, VEGFR-2, MET	RET	RET
Dose	300 mg/d	140 mg/d	160 mg twice a day	300 mg/d
Approval for MTC	2012 (FDA) 2012 (EMA)	2012 (FDA) 2013 (EMA)	2020 (FDA) 2021 (EMA)	2020 (FDA) NA (EMA)
Structure				
Study	Vandetanib vs. Placebo (ZETA) (NCT00410761)	Cabozantinib vs. placebo (EXAM) (NCT00704730)	Selpercatinib (LIBRETTO-001) (NCT04280081)	Pralsetinib (ARROW) (NCT03037385)
Trial design	Multicenter, double-blind, randomized, placebo-controlled	Cabozantinib vs. placebo	Open-label	Open-Label
Clinical trial phase	III	III	I/II	I/II
Participants	231 Vandetanib vs. 100 placebo	219 Cabozantinib vs. 111 placebo	55 pretreated 88 untreated All RET-mutated	55 pretreated 29 untreated All RET-mutated
Year	2006/12–2007/11	2008/9–2011/2	2017/5–2019/6	2017/3–2020/5
Primary endpoint	PFS	PFS	ORR	ORR
Secondary endpoint	ORR, DCR, OS, biochemical Response, time to worsening pain	OS, ORR	DOR, PFS, safety	DOR, CBR, DCR, PFS, OS, safety
PFS (Months)	30.5 (Vandetanib) vs 19.3 (placebo); HR: 0.46 95%CI: 0.31–0.69; p < 0.001	11.2 (Cabozantinib) vs 4.0 (placebo); HR: 0.28; 95%CI: 0.19–0.40; p < 0.001	1-year PFS rate: a. RET-mutant MTC previously received MKIs: 82%; 95% CI, 69%-90% b. RET-mutant MTC without previously received MKIs: 92%; 95% CI: 82%-97% c. RET-fusion-positive TC: 64%; 95% CI: 37%- 82%	1-year PFS rate: a. RET-mutant MTC received only Pralsetinib: 71%; 95%CI: 0.48–0.89 b. RET-mutant MTC received Pralsetinib plus Cabozantinib, Vandetanib or both: 60%; 95%CI: 46%–73%
ORR	45% (Vandetanib) vs 13% (placebo) OR: 5.48; 95%CI: 2.99–10.79; *p* < 0 .001	32% (Cabozantinib) vs 25% (placebo)	a. RET-mutant MTC previously received MKIs: 69%; 95%CI, 55%-81% b. RET-mutant MTC patients without previously received MKIs: 73%; 95% CI: 62%-82% c. RET fusion–positive TC: 79%; 95% CI: 54%-94%	a. RET-mutant MTC previously received MKIs: 60%; 95% CI: 46%–73% b. Treatment-naïve RET-mutant MTC: 71%; 95% CI: 48%–89% c. RET fusion-positive TC: 89%; 95%CI: 52%–100%
DCR	87%(Vandetanib) vs 71% (placebo); OR: 2.64; 95%CI: 1.48- 4.69; p < 0 .001	/	/	a. RET-mutant MTC previously received MKIs: 93%; 95%CI: 82%–98% b. Treatment-naïve RET-mutant MTC: 100%; 95%CI: 84%–100% c. RET fusion-positive TC: 100%; 95%CI: 66%–100%
OS	HR, 0.89; 95% CI, 0.48–1.65	HR, 0.98; 95% CI, 0.63–1.52	/	d. RET-mutant MTC previously received MKIs: 60%; 95%CI: 46%–73% e. Treatment-naïve RET-mutant MTC: 71%; 95%CI: 48%–89% f. RET fusion-positive TC: 89%; 95%CI: 52%–100%

Table 1 (continued)

	Multikinase Inhibitors, MKI		RET selective inhibitors	
	Vandetanib	**Cabozantinib**	**Selpercatinib**	**Pralsetinib**
CBR	/	/	/	a. RET-mutant MTC previously received MKIs: 80%; 95%CI: 67–90 b. Treatment-naïve RET-mutant MTC: 100%; 95%CI: 84–100 c. RET fusion-positive TC: 89%; 95%CI: 52–100

Table 2 Side effects data of clinical trials evaluating Vandetanib, Cabozantinib, Selpercatinib and Pralsetinib treatments in MTC patients

	Multikinase Inhibitors, MKI		RET selective inhibitors	
	Vandetanib	**Cabozantinib**	**Selpercatinib**	**Pralsetinib**
Most common AEs	Diarrhea Rash Nausea Hypertension Headache	Diarrhea Palmar-plantar Erythrodysesthesia Decreased weight Decreased appetite	Dry mouth Diarrhea Hypertension Fatigue Oedema	Anaemia Musculoskeletal pain Constipation Increased AST level
Dose modifications because of (all) AEs	Dose Reduction (35%) Interruption NA Discontinuation (12%)	Dose Reduction (79%) Interruption (65%) Discontinuation (16%)	Dose Reduction (31%) Interruption (5%) Discontinuation (2%)	Dose Reduction NA Interruption NA Discontinuation (4%)
Most common grade 3 or higher AEs	Diarrhea (11%) Hypertension (9%) ECG QT prolonged (8%) Fatigue (6%) Decreased appetite (4%)	Diarrhea (15.9%) Palmarplantar Erythrodysesthesia (12.6%) Fatigue (9.3%)	Hypertension (21%) Increased ALT level (11%) Increased AST level (9%) Hyponatremia (8%) Diarrhea (6%)	Hypertension (17%) Neutropenia (13%) Lymphopenia (12%) Anaemia (10%)

MKI Multi-kinase inhibitors, *PFS* Progression-free survival, *OS* Overall survival, *ORR* Objective response rate, *DCR* Disease-control rate, *DOR* Duration of response, *CBR* Clinical benefit rate, *AST* Aspartate aminotransferase, *ALT* Alanine aminotransferase

negative should undergo somatic RET testing [15]. Especially for recurrent, unresectable, or advanced MTC, genetic testing, including somatic mutations of RET gene, is suggested to guide the selection of targeted therapy drugs, and one algorithm was reported to help detect somatic RET mutation, which is able to optimize cost/benefit ratio [121].

Although several TKIs have been approved by FDA and EMA for treating advanced MTC, there is still an important proportion of patients who fail to respond to TKIs or who cannot tolerate adverse reactions, besides, resistance has also emerged to these newer TKIs. Therefore, novel therapeutic methods are still needed. Next-generation medical therapies, such as immunotherapy and peptide receptor radionuclide therapy (PRRT) have shown promise, while prospective randomized controlled trials are needed to verify the efficacy and safety in the future [170].

Resistance mechanisms of RET-targeted therapy

As described above, several TKIs have been approved for treating advanced MTC and showed considerable effect, however, resistance has also emerged to these TKIs. In clinical practice, both intrinsic resistance and acquired resistance to RET-targeted TKIs have been observed, and the mechanisms have been largely unknown to date. The efficacy of MKIs, such as Cabozantinib and Vandetanib, is hindered by low ORR, this can be attributed, at least in part, to the inadequate inhibition of the oncogenic RET kinase. Due to the occurrence of off-target AEs, a reduction in dosage was required for a significant proportion (ranging from 35 to 79%) of MTC patients taking Vandetanib or Cabozantinib [147]. Hence, attaining appropriate drug concentrations for RET inhibition using these MKIs is difficult [147]. Although the advent of selective RET inhibitors, including Pralsetinib and Selpercatinib, have greatly improved outcomes of these MKI-resistant

patients, which showed a better efficacy, significantly improved ORR and a milder toxicity profile [21, 149, 171], over 30% of patients fail to achieve partial response (PR) to these drugs (3% of patients had responded to prior MKI but not to Selpercatinib, and 28% of patients failed to respond to either Selpercatinib or Pralsetinib). And there are patients who experiment rapid progression or tumor recurrence after an early response to those TKIs, which suggest the existence of primary and acquired resistance. These data show the complexity of the RET resistance landscape and the necessity of understanding the physiopathology of the different mechanisms involved to overcome them [170]. Figure 5 summarizes the mechanisms of primary and acquired resistance to RET inhibitors, which contain tumor microenvironment (TME), coexisting RET alterations and coexisting mutations of oncogenic drivers that enable bypass signaling.

Before analyzing the resistance mechanism of RET inhibitors, it is important to understand how RET inhibitors bind to RET kinase. These small-molecule TKIs act by partially or completely binding to the nucleotide-binding pocket of the RET kinase domain, thereby blocking kinase activity. And kinases can adopt an active or inactive conformation according to the spatial orientation of the activation loop. For example, if the aspartate-phenylalanine-glycine (DFG) motif at the N-terminal is in the activation loop, it is called "DFG-in", while if DFG motif is flipped-out, it is called "DFG-out". TKIs are classified into three types, including typeI, type II and type III, the mechanism of action differs between these three types. Inhibitors of typeI(i.e. Sunitinib) act by competing with ATP to bind to the ATP binding pocket, thus blocking the active conformation of the kinase. TypeII TKIs (i.e. Sorafenib) indirectly compete with ATP by occupying the hydrophobic pocket adjacent to ATP-binding site that is only available in the DFG-out conformation, thereby stabilizing the inactive kinase [172]. Type III TKIs (e.g. Vandetanib) work by covalently binding to cysteines at specific sites of the kinase (variably located) and prevent the activation of the kinase [173].Of note, MKIs and selective RET inhibitors bind differently to RET. MKIs occupy both the front and back clefts of the drug-binding pockets by passing through the gate, while Selpercatinib binds to the front cleft and wrap around it to reach the back cleft without passing through the gate [174].

Intrinsic resistance mechanisms

Coexisting RET alteration

Coexisting RET alteration has been observed as one of mechanisms of primary resistance to MKIs. The RET M918T mutation, which affects the C-lobe of the kinase, is the most frequent mutation in MTC. It has been observed that the half maximal inhibitory concentrations (IC50s) of Vandetanib, Cabozantinib and Lenvatinib for RET M918T kinase were severalfold of that for the wild-type RET kinase, suggesting that a higher dose may be needed in patients with RET mutations [175]. Other aberrations, such as intrinsic gatekeeper mutations at RET V804L/M or other alterations typically acting as acquired resistance mechanisms, have occasionally been described as intrinsic mechanisms of resistance [172, 176–178].

Bypass signaling

Clinically, co-occurrence of driver oncogenes can be observed in RET-altered tumors, including RAS mutation, EGFR mutation, and MET amplification [179, 180], and acquired mutations of these driver oncogenes have also been found in preclinical experiments [181]. RET-targeted TKIs may lose efficacy due to the co-occurrence of the above driver genes, which could bypass the requirements of RET proto-oncogene. While it has not been identified in MTC.

For MKIs, AKT2 amplification has been thought to have a role in de novo and acquired resistance to targeted therapies such as Vandetanib, it is due to that in vitro studies have demonstrated the role of AKT2 amplification in tumorigenesis, and the AKT gene family encodes serine/threonine kinases that phosphorylate downstream protein effectors such as mTOR, which constitutively activate RET. Thus, the addition of mTOR inhibitors like Everolimus may overcome this resistance, as confirmed by the results of a phase I trial (NCT01582191), which showed a higher ORR and longer PFS when adding Everolimus to Vandetanib in RET-driven tumors [177, 182–184].

