UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

FACULDADE DE MEDICINA

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS MÉDICAS: ENDOCRINOLOGIA

PAPEL DO ANTIGENO CARBOIDRATO 19.9 COMO MARCADOR DE AGRESSIVIDADE NO CARCINOMA MEDULAR DE TIREOIDE

CARLA VAZ FERREIRA VARGAS

Porto Alegre, março de 2018.

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TESE DE DOUTORADO

CARLA VAZ FERREIRA VARGAS

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- **Artigo de revisão:** Advances and controversies in the management of medullary thyroid carcinoma; publicado no Current Opinion Oncology 2017, 29:25–32. Impact factor: 4.414.

- **Artigo original:** Role of antigen carbohydrate 19.9 as a marker of aggressiveness in thyroid medullary carcinoma

Dados preliminares do artigo original da presente tese foram apresentados nos seguintes eventos científicos:

- XVI Latin American Thyroid Congress, 2017, Rio de Janeiro/RJ
  Carbohydrate antigen 19.9 expression in tumor samples of medullary thyroid carcinoma is not associated with cellular dedifferentiation.
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- XVII Encontro Brasileiro de Tireoide, 2016, Gramado/RS
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Além dos artigos que fazem parte da presente tese, ao longo do período do doutorado foram desenvolvidos os seguintes manuscritos:

- Global DNA Methylation Profile in Medullary Thyroid Cancer Patients. Ceolin, L; Goularte, APP; **Ferreira, CV**; Romitti, M; Maia, AL. Experimental and Molecular Pathology. Em revisão.

- Effect of 3-UTR RET Variants on RET mRNA Secondary Structure and Disease Presentation in Medullary Thyroid Carcinoma. Ceolin, L ; Romitti, M ; Siqueira, DR ; **Ferreira, CV** ; Scapineli, JO ; Assis-Brazil, B ; Maximiano, RV ; Amarante, TD ; Nunes, MCS ;Weber, G ; Maia, AL . **Plos One**, v. 11, p. e0147840, 2016.

- MAPK and SHH pathways modulate type 3 deiodinase expression in papillary thyroid carcinoma. Romitti, M ; Wajner, SM ; Ceolin, L ; **Ferreira, CV** ; Ribeiro, RVP ; Rohenkohl, HC ; Weber, SS ; Lopez, PLC ; Fuziwara, CS ; Kimura, ET ; Maia, AL. **Endocrine Related Cancer**, v. 23, p. 135-146, 2016.

- Role of *RET* genetic variants in men 2-associated pheochromocytoma. Siqueira, DR ; Ceolin, L ; **Ferreira, CV** ; Romitti, M ; Maia, SC ; Maciel, LMZ ; Maia, AL. **European Journal of Endocrinology**, v. 170, p. 400, 2014.

- Novos medicamentos no tratamento clínico do carcinoma medular de tireoide. **Ferreira CV**, Siqueira DR, Maia AL. In: Sociedade Brasileira de Endocrinologia e Metabologia; Graf H, Czepielewski M, Meirelles R, organizadores. **PROENDOCRINO Programa de Atualização em Endocrinologia e Metabologia: Ciclo 5.** Porto Alegre: Artmed/Panamericana; 2014. p.31-48. (Sistema de Educação Médica Continuada a Distância, v.3).

LISTA DE ABREVIATURAS E SIGLAS

AKT	Protein kinase B
CA19.9	Carbohydrate antigen
CEA	Carcinoembryonic antigen
CI	Confidence interval
c-Kit	Hepatocyte growth factor
EBRT	External beam radiation therapy
EGF	Epidermal growth factor
ERK	Extracellular signal- regulated kinase
ERs	Estrogen-responsive elements
ESR2	Estrogen Receptor 2 gene
FGFR	Fibroblast growth factor receptor
GDNF	Glial-derived neurotrophic factor
Gli2	Hypoxia-inducible factor-1
MEN 2	Multiple endocrine neoplasia type 2
MEN 2A	Multiple endocrine neoplasia type 2 A
MEN 2B	Multiple endocrine neoplasia type 2 B
miRNA	Micro Ribonucleic acid
MTC	Medullary thyroid carcinoma
mTOR	Mammalian target of rapamycin
NFkB	Nuclear factor kB
NGS	Next-generation sequencing
ORR	Objective response rate
PDGFRα	Platelet-derived growth factor receptor α
PFS	Progression-free survival
PI3K	Phosphoinositide 3-kinase
RET	REarranged during Transfection
RNA	Ribonucleic acid
SHh	Sonic hedgehog
Smo	Smoothened
sMTC	Sporadic medullary thyroid carcinoma
TKIs	Tyrosine kinase inhibitors

UTR	Untranslated region
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor

SUMÁRIO

RESUMO

O carcinoma medular da tireoide (CMT) é um tumor maligno raro originário de células C parafoliculares da tireoide e corresponde a 4% das neoplasias malignas dessa glândula. O CMT apresenta-se como um tumor esporádico (75-80%) ou na forma hereditária (20-25%). O único tratamento curativo disponível no momento para o CMT é cirúrgico. No entanto, isso só é possível em casos em que o diagnóstico é realizado precocemente e a doença encontra-se restrita a glândula. Nos pacientes com doença avançada, onde as opções terapêuticas tradicionais como quimioterapia e radioterapia não são efetivas, os inibidores tirosino-quinase tem demonstrado eficácia na sobrevida livre de progessão da doença. Níveis de calcitonina sérica, um biomarcador específico para céluas C tireoideanas, e o antígeno-carcinoembrionário (CEA) são amplamente utilizados como marcadores no diagnóstico e seguimento dos pacientes com CMT. No entanto, estudos recentes têm indicado que níveis séricos elevados do antígeno carboidrato 19.9 (CA19.9), marcador tumoral bem estabelecido no em neoplasias pancreáticas, como um potencial marcador de agressividade e mortalidade em indivíduos com CMT avançado.

O objetivo desse trabalho foi avaliar o papel do CA19.9 como marcador de agressividade tumoral em pacientes com CMT.

Amostras tumorais de pacientes com CMT atendidos no Serviço de Endocrinologia do HCPA foram avaliados para expressão do CA19.9 por imunohistoquimica, através de anticorpo especifico. Para estudar a hipótese de os níveis de CA19.9 observados em pacientes com CMT estarem associados à desdiferenciação das células C, também avaliamos a expressão tecidual de CD133, um marcador para a identificação de células-tronco cancerígenas (CSC). A leitura das lâminas foi realizada por patologista, e quantificação da expressão foi inicialmente realizada pelo método de h-score. Adicionalmente as amostras foram classificadas de acordo com o padrão de expressão observado: células individuais, focos ou difuso.

Setenta pacientes com CMT foram incluídos no estudo, 57,1% apresentavam a forma hereditária e 42,9% a forma esporádica. A idade média ao diagnóstico foi 36.1 (±16.3) anos e 58,6% foram do sexo feminino. A mediana dos níveis de calcitonina e CEA foram de 536pg/ml (49,35-1300,5) e 21,3ng/ml (3,6-52,6), respectivamente. Aproximadamente 53% dos pacientes apresentavam metástases locais e 20% à distância ao diagnóstico. Das 64 amostras de tumor primário disponíveis para analise, 56 (87,5%) apresentaram expressão do CA19.9, com mediana de h-score 14 (2-30). De forma semelhante, o CD133 estava expresso em 90.5% das amostras de tumor primário, no entanto não se observou nenhuma correlação entre os dois marcadores estudados (r=-0.09; P=0.74). Não foram observadas diferenças na expressão de CA19.9 sobre idade, sexo, níveis

séricos calcitonina ou CEA (P>0,05). Curiosamente amostras de CMT hereditário tinham maior expressão de CA19.9 que amostras de CMT esporádico. Observamos três padrões de expressão distintos para o CA19.9: células individuais, focal e difuso. A maioria das amostras (64,3%) apresentaram o padrão de expressão focal. O padrão de células individuais foi observado em 17 (30,3%) das amostras e o padrão difuso em 3 (5,4%). As formas esporádica e hereditária da doença apresentaram diferentes padrões de expressão. De forma interessante, o CMT esporádico mostrou-se associado ao padrão de células individuais (70,6%), enquanto a forma hereditária foi associada ao padrão focal de expressão (63,9%) (P=0,04). Adicionalmente, o padrão de células individuais foi associado a metástases local (P=0,055) enquanto que o padrão difuso, a metástases à distância (P=0,032).