For selective RET inhibitors, a previous study reported that patients with RET fusion-positive and MET amplification positive NSCLC responded to the combination of Selpercatinib and Crizotinib, which is the MET/ALK/ROS1-targeted TKI, while were resistant to Selpercatinib [185]. The acquisition of KHDRBS1-NTRK3 fusion (K8; N14) has been identified as a mechanism of resistance to Selpercatinib in patients with KIF5B-RET fusion (K15; R12) positive lung cancer. Some reports have shown that acquired tertiary MET resistance to Capmatinib (a MET-targeted inhibitor) and Selpercatinib, which is characterized by MET D1228N mutation and LSM8-MET fusion, has been observed in patients with KIF5B-RET-positive NSCLC who previously developed initial resistance to Selpercatinib by generating secondary MET amplification [186, 187].

Another analysis included 70 pairs of matched samples of pre-treatment and post-progression tumor biopsies and plasma cell-free DNA (cfDNA) specimens for correlative genomic analyses, all these samples were from

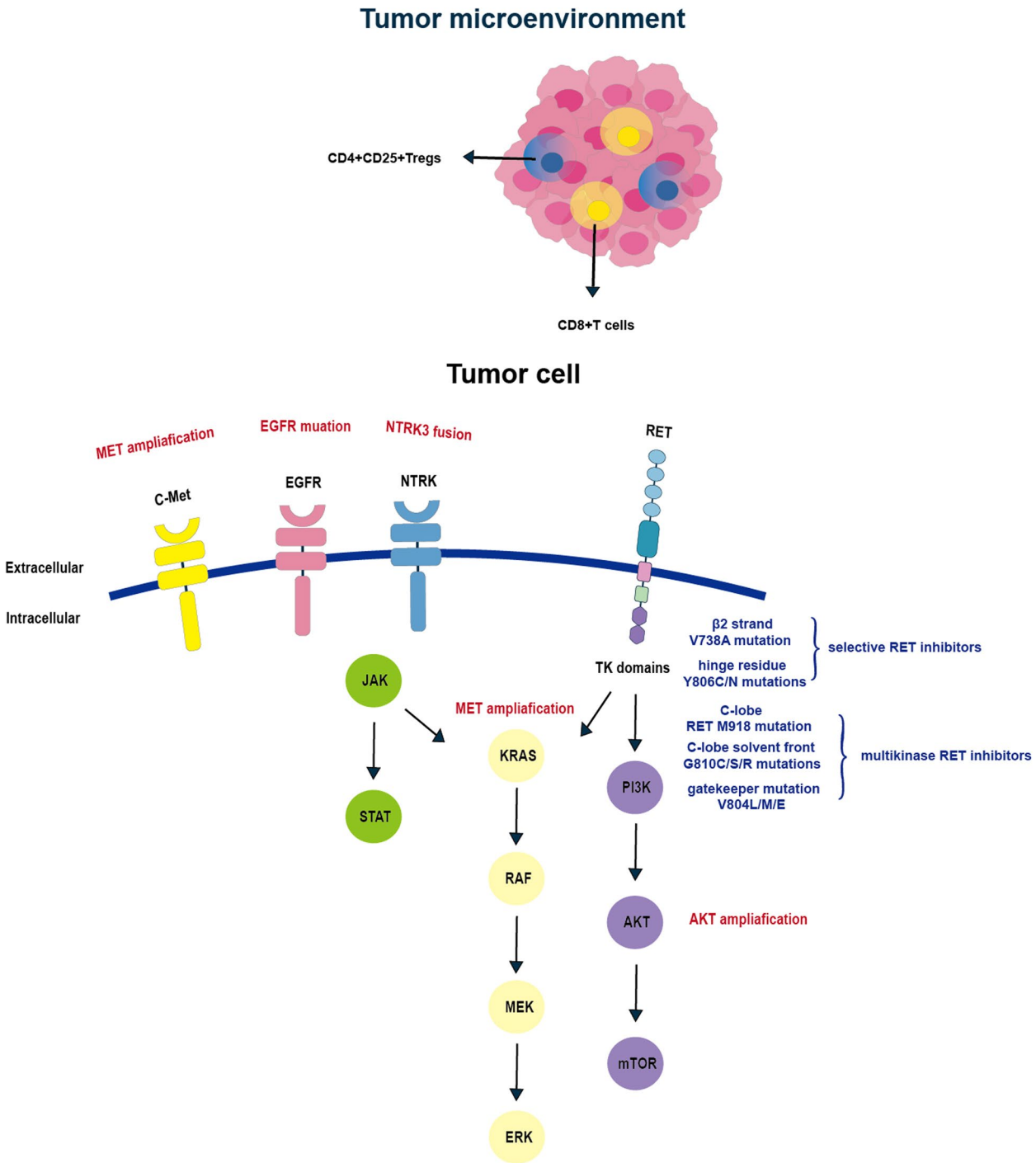


Fig. 5 Primary resistance and acquired resistance mechanisms to RET inhibitors in MTC. It shows the mechanisms of intrinsic resistance including tumor microenvironment (TME), co-existing RET alteration and bypass signaling; and mechanisms of acquired resistance include secondary RET alteration and bypass signaling

patients with confirmed RET activating fusion or mutation (including 19 MTC patients with RET mutation) and treated with Selpercatinib in the LIBRETTO-001 trial [188]. The authors sought to identify potential mechanisms of primary resistance to Selpercatinib, and found that prior exposure to MKIs did not alter subsequent outcomes with Selpercatinib. What's more, it was observed that 11 patients harbored PI3K pathway lesions

including 2 patients with PTEN loss-of-function mutations, and 9 patients with additional PTEN or PIK3CA mutations, while the patients harboring PI3K pathway alterations had a clinical benefit rate of 91% on Selpercatinib, indicating that these co-alterations of RET and PI3K did not preclude disease control. Moreover, it is revealed by pre-treatment plasma sequencing that 2 patients with primary resistance to Selpercatinib harbored KRAS mutations (G12D and G12V).

Tumor microenvironment and immune infiltration

The tumor microenvironment (TME) is composed of different cellular components, including tumor cells, immune cells, fibroblasts, extracellular matrix components, vessels and a variety of related cytokines and chemokines [189]. The TME has been recently found to be a key factor for the efficacy of targeted therapy. On the one hand, the TME may affect the response of the tumor to some TKIs, for example, the presence of $CD4^+$ and $CD8^+$ T cells in the TME may predict a better efficacy for EGFR-TKIs [190], TME stresses and autophagy may affect the resistance of EGFR-TKIs. On the other hand, TKIs may potentially affect TME, which in turn may have a significant impact on the choice of treatments later on, such as in clinical trials, even tumors without EGFR mutation responded well to EGFR-TKIs, strongly suggesting not only the tumor cells themselves but also potential tumor-specific immune responses might be the targets of EGFR-TKIs [191]. While the impact of TME on the effectiveness of RET inhibitors is undetermined and still to be established [192].

As is known, TME plays a central role in the relationship between tumor cells and the immune system, which is down-regulated by cancer cells so as to keep an immunosuppressive microenvironment, allowing tumor proliferation and protecting it from the immune system [193]. Due to the attraction of chemokines and other microenvironmental factors including hypoxia, both innate and adaptive immune cells are typically significantly infiltrated in the TME of MTC [194]. MTC express a low tumor mutation burden (TMB), which is associated with low response rates to immunotherapy. However, certain advanced MTC tumors develop a significant increase in T cell infiltration [195–197]. Thus, immunotherapy may play a role in advanced MTC and needs further investigation [197–200].

TME is locally infiltrated by different immune cell subsets, including TIL ($CD8^+$ T lymphocytes, $CD4^+$ T lymphocytes and B lymphocytes), tumor-associated macrophage (TAM), natural killer cell (NK cell), myeloid-derived suppressor cell (MDSC), etc., their different types and distributions constitute the complex immune characteristics of TME [201]. Among them, immunosuppressive regulatory T cells (Tregs), which are characterized by $CD4^+$ $CD25^+$ $FOXP3^+$are highly present in TME [202, 203]., and are related to a poor prognosis. Currently, there are several strategies involving Tregs to reduce initial resistance to RET inhibitors, like targeting FOXP3 or downregulating antigen presentation or MHCI by using RET inhibitors to revert this immunosuppressive activity [204], and CXC chemokine receptor 4 (CXCR4) inhibitors is one. CXCR4 is one of the main regulators of Tregs, and is a G-protein-coupled receptor activated through C-X-C motif chemokine ligand 12 (CXCL12). It plays a key part in TME by promoting tumor progression and recruiting immune cells and stromal cells. CXCR4 is highly expressed in thyroid cell lines expressing RET or MTC-associated RET mutants and is also expressed in endothelial cells and Tregs [198, 205, 206]. Hence, there are preclinical studies that demonstrate a downregulation of CXCR4 expression in RET-mutant cell lines treated with Vandetanib [205, 207], and strategies for combining CXCR4 inhibitors with classical therapies (including chemotherapy, immunotherapy and targeted therapies) are under development in both hematologic and solid tumors [206]. While to date, AMD3100 (Plerixafor or Mozobil) is the only CXCR4 inhibitor approved for patients with non-Hodgkin's lymphoma or multiple myeloma [208].

Acquired resistance mechanism

Currently, only a handful of reports have described patients who acquired resistance to prior RET MKIs, which may be attributed to inhibition of non-RET kinases, also known as non-kinase targets, by MKIs. Conversely, selective RET inhibitors including Pralsetinib and Selpercatinib decouple RET inhibition from the inhibition of non-RET kinases, and therefore might be more likely to explain the underlying mechanisms of acquired resistance.

Secondary RET alteration

Secondary RET alteration is one of the acquired resistance mechanisms for MKIs. Since MKIs present different conformational structures, a mutation in the RET kinase domain may confer resistance to a certain subset of TKIs, but not to others. Although infrequent, there may be more than one mutation in the RET kinase domain, resulting in a wide spectrum of resistances [175]. Usually, acquired mutations to MKIs occur at the gatekeeper position, which occurs at residue V804 and is the fundamental residue placed at the RET active site. It controls the access of drugs to a hydrophobic cavity that helps anchor MKIs to the active site. These gatekeeper mutations, originating by the substitution of valine for either leucine or methionine (V804L/M), lead to a disruption

of the hydrophobic pocket with a consequent resistance to drugs whose mechanisms of action involve this cavity. The appearance of gatekeeper mutations RET V804L/M/E typically leads to acquired resistance to MKIs such as Vandetanib, Cabozantininb or Lenvatinib. Ponatinib and sunitinib maintain partial activity against V804M. However, 75% of patients harboring a V804 mutation who were treated with Vandetanib showed clinical response, which can be explained by significant non-RET kinase activity [172, 176–178].

Acquired RET mutations occurred at other sites include RET S904F activation loop mutations, RET Y806C mutations at the hinge residue in the ATP binding pocket, and solvent front G810A/S mutations. The above three types of RER mutations confer resistance to Vandetanib, while G810A/S mutation is sensitive to Ponatinib and Lenvatinib, and S904F mutation is sensitive to Nintedanib. Another mutation, RET I788N, is resistant to Cabozantinib, Vandetanib and AD80, but sensitive to Ponatinib [58, 149, 176, 177, 209]. All these mutations have rarely been described as germline alterations [172, 176–178].

For selective RET inhibitors, secondary RET mutation also is one of the important acquired resistance mechanisms. One multicenter analysis collected post-treatment tissue and plasma samples obtained from 18 patients with RET fusion-positive NSCLC who treated with Selpercatinib, Pralsetinib, or a combination of both, with a median PFS of only 6.3 months in this group (CI: 3.6–10.8 months). And the results of NGS demonstrated that a subset of patients developed secondary mutations in RET, including two patients acquired mutations in the solvent-front of the kinase domain (G810C/S), and one patient got a mutation outside the kinase domain (G597V). In addition, three cases (15%) acquired MET amplification and one case developed KRAS amplification [210], suggesting that MET and KRAS amplifications may be more prevalent as resistance mechanisms within the population studied. Gatekeeper mutations appear to occur less frequently after selective RET inhibitors, as these mutations may minimally influence the binding of Selpercatinib and Pralsetinib. Although the sample size is small, the analysis suggests that the mechanisms of resistance to selective RET inhibitors may be more pleiotropic, at least at the genomic level [211].

These observations were largely confirmed by one analysis mentioned above [188]. Besides of primary resistance, the determinants of acquired resistance to Selpercatinib have also been established in this study. In 18 patients with initial response who subsequently recurred, 11 patients were identified with a genetically driven mechanism of resistance. Among them, 3 patients developed on-target resistance with the emergence of secondary RET mutations, including 2 patients with truncal KIF5B-RET fusions developed RET solvent front mutations (G810C or G810S), and 1 patient with RET M918T-mutant MTC in whom a gatekeeper V804M mutation was detected at baseline subsequently developed an acquired Y806C mutation in cis, which indicate that the serial genetic evolution of mutant RET can lead to Selpercatinib resistance.

Bypass signaling

The upregulation of other pathways implicated in cell proliferation is a recurrent escape mechanism across oncogenic drivers. Co-occurrence of driver oncogenes observed in RET-altered tumors can bypass the requirements of RET proto-oncogene and lead to not only primary but also acquired resistance to RET-targeted TKIs. The co-activation of the MAPK/ERK pathway has been associated with resistance to MKIs in RET-rearranged cells. In this situation, the addition of specific MEK inhibitor Trametinib to AD80, an MKI with potent activity against RET, is necessary to abrogate the resistant cells [149, 176, 212].In preclinical studies, the addition of EGFR inhibitor Gefitinib or Cetuximab to MKI therapy blocked the phosphorylation of AKT and ERK, resulting in downregulation of this pathway [192]. Other bypass mechanisms, such as the acquisition of MET D1228V in NSCLC or MET amplification in colorectal cancer, can be overcome by MET or EGFR inhibitors [177].

Modifications in the genes CCND1, CCND2, CDK4, CDK6, CDKN2A/B, or CDKN2C were observed in 21% of participants enrolled in the phase III EXAM study. These alterations have the potential to induce activation of cyclin D-dependent kinases CDK4 and CDK6. The role of CDK4/6 inhibitors in tumors harboring RET mutations is yet to be determined [89].

Yes-Associated Protein (YAP) regulates transcription factors, such as TEAD, β-catenin or STAT3, and acts as a transcription co-activator. It has been previously reported that deregulation of YAP contributes to resistance to RAF and ALK inhibitors. The role of YAP on Vandetanib resistance in MTC has been investigated by Wang. MTC cells were treated with Vandetanib for three months to generate a Vandetanib-resistant cell line. Overexpression of YAP was associated with Vandetanib resistance, and targeting these cells with a YAP inhibitor restored sensitivity to Vandetanib, providing a rationale for further studies [213]

As for selective RET inhibitors, another resistance mechanism that has been described in a RET fusion-positive lung cancer treated with Selpercatinib is an acquired NTRK3 fusion. The association of NTRK selective inhibitors Larotrectinib or Entrectinib could be a successful approach in this case [186]. KRAS amplification, as well

as variants of unknown significance in BRAF and ROS1, have also been reported [210, 214].

According to Rosen's study previously mentioned, besides of secondary RET mutation, emergent MAPK-activating alterations similar to those mediating primary resistance indicated that bypass signaling was another important mechanism of Selpercatinib resistance [188]. 7 in 18 patients acquired KRAS (G12A/R/V, G13D, A59del), NRAS (G13D, Q61R), or BRAF activating mutations or MET or FGFR1 amplifications. This diversity of MAPK-driven mechanisms of primary or acquired resistance was also evident within individual patients, indicating a complex pattern of polyclonal resistance to Selpercatinib therapy can emerge, consistent with a polyclonal resistance hypothesis.

Challenges, strategies, and auspicious orientations

Should surgical resection be performed in those patients who respond and have a persistent low-volume disease?

As a result of exceptional responses observed with targeted therapy (especially for Selpercatinib and Pralsetinib) in locally advanced or metastatic MTC, a clinical question that may be encountered is whether surgical resection should be performed in those patients who respond and have a persistent low-volume disease, or whether neoadjuvant therapy should be considered in those with borderline resectable MTC.[80,81] At present, there are also reports of cases of locally advanced MTC treated with Selpercatinib neoadjuvant therapy. The patient had undergone radical resection, and there was no recurrence on biochemical and imaging examinations [131]. Currently, there is a phase II trial studying the neoadjuvant use of Selpercatinib in locally advanced RET-mutated MTC. The research team is currently recruiting patients to determine if this will improve the rate of R0 resection, PFS, and OS (NCT04759911).

It is recommended to assess the efficacy after four cycles of induction therapy with small molecule tyrosine kinase inhibitors based on surgical treatment to determine if the patient is eligible for surgery. Considering the overall condition of the patient, the recommended prioritization criteria are as follows: (1) preservation of the carotid artery and internal carotid artery; (2) preservation of the esophagus; (3) preservation of the trachea and larynx; (4) preservation of the internal jugular vein. If these criteria are not met, a delay of 2 to 4 cycles of induction therapy may be considered. Additionally, a comprehensive assessment based on biochemical markers should be used to make a decision before the tumor shows signs of growth. With advances in targeted therapies, it is foreseeable that neoadjuvant targeted therapy will provide better treatment opportunities for more advanced TC cases, and we anticipate that the 10-year survival rate for thyroid cancer will continue to improve.

Can we achieve more durable and better response with combinatorial approaches utilizing selective RET inhibitors?

Now it has been established that concurrent activation of RET and other oncogenic drivers, or genomic alterations of other pathways are present, thus underlining the rationale of combination therapies.

Additional next steps to be addressed include developing combination targeted therapies for common co-alterations that occur with selective and multi-kinase inhibitors used for the treatment of advanced MTC [215]. In a lung cancer cell line with a CCDC6-RET fusion, epidermal growth factor (EGF) produced by endothelial cells activated EGFR and triggered resistance to MKIs by activating bypass survival signals through ERK and AKT. This suggests that, in patients where EGFR activation is a predominant mechanism of TKI-induced resistance, dual inhibition of EGFR and RET may be positive [192]. In fact, Rosen et al. isolated tumor cells from a patient with MET amplification and exposed them to Crizotinib and Selpercatinib, causing a cytotoxic effect. They later treated 4 patients with this combination after progression to Selpercatinib, achieving 3 partial responses and a maximum PFS of 10 months. These preliminary results should encourage the development of prospective trials in patients with RET-mutated tumors with MET amplification as mechanism of acquired resistance, preferably using type Ib selective MET inhibitors such as Capmatinib or Tepotinib [185].

As for acquired NTRK3 fusion, another resistance mechanism of Selpercatinib, the association of NTRK selective inhibitors Larotrectinib or Entrectinib could be a successful approach in this case [186]. KRAS amplification, as well as variants of unknown significance in BRAF and ROS1, have also been reported [210, 214].

Activation of FGFR signaling is also found to be a mechanism of adaptive resistance to RET inhibitors that activates ERK signaling. In cellular and animal models of CCDC6-RET-rearranged TC, combined inhibition of FGFR and RET prevented the development of adaptive resistance to RET inhibitors, reduced cell viability, and decreased tumor growth [216].

Development of next generation selective RET inhibitors

G810 is the C-lobe residue at the solvent front or "floor" region of the ATP binding site, and substitutions of glycine with cysteine, serine or arginine result in solvent front RET G810C/S/R mutations, which constitute the main mechanism of resistance to selective RET inhibitors. The IC50 fold changes of these mutants are higher

for Selpercatinib, which is suggested to have a greater impact compared to Pralsetinib. Apart from the C-lobe residue, the mutations may arise in other locations such as the hinge region (RET Y806C/N) and the β2 strand (RET V738A). They all confer resistance to Pralsetinib and Selpercatinib, which may be overcome by next generation selective RET inhibitors which are currently under development.

Next-generation selective RET inhibitors (includingTPX-0046, BOS172738, TAS0953/HM06, LOX-18228, LOX-19260 and APS03118) are under development, with a wider spectrum of targets to overcome the above-mentioned solvent front mutations [149, 177]. Nonetheless, the potential relevance of gatekeeper mutations as mediators of resistance to selective RET inhibitors suggests that simultaneous inhibition of "solvent front" and gatekeeper mutations may be the optimal approach. Furthermore, detection of these resistance mutations through ctDNA monitoring before clinical or radiographic progression may enable early identification of patients likely to develop refractory disease [177, 197, 217]. TPX-0046 is a selective next-generation RET/SRC inhibitor with proven efficacy against solvent-front mutations in BaF3/KR cells with a mean IC50 of 17 nM (while Pralsetinib and Selpercatinib exhibit IC50s > 500 nM) [158]. While the clinical trial of TPX-0046 for RET inhibitor-resistant and naive RET-driven cancers (NCT04161391) is terminated due to adverse change in the risk/benefit. BOS172738 [NCT03780517], which also presents a high selectivity for VEGFR2, has been studied in a phase I clinical trial showing an adequate safety profile and an ORR of 33% [218]. LOX-18228 has been assessed in cell lines with M918T RET mutation or KIF5B-RET fusion along with G810S or V804M resistance mutations. LOX-18228 has demonstrated activity against several types of cell lines, with promising results in a CCDC6-RET G810S and a CCDC6-RET V804M mutated patient-derived xenograft (PDX) model, reaching complete regression of the tumor [219]. APS03118 is a novel highly selective next-generation RET inhibitor that possesses potent in vitro and in vivo activity against a diverse range of RET alterations, including solvent front mutations (SFMs)-mediated resistance. APS03118 has received IND approval and Fast Track Designation from FDA, and a first-in-human phaseItrial for patients with RET-driven solid tumors with activating RET alterations [220].

Other Systemic Therapy

Peptide receptor radionuclide therapy (PRRT) targeting the somatostatin receptor

Peptide receptor radionuclide therapy (PRRT) targeting the somatostatin receptor (SSTR) was developed for the management of progressive gastroenteropancreatic and lung neuroendocrine tumors. Lutetium-177 DOTATATE was approved for this indication in 2018. Targeted radiotherapy has also been investigated for the treatment of advanced and progressive MTC, with a focus on SSTR and the cholecystokinin 2 receptor (CCK2R), two receptors commonly expressed on MTC cells in vitro and in vivo [221]. A systematic review of PRRT therapy in MTC reported on the results of 186 patients treated with PRRT targeting the SSTR with lutetium-177 and yttrium-90 [221]. With heterogeneous evaluation and reporting of results within studies, they described radiographic responses, with 44 out of 117 (37.6%) of patients showing progressive disease, 64 (54.7%) having stable disease, and 6 (5.1%) patients showing a partial response. AEs of therapy requiring discontinuation of PRRT occurred in 2 out of 154 (1.3%) patients with available data due to kidney toxicity. A meta-analysis of PRRT published in 2020 involving four studies with 98 MTC patients with any uptake on SSTR scintigraphy or PET/CT reported an objective response rate of 8.5%, a disease control rate of 54%, and a serious AE rate of 2.8% [222]. Based on these early data and experience, PRRT is a potential treatment option that can be considered under investigational protocols for patients with advanced, progressive MTC and uptake on SSTR-based imaging studies.

Immunotherapy

Immune-based therapies are currently being investigated as additional treatment options for patients with advanced MTC. A recent study looking at tissue from 46 MTC patients found that 49% and 90% of primary and metastatic tumors had organized immune infiltration, respectively, and a subset of patients had low-level PD-L1 expression. These results suggest that MTC may be an immunologically active tumor with the potential to be treated with immune checkpoint inhibitors (ICIs) or T-cell therapies targeting tumor-associated proteins [195, 204]. Several small studies have shown partial responses in a subset of patients who received tumor vaccines developed to stimulate dendritic cells (DCs) to present tumor-associated antigens (TAAs), like CEA, Ctn, and tumor lysate, resulting in cytotoxic T cells targeting MTC cells [223–225]. Although there are clinical trials that have included or are enrolling patients with MTC for investigational protocols involving anti-PD1 and anti-CTLA4 therapies and tumor vaccines [204], there are currently no clinical trial data to guide the use of immunotherapy in MTC patients. It is known that the use of MKIs targeting VEGFR can reverse the immune escape. Therefore, the combination of MKI or RET-selective inhibitors with ICIs is currently being investigated in aggressive MTC, aiming at limiting tumor growth while maximizing the endogenous antitumor immune response

[204]. And there are also several ongoing trials of immunotherapy in NSCLC patients with RET alterations, including a Chinese trial currently recruiting patients with the goal of comparing chemotherapy to chemoimmunotherapy (NCT04322591), and the LIBRETTO-431 trial (NCT04194944), which was designed to compare responses of Selpercatinib to two standard-of-care arms, one consisting of platinum and pemetrexed chemotherapy, the other chemotherapy and ICI pembrolizumab. In addition, the T-cell induced response caused by RET inhibitors opens the possibility of combination approaches with adoptive cell therapy [204]. Adoptive T cell immunotherapy used chimeric antigen receptor (CAR)-modified T cells (CAR-Ts), and one research found that GFRα4-specific CAR-Ts trigger antigen-dependent cytotoxicity and cytokine production in vitro, and they are able to eliminate tumors derived from the MTC TT cell line in an immunodeficient mouse xenograft model of MTC, which demonstrated the feasibility of targeting GFRα4 by CAR-T and support this antigen as a promising target for adoptive T cell immunotherapy and other antibody-based therapies for MTC [226].

Conclusions

With in-depth research into the molecular mechanisms of MTC, the recent development of more selective RET inhibitors has opened a new era in the management of these infrequent tumors. Results of various clinical trials indicate the promise that treatment can improve gradually with the passage of time. For patients with locally advanced disease that is difficult to surgically manage, or has distant metastasis, targeted therapies, immunotherapies, and neoadjuvant treatments may offer new options. Although significant therapeutic effects have been achieved during treatment with RET inhibitors, adverse events and drug resistance still cannot be ignored. There is an urgent need to develop a new generation of therapies and new combination therapy strategies to overcome resistance to RET-targeted TKIs. Furthermore, for several patients who do not carry RET mutations, the need to search for effective treatment strategies and develop new drugs is the key to leaving no one behind.

Acknowledgements
Not applicable.

Authors' contributions
Ying Zhang and Wei-Hui Zheng wrote the main manuscript text; Ying Zhang prepared Figs. 1, 2, 3 and 5, Wei-Hui Zheng prepared Fig. 4; Shi-Hong Zhou prepared Tables 1, and 2 and revised the manuscript; Jia-Lei Gu, Qing Yu, Yi-Zhou Zhu and Yu-Jie Yan searched and organized references; Jin Biao Shang design the work; Zhi Zhu substantively revised the draft; All authors reviewed the manuscript.

Funding
This work was been supported by Medical and Health Science and Technology Project of Zhejiang Province, China (2021KY110, 2024KY824).

Availability of data and materials
Not applicable.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Thyroid Surgery, Zhejiang Cancer Hospital, No. 1 East Banshan Road, Gongshu District, Hangzhou 310022, Zhejiang, China. [2]Department of Radiation Oncology, Shanghai Pulmonary Hospital, Tongji University Medical School Cancer Institute, Tongji University School of Medicine, Shanghai, China. [3]Key Laboratory of Head & Neck Cancer Translational Research of Zhejiang Province, Hangzhou, Zhejiang, China. [4]Department of Thoracic Surgery, The First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China. [5]Zhejiang Provincial Clinical Research Center for Malignant Tumor, Hangzhou, Zhejiang, China. [6]The Second Clinical Medical College, Zhejiang Chinese Medical University, Hangzhou, Zhejiang, China. [7]The MOE Key Laboratory of Spectrochemical Analysis & Instrumentation, The Key Laboratory of Chemical Biology of Fujian Province, State Key Laboratory of Physical Chemistry of Solid Surfaces, Department of Chemical Biology, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen 361005, China.

Received: 23 June 2024 Accepted: 18 September 2024
Published online: 28 September 2024

References

1. Siegel RL, Giaquinto AN, Jemal A. Cancer statistics, 2024. CA Cancer J Clin. 2024;74(1):12–49.
2. Barletta JA, Nose V, Sadow PM. Genomics and Epigenomics of medullary thyroid carcinoma: from sporadic disease to familial manifestations. Endocr Pathol. 2021;32(1):35–43.
3. Miranda-Filho A, Lortet-Tieulent J, Bray F, Cao B, Franceschi S, Vaccarella S, et al. Thyroid cancer incidence trends by histology in 25 countries: a population-based study. Lancet Diabetes Endocrinol. 2021;9(4):225–34.
4. Roman S, Lin R, Sosa JA. Prognosis of medullary thyroid carcinoma: demographic, clinical, and pathologic predictors of survival in 1252 cases. Cancer. 2006;107(9):2134–42.
5. Wells SA Jr, Asa SL, Dralle H, Elisei R, Evans DB, Gagel RF, et al. Revised American Thyroid Association guidelines for the management of medullary thyroid carcinoma. Thyroid. 2015;25(6):567–610.
6. Konstantinidis A, Stang M, Roman SA, Sosa JA. Surgical management of medullary thyroid carcinoma. Updates Surg. 2017;69(2):151–60.
7. Ceolin L, Duval M, Benini AF, Ferreira CV, Maia AL. Medullary thyroid carcinoma beyond surgery: advances, challenges, and perspectives. Endocr Relat Cancer. 2019;26(9):R499–518.
8. Bai Y, Niu D, Yao Q, Lin D, Kakudo K. Updates in the advances of sporadic medullary thyroid carcinoma: from the molecules to the clinic. Gland Surg. 2020;9(5):1847–56.
9. Mulligan LM. 65 YEARS OF THE DOUBLE HELIX: Exploiting insights on the RET receptor for personalized cancer medicine. Endocr Relat Cancer. 2018;25(8):T189–200.
10. Randle RW, Balentine CJ, Leverson GE, Havlena JA, Sippel RS, Schneider DF, et al. Trends in the presentation, treatment, and survival of patients with medullary thyroid cancer over the past 30 years. Surgery. 2017;161(1):137–46.

11. Jayarajah U, Wijekoon M, Seneviratne SA. Prophylactic prednisolone for the prevention of early and intermediate adverse effects of radioactive iodine therapy in patients with thyroid cancer: study protocol for a single-centre, phase II/III, randomized, double-blinded, placebo-controlled clinical trial. Trials. 2020;21(1):812.
12. Nocera M, Baudin E, Pellegriti G, Cailleux AF, Mechelany-Corone C, Schlumberger M. Treatment of advanced medullary thyroid cancer with an alternating combination of doxorubicin-streptozocin and 5 FU-dacarbazine. Groupe d'Etude des Tumeurs a Calcitonine (GETC). Br J Cancer. 2000;83(6):715–8.
13. Hadoux J, Schlumberger M. Chemotherapy and tyrosine-kinase inhibitors for medullary thyroid cancer. Best Pract Res Clin Endocrinol Metab. 2017;31(3):335–47.
14. Shimaoka K, Schoenfeld DA, DeWys WD, Creech RH, DeConti R. A randomized trial of doxorubicin versus doxorubicin plus cisplatin in patients with advanced thyroid carcinoma. Cancer. 1985;56(9):2155–60.
15. Haddad RI, Bischoff L, Ball D, Bernet V, Blomain E, Busaidy NL, et al. Thyroid carcinoma, Version 2.2022, NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw. 2022;20(8):925–51.
16. Filetti S, Durante C, Hartl D, Leboulleux S, Locati LD, Newbold K, et al. Thyroid cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-updagger. Ann Oncol. 2019;30(12):1856–83.
17. Filetti S, Durante C, Hartl DM, Leboulleux S, Locati LD, Newbold K, et al. ESMO Clinical Practice Guideline update on the use of systemic therapy in advanced thyroid cancer. Ann Oncol. 2022;33(7):674–84.
18. Elisei R, Schlumberger MJ, Muller SP, Schoffski P, Brose MS, Shah MH, et al. Cabozantinib in progressive medullary thyroid cancer. J Clin Oncol. 2013;31(29):3639–46.
19. Wells SA Jr, Robinson BG, Gagel RF, Dralle H, Fagin JA, Santoro M, et al. Vandetanib in patients with locally advanced or metastatic medullary thyroid cancer: a randomized, double-blind phase III trial. J Clin Oncol. 2012;30(2):134–41.
20. Sherman SI, Clary DO, Elisei R, Schlumberger MJ, Cohen EE, Schoffski P, et al. Correlative analyses of RET and RAS mutations in a phase 3 trial of cabozantinib in patients with progressive, metastatic medullary thyroid cancer. Cancer. 2016;122(24):3856–64.
21. Wirth LJ, Sherman E, Robinson B, Solomon B, Kang H, Lorch J, et al. Efficacy of Selpercatinib in RET-Altered Thyroid Cancers. N Engl J Med. 2020;383(9):825–35.
22. Zheng X, Ji Q, Sun Y, Ge M, Zhang B, Cheng Y, et al. Efficacy and safety of selpercatinib in Chinese patients with advanced RET-altered thyroid cancers: results from the phase II LIBRETTO-321 study. Ther Adv Med Oncol. 2022;14:17588359221119318.
23. US Food and Drug Administration. Retevmo (selpercatinib); 2022. www.accessdata.fda.gov/drugsatfdadocs/label/2020/213246s000lbl.pdf.
24. Zhou C, Solomon B, Loong HH, Park K, Pérol M, Arriola E, et al. First-Line Selpercatinib or Chemotherapy and Pembrolizumab in RET Fusion-Positive NSCLC. N Engl J Med. 2023;389(20):1839–50.
25. Subbiah V, Gainor JF, Rahal R, Brubaker JD, Kim JL, Maynard M, et al. Precision targeted therapy with BLU-667 for RET-driven cancers. Cancer Discov. 2018;8(7):836–49.
26. Subbiah V, Hu MI, Wirth LJ, Schuler M, Mansfield AS, Curigliano G, et al. Pralsetinib for patients with advanced or metastatic RET-altered thyroid cancer (ARROW): a multi-cohort, open-label, registrational, phase 1/2 study. Lancet Diabetes Endocrinol. 2021;9(8):491–501.
27. Us Food and Drug Administration. Gavreto (Pralsetinib). www.accessdata.fda.gov/drugsatfdadocs/label/2020/213721s000lbl.pdf.
28. Markham A. Pralsetinib: first approval. Drugs. 2020;80(17):1865–70.
29. Markham A. Selpercatinib: first approval. Drugs. 2020;80(11):1119–24.
30. Della Corte CM, Morgillo F. Rethinking treatment for RET-altered lung and thyroid cancers: selpercatinib approval by the EMA. ESMO Open. 2021;6(1):100041.
31. Ltd FH-LR. European Commission approves Roche's Gavreto (pralsetinib) for the treatment of adults with RET fusion-positive advanced non-small cell lung cancer. https://www.roche.com/media/releases/med-cor-2021-11-19.htm. November 19, 2021.
32. Fugazzola L. Medullary thyroid cancer - An update. Best Pract Res Clin Endocrinol Metab. 2023;37(1):101655.
33. Eng C. RET proto-oncogene in the development of human cancer. J Clin Oncol. 1999;17(1):380–93.
34. Wells SA Jr, Pacini F, Robinson BG, Santoro M. Multiple endocrine neoplasia type 2 and familial medullary thyroid carcinoma: an update. J Clin Endocrinol Metab. 2013;98(8):3149–64.
35. Li SY, Ding YQ, Si YL, Ye MJ, Xu CM, Qi XP. 5P Strategies for Management of Multiple Endocrine Neoplasia Type 2: a paradigm of precision medicine. Front Endocrinol (Lausanne). 2020;11:543246.
36. Hofstra RM, Landsvater RM, Ceccherini I, Stulp RP, Stelwagen T, Luo Y, et al. A mutation in the RET proto-oncogene associated with multiple endocrine neoplasia type 2B and sporadic medullary thyroid carcinoma. Nature. 1994;367(6461):375–6.
37. Ciampi R, Romei C, Ramone T, Prete A, Tacito A, Cappagli V, et al. Genetic landscape of somatic mutations in a large cohort of sporadic medullary thyroid carcinomas studied by next-generation targeted sequencing. iScience. 2019;20:324–36.
38. Jung CK, Agarwal S, Hang JF, Lim DJ, Bychkov A, Mete O. Update on C-Cell Neuroendocrine Neoplasm: prognostic and predictive histopathologic and molecular features of medullary thyroid carcinoma. Endocr Pathol. 2023;34(1):1–22.
39. Bugalho MJ, Frade JP, Santos JR, Limbert E, Sobrinho L. Molecular analysis of the RET proto-oncogene in patients with sporadic medullary thyroid carcinoma: a novel point mutation in the extracellular cysteine-rich domain. Eur J Endocrinol. 1997;136(4):423–6.
40. Jhiang SM, Fithian L, Weghorst CM, Clark OH, Falko JM, O'Dorisio TM, et al. RET mutation screening in MEN2 patients and discovery of a novel mutation in a sporadic medullary thyroid carcinoma. Thyroid. 1996;6(2):115–21.
41. Marsh DJ, Learoyd DL, Andrew SD, Krishnan L, Pojer R, Richardson AL, et al. Somatic mutations in the RET proto-oncogene in sporadic medullary thyroid carcinoma. Clin Endocrinol (Oxf). 1996;44(3):249–57.
42. Moura MM, Cavaco BM, Pinto AE, Domingues R, Santos JR, Cid MO, et al. Correlation of RET somatic mutations with clinicopathological features in sporadic medullary thyroid carcinomas. Br J Cancer. 2009;100(11):1777–83.
43. Romei C, Casella F, Tacito A, Bottici V, Valerio L, Viola D, et al. New insights in the molecular signature of advanced medullary thyroid cancer: evidence of a bad outcome of cases with double RET mutations. J Med Genet. 2016;53(11):729–34.
44. Takahashi M, Ritz J, Cooper GM. Activation of a novel human transforming gene, ret, by DNA rearrangement. Cell. 1985;42(2):581–8.
45. Arighi E, Borrello MG, Sariola H. RET tyrosine kinase signaling in development and cancer. Cytokine Growth Factor Rev. 2005;16(4–5):441–67.
46. Vodopivec DM, Hu MI. RET kinase inhibitors for RET-altered thyroid cancers. Ther Adv Med Oncol. 2022;14:17588359221101692.
47. Pachnis V, Mankoo B, Costantini F. Expression of the c-ret proto-oncogene during mouse embryogenesis. Development. 1993;119(4):1005–17.
48. Shakya R, Watanabe T, Costantini F. The role of GDNF/Ret signaling in ureteric bud cell fate and branching morphogenesis. Dev Cell. 2005;8(1):65–74.
49. Jain S, Naughton CK, Yang M, Strickland A, Vij K, Encinas M, et al. Mice expressing a dominant-negative Ret mutation phenocopy human Hirschsprung disease and delineate a direct role of Ret in spermatogenesis. Development. 2004;131(21):5503–13.
50. Fonseca-Pereira D, Arroz-Madeira S, Rodrigues-Campos M, Barbosa IA, Domingues RG, Bento T, et al. The neurotrophic factor receptor RET drives haematopoietic stem cell survival and function. Nature. 2014;514(7520):98–101.
51. Takaya K, Yoshimasa T, Arai H, Tamura N, Miyamoto Y, Itoh H, et al. Expression of the RET proto-oncogene in normal human tissues, pheochromocytomas, and other tumors of neural crest origin. J Mol Med (Berl). 1996;74(10):617–21.
52. Drilon A, Hu ZI, Lai GGY, Tan DSW. Targeting RET-driven cancers: lessons from evolving preclinical and clinical landscapes. Nat Rev Clin Oncol. 2018;15(3):151–67.
53. Takahashi M, Cooper GM. ret transforming gene encodes a fusion protein homologous to tyrosine kinases. Mol Cell Biol. 1987;7(4):1378–85.
54. Takahashi M, Buma Y, Iwamoto T, Inaguma Y, Ikeda H, Hiai H. Cloning and expression of the ret proto-oncogene encoding a tyrosine kinase with two potential transmembrane domains. Oncogene. 1988;3(5):571–8.

55. Takahashi M, Buma Y, Hiai H. Isolation of ret proto-oncogene cDNA with an amino-terminal signal sequence. Oncogene. 1989;4(6):805–6.
56. Iwamoto T, Taniguchi M, Asai N, Ohkusu K, Nakashima I, Takahashi M. cDNA cloning of mouse ret proto-oncogene and its sequence similarity to the cadherin superfamily. Oncogene. 1993;8(4):1087–91.
57. Anders J, Kjar S, Ibanez CF. Molecular modeling of the extracellular domain of the RET receptor tyrosine kinase reveals multiple cadherin-like domains and a calcium-binding site. J Biol Chem. 2001;276(38):35808–17.
58. Salvatore D, Santoro M, Schlumberger M. The importance of the RET gene in thyroid cancer and therapeutic implications. Nat Rev Endocrinol. 2021;17(5):296–306.
59. Baloh RH, Enomoto H, Johnson EM Jr, Milbrandt J. The GDNF family ligands and receptors - implications for neural development. Curr Opin Neurobiol. 2000;10(1):103–10.
60. Pasini B, Borrello MG, Greco A, Bongarzone I, Luo Y, Mondellini P, et al. Loss of function effect of RET mutations causing Hirschsprung disease. Nat Genet. 1995;10(1):35–40.
61. Amiel J, Sproat-Emison E, Garcia-Barcelo M, Lantieri F, Burzynski G, Borrego S, et al. Hirschsprung disease, associated syndromes and genetics: a review. J Med Genet. 2008;45(1):1–14.
62. Fusco A, Grieco M, Santoro M, Berlingieri MT, Pilotti S, Pierotti MA, et al. A new oncogene in human thyroid papillary carcinomas and their lymph-nodal metastases. Nature. 1987;328(6126):170–2.
63. Li AY, McCusker MG, Russo A, Scilla KA, Gittens A, Arensmeyer K, et al. RET fusions in solid tumors. Cancer Treat Rev. 2019;81:101911.
64. Romei C, Ciampi R, Elisei R. A comprehensive overview of the role of the RET proto-oncogene in thyroid carcinoma. Nat Rev Endocrinol. 2016;12(4):192–202.
65. Novello S, Califano R, Reinmuth N, Tamma A, Puri T. RET fusion-positive non-small cell lung cancer: the evolving treatment landscape. Oncologist. 2023;28(5):402–13.
66. Mulligan LM, Kwok JB, Healey CS, Elsdon MJ, Eng C, Gardner E, et al. Germ-line mutations of the RET proto-oncogene in multiple endocrine neoplasia type 2A. Nature. 1993;363(6428):458–60.
67. Santoro M, Carlomagno F, Romano A, Bottaro DP, Dathan NA, Grieco M, et al. Activation of RET as a dominant transforming gene by germline mutations of MEN2A and MEN2B. Science. 1995;267(5196):381–3.
68. Skinner MA, Moley JA, Dilley WG, Owzar K, Debenedetti MK, Wells SA Jr. Prophylactic thyroidectomy in multiple endocrine neoplasia type 2A. N Engl J Med. 2005;353(11):1105–13.
69. Frank-Raue K, Rondot S, Raue F. Molecular genetics and phenomics of RET mutations: Impact on prognosis of MTC. Mol Cell Endocrinol. 2010;322(1–2):2–7.
70. Plaza-Menacho I, Burzynski GM, de Groot JW, Eggen BJ, Hofstra RM. Current concepts in RET-related genetics, signaling and therapeutics. Trends Genet. 2006;22(11):627–36.
71. Mologni L. Development of RET kinase inhibitors for targeted cancer therapy. Curr Med Chem. 2011;18(2):162–75.
72. Goodman KM, Kjaer S, Beuron F, Knowles PP, Nawrotek A, Burns EM, et al. RET recognition of GDNF-GFRalpha1 ligand by a composite binding site promotes membrane-proximal self-association. Cell Rep. 2014;8(6):1894–904.
73. Marx SJ. Molecular genetics of multiple endocrine neoplasia types 1 and 2. Nat Rev Cancer. 2005;5(5):367–75.
74. Brauckhoff M, Gimm O, Weiss CL, Ukkat J, Sekulla C, Brauckhoff K, et al. Multiple endocrine neoplasia 2B syndrome due to codon 918 mutation: clinical manifestation and course in early and late onset disease. World J Surg. 2004;28(12):1305–11.
75. Elisei R, Matrone A, Valerio L, Molinaro E, Agate L, Bottici V, et al. Fifty years after the first description, MEN 2B syndrome diagnosis is still late: descriptions of two recent cases. J Clin Endocrinol Metab. 2019;104(7):2520–6.
76. Plaza-Menacho I, Barnouin K, Goodman K, Martinez-Torres RJ, Borg A, Murray-Rust J, et al. Oncogenic RET kinase domain mutations perturb the autophosphorylation trajectory by enhancing substrate presentation in trans. Mol Cell. 2014;53(5):738–51.
77. Kouvaraki MA, Shapiro SE, Perrier ND, Cote GJ, Gagel RF, Hoff AO, et al. RET proto-oncogene: a review and update of genotype-phenotype correlations in hereditary medullary thyroid cancer and associated endocrine tumors. Thyroid. 2005;15(6):531–44.
78. Maciel RMB, Maia AL. GLOBAL ENDOCRINOLOGY: Geographical variation in the profile of RET variants in patients with medullary thyroid cancer: a comprehensive review. Eur J Endocrinol. 2021;186(1):R15–30.
79. Machens A. Early malignant progression of hereditary medullary thyroid cancer. N Engl J Med. 2004;350(9):943.
80. Garces Visier C, Espinoza Vega M, Guillen Redondo P, Ollero Fresno JC, Souto Romero H, Luis Huertas A, et al. Prophylactic thyroidectomy in multiple endocrine neoplasia type 2A in children: a single centre experience. J Pediatr Endocrinol Metab. 2019;32(8):889–93.
81. Castinetti F, Qi XP, Walz MK, Maia AL, Sanso G, Peczkowska M, et al. Outcomes of adrenal-sparing surgery or total adrenalectomy in phaeochromocytoma associated with multiple endocrine neoplasia type 2: an international retrospective population-based study. Lancet Oncol. 2014;15(6):648–55.
82. Kebebew E, Ituarte PH, Siperstein AE, Duh QY, Clark OH. Medullary thyroid carcinoma: clinical characteristics, treatment, prognostic factors, and a comparison of staging systems. Cancer. 2000;88(5):1139–48.
83. Kebebew E, Tresler PA, Siperstein AE, Duh QY, Clark OH. Normal thyroid pathology in patients undergoing thyroidectomy for finding a RETgene germline mutation: a report of three cases and review of the literature. Thyroid. 1999;9(2):127–31.
84. Machens A, Elwerr M, Lorenz K, Weber F, Dralle H. Long-term outcome of prophylactic thyroidectomy in children carrying RET germline mutations. Br J Surg. 2018;105(2):e150–7.
85. Brauckhoff M, Machens A, Lorenz K, Bjoro T, Varhaug JE, Dralle H. Surgical curability of medullary thyroid cancer in multiple endocrine neoplasia 2B: a changing perspective. Ann Surg. 2014;259(4):800–6.
86. McDonnell JE, Gild ML, Clifton-Bligh RJ, Robinson BG. Multiple endocrine neoplasia: an update. Intern Med J. 2019;49(8):954–61.
87. Romei C, Cosci B, Renzini G, Bottici V, Molinaro E, Agate L, 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). 2011;74(2):241–7.
88. Elisei R, Romei C, Renzini G, Bottici V, Cosci B, Molinaro E, et al. The timing of total thyroidectomy in RET gene mutation carriers could be personalized and safely planned on the basis of serum calcitonin: 18 years experience at one single center. J Clin Endocrinol Metab. 2012;97(2):426–35.
89. Heilmann AM, Subbiah V, Wang K, Sun JX, Elvin JA, Chmielecki J, et al. Comprehensive Genomic Profiling of Clinically Advanced Medullary Thyroid Carcinoma. Oncology. 2016;90(6):339–46.
90. Wang S, Wang B, Xie C, Ye D. RET Proto-oncogene Gene Mutation Is Related to Cervical Lymph Node Metastasis in Medullary Thyroid Carcinoma. Endocr Pathol. 2019;30(4):297–304.
91. Elisei R, Cosci B, Romei C, Bottici V, Renzini G, Molinaro E, et al. Prognostic significance of somatic RET oncogene mutations in sporadic medullary thyroid cancer: a 10-year follow-up study. J Clin Endocrinol Metab. 2008;93(3):682–7.
92. Agrawal N, Jiao Y, Sausen M, Leary R, Bettegowda C, Roberts NJ, et al. Exomic sequencing of medullary thyroid cancer reveals dominant and mutually exclusive oncogenic mutations in RET and RAS. J Clin Endocrinol Metab. 2013;98(2):E364-369.
93. Nikiforova MN, Wald AI, Roy S, Durso MB, Nikiforov YE. Targeted next-generation sequencing panel (ThyroSeq) for detection of mutations in thyroid cancer. J Clin Endocrinol Metab. 2013;98(11):E1852-1860.
94. Moura MM, Cavaco BM, Pinto AE, Leite V. High prevalence of RAS mutations in RET-negative sporadic medullary thyroid carcinomas. J Clin Endocrinol Metab. 2011;96(5):E863-868.
95. Boichard A, Croux L, Al Ghuzlan A, Broutin S, Dupuy C, Leboulleux S, 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. 2012;97(10):E2031-2035.
96. Simbolo M, Mian C, Barollo S, Fassan M, Mafficini A, Neves D, et al. High-throughput mutation profiling improves diagnostic stratification of sporadic medullary thyroid carcinomas. Virchows Arch. 2014;465(1):73–8.
97. Shirali AS, Hu MI, Chiang YJ, Graham PH, Fisher SB, Sosa JA, et al. Next-generation sequencing in sporadic medullary thyroid cancer patients: mutation profile and disease aggressiveness. J Endocr Soc. 2024;8(6):bvae048.

98. Elisei R, Ciampi R, Matrone A, Prete A, Gambale C, Ramone T, et al. Somatic RET Indels in sporadic medullary thyroid cancer: prevalence and response to Selpercatinib. J Clin Endocrinol Metab. 2022;107(8):2195–202.
99. Ciampi R, Ramone T, Romei C, Casalini R, Matrone A, Prete A, et al. NF1 Gene inactivation acts as tumor driver in RET/RAS negative medullary thyroid carcinomas. Eur J Endocrinol. 2023;188(5):430–7.
100. Eng C, Mulligan LM, Smith DP, Healey CS, Frilling A, Raue F, et al. Low frequency of germline mutations in the RET proto-oncogene in patients with apparently sporadic medullary thyroid carcinoma. Clin Endocrinol (Oxf). 1995;43(1):123–7.
101. Elisei R, Tacito A, Ramone T, Ciampi R, Bottici V, Cappagli V, et al. Twenty-Five Years Experience on RET genetic screening on hereditary MTC: an update on the prevalence of germline RET Mutations. Genes (Basel). 2019;10(9):698.
102. Maxwell JE, Sherman SK, O'Dorisio TM, Howe JR. Medical management of metastatic medullary thyroid cancer. Cancer. 2014;120(21):3287–301.
103. Lorusso L, Cappagli V, Valerio L, Giani C, Viola D, Puleo L, et al. Thyroid cancers: from surgery to current and future systemic therapies through their molecular identities. Int J Mol Sci. 2021;22(6):3117.
104. Zhang D, Colombo C, Sun H, Kim HY, Pino A, De Leo S, et al. Unilateral surgery for medullary thyroid carcinoma: seeking for clinical practice guidelines. Front Endocrinol (Lausanne). 2022;13:875875.
105. Kouvaraki MA, Shapiro SE, Fornage BD, Edeiken-Monro BS, Sherman SI, Vassilopoulou-Sellin R, et al. Role of preoperative ultrasonography in the surgical management of patients with thyroid cancer. Surgery. 2003;134(6):946–54 discussion 954-945.
106. Matrone A, Gambale C, Biagini M, Prete A, Vitti P, Elisei R. Ultrasound features and risk stratification systems to identify medullary thyroid carcinoma. Eur J Endocrinol. 2021;185(2):193–200.
107. Essig GF Jr, Porter K, Schneider D, Arpaia D, Lindsey SC, Busonero G, et al. Multifocality in sporadic medullary thyroid carcinoma: an international multicenter study. Thyroid. 2016;26(11):1563–72.
108. Elisei R, Bottici V, Luchetti F, Di Coscio G, Romei C, Grasso L, et al. Impact of routine measurement of serum calcitonin on the diagnosis and outcome of medullary thyroid cancer: experience in 10,864 patients with nodular thyroid disorders. J Clin Endocrinol Metab. 2004;89(1):163–8.
109. Fugazzola L, Di Stefano M, Censi S, Repaci A, Colombo C, Grimaldi F, et al. Basal and stimulated calcitonin for the diagnosis of medullary thyroid cancer: updated thresholds and safety assessment. J Endocrinol Invest. 2021;44(3):587–97.
110. Trimboli P, Treglia G, Guidobaldi L, Romanelli F, Nigri G, Valabrega S, et al. Detection rate of FNA cytology in medullary thyroid carcinoma: a meta-analysis. Clin Endocrinol (Oxf). 2015;82(2):280–5.
111. Workman AD, Soylu S, Kamani D, Nourmahnad A, Kyriazidis N, Saade R, et al. Limitations of preoperative cytology for medullary thyroid cancer: Proposal for improved preoperative diagnosis for optimal initial medullary thyroid carcinoma specific surgery. Head Neck. 2021;43(3):920–7.
112. Censi S, Manso J, Mian C. Other markers of medullary thyroid cancer, not only calcitonin. Eur J Endocrinol. 2023;188(1):lvac009.
113. Green K, Hintze J, O'Neill JP. Surgical aspects and controversies in the management of medullary thyroid cancer. Ir J Med Sci. 2022;191(6):2461–6.
114. Mazeh H, Sippel RS. Surgical management of medullary thyroid cancer. Minerva Endocrinol. 2012;37(4):329–34.
115. Fussey JM, Bradley PJ, Smith JA. Controversies in the surgical management of sporadic medullary thyroid carcinoma. Curr Opin Otolaryngol Head Neck Surg. 2020;28(2):68–73.
116. Mitchell AL, Gandhi A, Scott-Coombes D, Perros P. Management of thyroid cancer: United Kingdom National Multidisciplinary Guidelines. J Laryngol Otol. 2016;130(S2):S150–60.
117. Patel KN, Yip L, Lubitz CC, Grubbs EG, Miller BS, Shen W, et al. The American association of endocrine surgeons guidelines for the definitive surgical management of thyroid disease in adults. Ann Surg. 2020;271(3):e21–93.
118. Machens A, Dralle H. Biomarker-based risk stratification for previously untreated medullary thyroid cancer. J Clin Endocrinol Metab. 2010;95(6):2655–63.
119. Machens A, Lorenz K, Weber F, Dralle H. Exceptionality of distant metastasis in node-negative hereditary and sporadic medullary thyroid cancer: lessons learned. J Clin Endocrinol Metab. 2021;106(8):e2968–79.
120. McMullen C, Rocke D, Freeman J. Complications of bilateral neck dissection in thyroid cancer from a single high-volume center. JAMA Otolaryngol Head Neck Surg. 2017;143(4):376–81.
121. Matrone A, Prete A, Gambale C, Elisei R. Timing and Ideal Patient for an Appropriate Search for Somatic RET Mutation in Medullary Thyroid Cancer. JCO Precis Oncol. 2024;8:e2400017.
122. Pellegriti G, Leboulleux S, Baudin E, Bellon N, Scollo C, Travagli JP, et al. Long-term outcome of medullary thyroid carcinoma in patients with normal postoperative medical imaging. Br J Cancer. 2003;88(10):1537–42.
123. Prete A, Gambale C, Torregrossa L, Ciampi R, Romei C, Ramone T, et al. Clinical evolution of sporadic medullary thyroid carcinoma with biochemical incomplete response after initial treatment. J Clin Endocrinol Metab. 2023;108(8):e613–22.
124. Liu CQ, Shen CK, Du YX, Li ZM, Shi X, Wang Y, et al. Survival outcome and optimal candidates of primary tumor resection for patients with metastatic medullary thyroid cancer. J Clin Endocrinol Metab. 2024;0(0):1–7.
125. Fersht N, Vini L, A'Hern R, Harmer C. The role of radiotherapy in the management of elevated calcitonin after surgery for medullary thyroid cancer. Thyroid. 2001;11(12):1161–8.
126. Brierley J, Tsang R, Simpson WJ, Gospodarowicz M, Sutcliffe S, Panzarella T. Medullary thyroid cancer: analyses of survival and prognostic factors and the role of radiation therapy in local control. Thyroid. 1996;6(4):305–10.
127. Schwartz DL, Rana V, Shaw S, Yazbeck C, Ang KK, Morrison WH, et al. Postoperative radiotherapy for advanced medullary thyroid cancer–local disease control in the modern era. Head Neck. 2008;30(7):883–8.
128. Jin M, Megwalu UC, Noel JE. External beam radiotherapy for medullary thyroid cancer following total or near-total thyroidectomy. Otolaryngol Head Neck Surg. 2021;164(1):97–103.
129. Rowell NP. The role of external beam radiotherapy in the management of medullary carcinoma of the thyroid: a systematic review. Radiother Oncol. 2019;136:113–20.
130. He J, Li J, Cheng Y, Fan J, Guo J, Jiang Z, et al. Guidelines of Chinese Society of Clinical Oncology (CSCO) Differentiated Thyroid Cancer. J Cancer Control Treat. 2021;34(12):1164–201.
131. Jozaghi Y, Zafereo M, Williams MD, Gule-Monroe MK, Wang J, Grubbs EG, et al. Neoadjuvant selpercatinib for advanced medullary thyroid cancer. Head Neck. 2021;43(1):E7–12.
132. Wang Y, Tian W, Li C, Guo Z. Chinese expert consensus on neoadjuvant treatment of locally advanced thyroid cancer (2023 edition). Chin J Pract Surg. 2023;43(08):841–8.
133. Selpercatinib Before Surgery for the Treatment of RET-Altered Thyroid Cancer. ClinicalTrials.gov identifier: NCT04759911.2024. https://classic.clinicaltrials.gov/ct2/show/NCT04759911?term5NCT04759911&draw52&rank51. In.
134. De Leo S, Trevisan M, Re Sarto GV, Moneta C, Pirovano M, Colombo C, et al. Letter to the Editor: tumor lysis syndrome during Selpercatinib treatment in a patient with medullary thyroid cancer. Thyroid. 2023;33(9):1130–2.
135. Chiacchiarini M, Trocchianesi S, Besharat ZM, Po A, Ferretti E. Role of tissue and circulating microRNAs and DNA as biomarkers in medullary thyroid cancer. Pharmacol Ther. 2021;219:107708.
136. Deutschbein T, Matuszczyk A, Moeller LC, Unger N, Yuece A, Lahner H, et al. Treatment of advanced medullary thyroid carcinoma with a combination of cyclophosphamide, vincristine, and dacarbazine: a single-center experience. Exp Clin Endocrinol Diabetes. 2011;119(9):540–3.
137. Rodriguez-Antona C, Pallares J, Montero-Conde C, Inglada-Perez L, Castelblanco E, Landa I, et al. Overexpression and activation of EGFR and VEGFR2 in medullary thyroid carcinomas is related to metastasis. Endocr Relat Cancer. 2010;17(1):7–16.
138. Wells SA Jr, Gosnell JE, Gagel RF, Moley J, Pfister D, Sosa JA, et al. Vandetanib for the treatment of patients with locally advanced or metastatic hereditary medullary thyroid cancer. J Clin Oncol. 2010;28(5):767–72.
139. Schlumberger M, Elisei R, Muller S, Schoffski P, Brose M, Shah M, et al. Overall survival analysis of EXAM, a phase III trial of cabozantinib in patients with radiographically progressive medullary thyroid carcinoma. Ann Oncol. 2017;28(11):2813–9.
140. Schlumberger M, Jarzab B, Cabanillas ME, Robinson B, Pacini F, Ball DW, et al. A Phase II Trial of the Multitargeted Tyrosine Kinase Inhibitor

Lenvatinib (E7080) in Advanced Medullary Thyroid Cancer. Clin Cancer Res. 2016;22(1):44–53.
141. Matrone A, Prete A, Nervo A, Ragni A, Agate L, Molinaro E, et al. Lenvatinib as a salvage therapy for advanced metastatic medullary thyroid cancer. J Endocrinol Invest. 2021;44(10):2139–51.
142. Lam ET, Ringel MD, Kloos RT, Prior TW, Knopp MV, Liang J, et al. Phase II clinical trial of sorafenib in metastatic medullary thyroid cancer. J Clin Oncol. 2010;28(14):2323–30.
143. Ravaud A, de la Fouchardiere C, Caron P, Doussau A, Do Cao C, Asselineau J, 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. 2017;76:110–7.
144. Sun Y, Du F, Gao M, Ji Q, Li Z, Zhang Y, et al. Anlotinib for the Treatment of Patients with Locally Advanced or Metastatic Medullary Thyroid Cancer. Thyroid. 2018;28(11):1455–61.
145. Ito Y, Onoda N, Ito KI, Sugitani I, Takahashi S, Yamaguchi I, et al. Sorafenib in Japanese Patients with Locally Advanced or Metastatic Medullary Thyroid Carcinoma and Anaplastic Thyroid Carcinoma. Thyroid. 2017;27(9):1142–8.
146. Chougnet CN, Borget I, Leboulleux S, de la Fouchardiere C, Bonichon F, Criniere L, et al. Vandetanib for the treatment of advanced medullary thyroid cancer outside a clinical trial: results from a French cohort. Thyroid. 2015;25(4):386–91.
147. Drilon A, Hu ZI, Lai GGY, Tan DSW. Targeting RET-driven cancers: lessons from evolving preclinical and clinical landscapes. Nat Rev Clin Oncol. 2018;15(3):150.
148. Kesby NL, Papachristos AJ, Gild M, Aniss A, Sywak MS, Clifton-Bligh R, et al. Outcomes of advanced medullary thyroid carcinoma in the Era of targeted therapy. Ann Surg Oncol. 2022;29(1):64–71.
149. Subbiah V, Yang D, Velcheti V, Drilon A, Meric-Bernstam F. State-of-the-Art Strategies for Targeting RET-Dependent Cancers. J Clin Oncol. 2020;38(11):1209–21.
150. Hadoux J, Elisei R, Brose MS, Hoff AO, Robinson BG, Gao M, et al. Phase 3 Trial of Selpercatinib in Advanced RET-Mutant Medullary Thyroid Cancer. N Engl J Med. 2023;389(20):1851–61.
151. Wirth LJ, Brose MS, Elisei R, Capdevila J, Hoff AO, Hu MI, et al. LIBRETTO-531: a phase III study of selpercatinib in multikinase inhibitor-naive RET-mutant medullary thyroid cancer. Future Oncol. 2022;18(28):3143–50.
152. Pu X, Xu C, Wang Q, Wang W, Wu F, Cai X, et al. Expert consensus on the diagnosis and treatment of RET gene fusion non-small cell lung cancer in China. Thorac Cancer. 2023;14(31):3166–77.
153. Ortiz MV, Gerdemann U, Raju SG, Henry D, Smith S, Rothenberg SM, et al. Activity of the Highly Specific RET Inhibitor Selpercatinib (LOXO-292) in Pediatric Patients With Tumors Harboring RET Gene Alterations. JCO Precis Oncol. 2020;4:PO.19.00401.
154. Subbiah VHMN, Gainor JF, et al. Clinical activity of the RET inhibitor pralse tinib (BLU-667) in patients with RET fusion1 solid tumors. J Clin Oncol. 2020;38(15 Suppl):109.
155. Griesinger F, Curigliano G, Thomas M, Subbiah V, Baik CS, Tan DSW, et al. Safety and efficacy of pralsetinib in RET fusion-positive non-small-cell lung cancer including as first-line therapy: update from the ARROW trial. Ann Oncol. 2022;33(11):1168–78.
156. Fischer L. Pralsetinib Will No Longer Be Available for RET-Mutated Medullary Thyroid Cancer. July 6, 2023. https://www.oncnursingnews.com/view/pralsetinib-will-no-longer-be-available-for-ret-mutated-medullary-thyroid-cancer. In.
157. Schoffski P, Aftimos PG, Massard C, Italiano A, Jungels C, Andreas K, 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. 2019;37(15_suppl):TPS3162.
158. Drilon ARE, Zhai D, Deng W, Zhang X, Lee D, et al. TPX-0046 is a novel and potent RET/SRC inhibitor for RET-driven cancers. Ann Oncol. 2019;30:v190-1.
159. Product Monograph Including Patient Medication Information: RETEVMO (Selpercatinib capsules); 2022. https://pdf.hres.ca/dpdpm/00065504.PDF.
160. Highlights of Prescribing Information: RETEVMO (selpercatinib) capsules, for oral use; 2020. https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/213246s000lbl.pdf
161. Selpercatinib: Drug information; 2022. https://www-uptodate-com.uml.idm.oclc.org/contents/selpercatinib-drug-information?source=auto_suggest&selectedTitle=1~1—1~4selpercatini&search=selpercatinib#F54397849
162. Assessment report: Gavreto; 2021. https://www.ema.europa.eu/en/documents/assessment-report/gavreto-epar-public-assessmentreport_en.pdf
163. Product Monograph Including Patient Medication Information: GAVRETO (pralsetinib); 2021. https://pdf.hres.ca/dpd_pm/00062013.PDF
164. Tsang V, Gill A, Gild M, Lurie B, Blumer L, Siddall R, et al. Selpercatinib Treatment of RET-mutated thyroid cancers is associated with gastrointestinal adverse effects. J Clin Endocrinol Metab. 2022;107(9):e3824–9.
165. Wu K, Kumar S, Hsiao E, Kerridge I, Qiu MR, Siddall R, et al. Pulmonary BRAF-driven Langerhans cell histiocytosis following selpercatinib use in metastatic medullary thyroid cancer. Endocrinol Diabetes Metab Case Rep. 2024;2024(2):23–0079.
166. Gambale C, Prete A, Romei C, Celi A, Elisei R, Matrone A. Fluctuating obliterative bronchiolitis in RET-mutant medullary thyroid cancer patient treated with selpercatinib. Eur Thyroid J. 2024;13(5):e240189.
167. Kalchiem-Dekel O, Falcon CJ, Bestvina CM, Liu D, Kaplanis LA, Wilhelm C, et al. Brief Report: Chylothorax and Chylous Ascites During RET Tyrosine Kinase Inhibitor Therapy. J Thorac Oncol. 2022;17(9):1130–6.
168. Prete A, Gambale C, Cappagli V, Bottici V, Rossi P, Caciagli M, et al. Chylous effusions in advanced medullary thyroid cancer patients treated with selpercatinib. Eur J Endocrinol. 2022;187(6):905–15.
169. Matrone A, Kroiss M, Gild ML, Hamidi S, Sayehli CM, Siddal R, et al. Erectile Dysfunction in Patients Treated with Selpercatinib for RET-Altered Thyroid Cancer. Thyroid. 2024;34:1177–80.
170. Roman-Gil MS, Pozas J, Rosero-Rodriguez D, Chamorro-Perez J, Ruiz-Granados A, Caracuel IR, et al. Resistance to RET targeted therapy in Thyroid Cancer: Molecular basis and overcoming strategies. Cancer Treat Rev. 2022;105:102372.
171. Drilon A, Subbiah V, Gautschi O, Tomasini P, de Braud F, Solomon BJ, et al. Selpercatinib in Patients With RET Fusion-Positive Non-Small-Cell Lung Cancer: Updated Safety and Efficacy From the Registrational LIBRETTO-001 Phase I/II Trial. J Clin Oncol. 2023;41(2):385–94.
172. De Falco V, Carlomagno F, Li HY, Santoro M. The molecular basis for RET tyrosine-kinase inhibitors in thyroid cancer. Best Pract Res Clin Endocrinol Metab. 2017;31(3):307–18.
173. Matrone A, Valerio L, Pieruzzi L, Giani C, Cappagli V, Lorusso L, et al. Protein kinase inhibitors for the treatment of advanced and progressive radiorefractory thyroid tumors: From the clinical trials to the real life. Best Pract Res Clin Endocrinol Metab. 2017;31(3):319–34.
174. Subbiah V, Shen T, Terzyan SS, Liu X, Hu X, Patel KP, et al. Structural basis of acquired resistance to selpercatinib and pralsetinib mediated by non-gatekeeper RET mutations. Ann Oncol. 2021;32(2):261–8.
175. Liu X, Shen T, Mooers BHM, Hilberg F, Wu J. Drug resistance profiles of mutations in the RET kinase domain. Br J Pharmacol. 2018;175(17):3504–15.
176. Fancelli S, Caliman E, Mazzoni F, Brugia M, Castiglione F, Voltolini L, et al. Chasing the target: new phenomena of resistance to novel selective RET inhibitors in lung cancer. Updated Evidence and Future Perspectives. Cancers (Basel). 2021;13(5):1091.
177. Subbiah V, Cote GJ. Advances in Targeting RET-Dependent Cancers. Cancer Discov. 2020;10(4):498–505.
178. Subbiah V, Velcheti V, Tuch BB, Ebata K, Busaidy NL, Cabanillas ME, et al. Selective RET kinase inhibition for patients with RET-altered cancers. Ann Oncol. 2018;29(8):1869–76.
179. Kato S, Subbiah V, Marchlik E, Elkin SK, Carter JL, Kurzrock R. RET Aberrations in Diverse Cancers: Next-Generation Sequencing of 4,871 Patients. Clin Cancer Res. 2017;23(8):1988–97.
180. Rich TA, Reckamp KL, Chae YK, Doebele RC, Iams WT, Oh M, 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. 2019;25(19):5832–42.
181. Nelson-Taylor SK, Le AT, Yoo M, Schubert L, Mishall KM, Doak A, et al. Resistance to RET-Inhibition in RET-Rearranged NSCLC Is Mediated By Reactivation of RAS/MAPK Signaling. Mol Cancer Ther. 2017;16(8):1623–33.

182. Xing M. Genetic alterations in the phosphatidylinositol-3 kinase/Akt pathway in thyroid cancer. Thyroid. 2010;20(7):697–706.
183. Subbiah V, Berry J, Roxas M, Guha-Thakurta N, Subbiah IM, Ali SM, et al. Systemic and CNS activity of the RET inhibitor vandetanib combined with the mTOR inhibitor everolimus in KIF5B-RET re-arranged non-small cell lung cancer with brain metastases. Lung Cancer. 2015;89(1):76–9.
184. Koh YW, Shah MH, Agarwal K, McCarty SK, Koo BS, Brendel VJ, et al. Sorafenib and Mek inhibition is synergistic in medullary thyroid carcinoma in vitro. Endocr Relat Cancer. 2012;19(1):29–38.
185. Rosen EY, Johnson ML, Clifford SE, Somwar R, Kherani JF, Son J, et al. Overcoming MET-Dependent Resistance to Selective RET Inhibition in Patients with RET Fusion-Positive Lung Cancer by Combining Selpercatinib with Crizotinib. Clin Cancer Res. 2021;27(1):34–42.
186. Subbiah V, Shen T, Tetzlaff M, Weissferdt A, Byers LA, Cascone T, et al. Patient-driven discovery and post-clinical validation of NTRK3 fusion as an acquired resistance mechanism to selpercatinib in RET fusion-positive lung cancer. Ann Oncol. 2021;32(6):817–9.
187. Zhu VW, Zhang SS, Zhang J, Swensen J, Xiu J, Ou SI. Acquired Tertiary MET Resistance (MET D1228N and a Novel LSM8-MET Fusion) to Selpercatinib and Capmatinib in a Patient With KIF5B-RET-positive NSCLC With Secondary MET Amplification as Initial Resistance to Selpercatinib. J Thorac Oncol. 2021;16(7):e51–4.
188. Rosen EY, Won HH, Zheng Y, Cocco E, Selcuklu D, Gong Y, et al. The evolution of RET inhibitor resistance in RET-driven lung and thyroid cancers. Nat Commun. 2022;13(1):1450.
189. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature. 2008;454(7203):436–44.
190. Zhang B, Zhang Y, Zhao J, Wang Z, Wu T, Ou W, et al. M2-polarized macrophages contribute to the decreased sensitivity of EGFR-TKIs treatment in patients with advanced lung adenocarcinoma. Med Oncol. 2014;31(8):127.
191. Chen S, Tang J, Liu F, Li W, Yan T, Shangguan D, et al. Changes of tumor microenvironment in non-small cell lung cancer after TKI treatments. Front Immunol. 2023;14:1094764.
192. Chang H, Sung JH, Moon SU, Kim HS, Kim JW, Lee JS. EGF Induced RET Inhibitor Resistance in CCDC6-RET Lung Cancer Cells. Yonsei Med J. 2017;58(1):9–18.
193. Palucka AK, Coussens LM. The Basis of Oncoimmunology. Cell. 2016;164(6):1233–47.
194. Ward LS. Immune response in thyroid cancer: widening the boundaries. Scientifica (Cairo). 2014;2014:125450.
195. Pozdeyev N, Erickson TA, Zhang L, Ellison K, Rivard CJ, Sams S, et al. Comprehensive Immune Profiling of Medullary Thyroid Cancer. Thyroid. 2020;30(9):1263–79.
196. Adashek JJ, Desai AP, Andreev-Drakhlin AY, Roszik J, Cote GJ, Subbiah V. Hallmarks of RET and Co-occuring Genomic Alterations in RET-aberrant Cancers. Mol Cancer Ther. 2021;20(10):1769–76.
197. Thein KZ, Velcheti V, Mooers BHM, Wu J, Subbiah V. Precision therapy for RET-altered cancers with RET inhibitors. Trends Cancer. 2021;7(12):1074–88.
198. Castellone MD, Melillo RM. RET-mediated modulation of tumor microenvironment and immune response in multiple endocrine neoplasia type 2 (MEN2). Endocr Relat Cancer. 2018;25(2):T105–19.
199. Addeo A, Passaro A, Malapelle U, Banna GL, Subbiah V, Friedlaender A. Immunotherapy in non-small cell lung cancer harbouring driver mutations. Cancer Treat Rev. 2021;96:102179.
200. Angell TE, Lechner MG, Jang JK, Correa AJ, LoPresti JS, Epstein AL. BRAF V600E in papillary thyroid carcinoma is associated with increased programmed death ligand 1 expression and suppressive immune cell infiltration. Thyroid. 2014;24(9):1385–93.
201. Trujillo JA, Sweis RF, Bao R, Luke JJ. T Cell-Inflamed versus Non-T Cell-Inflamed Tumors: a conceptual framework for cancer immunotherapy drug development and combination therapy selection. Cancer Immunol Res. 2018;6(9):990–1000.
202. Yin H, Tang Y, Guo Y, Wen S. Immune Microenvironment of Thyroid Cancer. J Cancer. 2020;11(16):4884–96.
203. Li Z, Li D, Tsun A, Li B. FOXP3+ regulatory T cells and their functional regulation. Cell Mol Immunol. 2015;12(5):558–65.
204. French JD. Immunotherapy for advanced thyroid cancers - rationale, current advances and future strategies. Nat Rev Endocrinol. 2020;16(11):629–41.
205. Castellone MD, Guarino V, De Falco V, Carlomagno F, Basolo F, Faviana P, et al. Functional expression of the CXCR4 chemokine receptor is induced by RET/PTC oncogenes and is a common event in human papillary thyroid carcinomas. Oncogene. 2004;23(35):5958–67.
206. Santagata S, Ierano C, Trotta AM, Capiluongo A, Auletta F, Guardascione G, et al. CXCR4 and CXCR7 signaling pathways: a focus on the cross-talk between cancer cells and tumor microenvironment. Front Oncol. 2021;11:591386.
207. Ding X, Xiang L, Wang N, Zhao Z, Jin X, Sun Y, et al. Vandetanib-induced inhibition of neuroblastoma cell migration and invasion is associated with downregulation of the SDF-1/CXCR4 axis and matrix metalloproteinase 14. Oncol Rep. 2014;31(3):1165–74.
208. De Clercq E. AMD3100/CXCR4 Inhibitor. Front Immunol. 2015;6:276.
209. Terzyan SS, Shen T, Liu X, Huang Q, Teng P, Zhou M, et al. Structural basis of resistance of mutant RET protein-tyrosine kinase to its inhibitors nintedanib and vandetanib. J Biol Chem. 2019;294(27):10428–37.
210. Lin JJ, Liu SV, McCoach CE, Zhu VW, Tan AC, Yoda S, et al. Mechanisms of resistance to selective RET tyrosine kinase inhibitors in RET fusion-positive non-small-cell lung cancer. Ann Oncol. 2020;31(12):1725–33.
211. Shabbir A, Kojadinovic A, Shafiq T, Mundi PS. Targeting RET alterations in cancer: Recent progress and future directions. Crit Rev Oncol Hematol. 2023;181:103882.
212. Plenker D, Riedel M, Bragelmann J, Dammert MA, Chauhan R, Knowles PP, et al. Drugging the catalytically inactive state of RET kinase in RET-rearranged tumors. Sci Transl Med. 2017;9(394):eaah6144.
213. Wang H, Tang J, Su Z. YAP confers resistance to vandetanib in medullary thyroid cancer. Biochem Cell Biol. 2020;98(3):443–8.
214. Gainor JF, Curigliano G, Kim DW, Lee DH, Besse B, Baik CS, et al. Pralsetinib for RET fusion-positive non-small-cell lung cancer (ARROW): a multi-cohort, open-label, phase 1/2 study. Lancet Oncol. 2021;22(7):959–69.
215. Kurzrock R. Selpercatinib Aimed at RET-Altered Cancers. N Engl J Med. 2020;383(9):868–9.
216. Raman R, Villefranc JA, Ullmann TM, Thiesmeyer J, Anelli V, Yao J, et al. Inhibition of FGF receptor blocks adaptive resistance to RET inhibition in CCDC6-RET-rearranged thyroid cancer. J Exp Med. 2022;219(6):e20210390.
217. Solomon BJ, Tan L, Lin JJ, Wong SQ, Hollizeck S, Ebata K, et al. RET Solvent Front Mutations Mediate Acquired Resistance to Selective RET Inhibition in RET-Driven Malignancies. J Thorac Oncol. 2020;15(4):541–9.
218. Schoffski P, Cho BC, Italiano A, Loong HHF, Massard C, Rodriguez LM, et al. BOS172738, a highly potent and selective RET inhibitor, for the treatment of RET-altered tumors including RET-fusion+ NSCLC and RET-mutant MTC: Phase 1 study results. JCO. 2021;39(15_suppl):3008–3008.
219. Nisha GRKEDAJABBJBKRCEBGTCIMK. Pre-clinical characterization of potent and selective next-generation RET inhibitors. Cancer Res 2021; 81(13_Supplement):1464.
220. Vivek Subbiah JZ, Lu Y, Liu Y, Chen M, Chen X, Wang H, Zhu J, Lu S, Drilon AE. The development of APS03118, a potent next-generation RET inhibitor for treating RET-inhibitor-resistant patients. J Clin Oncol. 2022;40(16_suppl):e15107.
221. Grossrubatscher E, Fanciulli G, Pes L, Sesti F, Dolci C, de Cicco F, et al. Advances in the Management of Medullary Thyroid Carcinoma: Focus on Peptide Receptor Radionuclide Therapy. J Clin Med. 2020;9(11):3507.
222. Lee DY, Kim YI. Peptide receptor radionuclide therapy in patients with differentiated thyroid cancer: a meta-analysis. Clin Nucl Med. 2020;45(8):604–10.
223. Bachleitner-Hofmann T, Friedl J, Hassler M, Hayden H, Dubsky P, Sachet M, et al. Pilot trial of autologous dendritic cells loaded with tumor lysate(s) from allogeneic tumor cell lines in patients with metastatic medullary thyroid carcinoma. Oncol Rep. 2009;21(6):1585–92.
224. Schott M, Feldkamp J, Klucken M, Kobbe G, Scherbaum WA, Seissler J. Calcitonin-specific antitumor immunity in medullary thyroid

carcinoma following dendritic cell vaccination. Cancer Immunol Immunother. 2002;51(11–12):663–8.
225. Schott M, Seissler J, Lettmann M, Fouxon V, Scherbaum WA, Feldkamp J. Immunotherapy for medullary thyroid carcinoma by dendritic cell vaccination. J Clin Endocrinol Metab. 2001;86(10):4965–9.
226. Bhoj VG, Li L, Parvathaneni K, Zhang Z, Kacir S, Arhontoulis D, et al. Adoptive T cell immunotherapy for medullary thyroid carcinoma targeting GDNF family receptor alpha 4. Mol Ther Oncolytics. 2021;20:387–98.

Publisher's Note