Nossos resultados demonstram expressão do CA19.9 na maioria das amostras de CMT. Diferenças nos níveis de expressão do CA19.9 não foram associadas às características clínicas ou oncológicas, sendo no entanto significativamente mais elevados em amostras de CMT hereditário. Três padrões de expressão distintos foram observados, sendo que o padrão difuso foi associado à presença de metástases à distância ao diagnóstico. Em conclusão, o CA19.9 é amplamente expresso no CMT e apresenta características distintas de outros marcadores atualmente utilizados. Estudos adicionais podem definir o papel desse marcador no manejo de pacientes de CMT.

ABSTRACT

Medullary thyroid carcinoma (MTC) is a rare malignant tumor originating from parafollicular C-cell of the thyroid and corresponds to 4% of malignant neoplasms of this gland. MTC presents as a sporadic tumor (75-80%) or in hereditary form (20-25%). The only curative treatment currently available for MTC is surgical. However, this is only possible in cases of early diagnosis and the disease is restricted to the gland. In patients with advanced disease, where traditional therapeutic options such as chemotherapy and radiotherapy are not effective, tyrosine kinase inhibitors have demonstrated efficacy in disease-free survival. Levels of serum calcitonin, a specific biomarker for thyroid C-cells, and carcinoembryonic antigen (CEA) are widely used as markers in the diagnosis and follow-up of patients with MTC. However, recent studies have indicated that elevated serum levels of carbohydrate antigen 19.9 (CA19.9), a well established tumor marker in pancreatic neoplasms, are a potential marker of aggression and mortality in individuals with advanced MTC.

The objective of this study was to evaluate the role of CA19.9 as a marker of tumor aggressiveness in patients with MTC.

Tumor samples from MTC patients treated at the HCPA Endocrinology Service were evaluated for expression of CA19.9 by immunohistochemistry using a specific antibody. To study the hypothesis that CA19.9 levels observed in patients with MTC are associated with C-cell de-differentiation, we also assessed the tissue expression of CD133, a marker for the identification of cancer stem cells (CSC). The reading of the slides was performed by a pathologist, and quantification of the expression was initially performed by the h-score method. Additionally, the samples were classified according to the observed expression pattern: individual cells, focal or diffuse.

Seventy patients with MTC were included in the study, 57.1% presented the hereditary form and 42.9% presented sporadic form. The mean age at diagnosis was 36.1 (± 16.3) years and 58.6% were female. The median levels of calcitonin and CEA were 536pg/ml (49.35-1300.5) and 21.3ng/ml (3.6-52.6), respectively. Approximately 53% of the patients had local metastases and 20% at a distance at diagnosis. Of the 64 primary tumor samples available for analysis, 56 (87.5%) presented CA19.9 expression, with median h-score 14 (2-30). Similarly, CD133 was expressed in 90.5% of the primary tumor samples. However, no correlation was observed between the two markers studied ($r=-0.09$; $P=0.74$). No differences in CA19.9 expression were observed on age, sex, serum calcitonin or CEA levels ($P>0.05$). Curiously, samples of hereditary MTC had higher CA19.9 expression than sporadic MTC samples. We observed three distinct expression patterns for

CA19.9: individual cells, focal and diffuse. Most of the samples (64.3%) had the focal expression pattern. The individual cell pattern was observed in 17 (30.3%) of the samples and the diffuse pattern in 3 (5.4%). The sporadic and hereditary forms of the disease presented different patterns of expression. Interestingly, sporadic CMT was associated with the individual cell pattern (70.6%), while the hereditary form was associated with the focal expression pattern (63.9%) (P=0.04). In addition, the individual cell pattern was associated with local metastases (P=0.055) while the diffuse pattern, with distant metastases (P=0.032).

Our results demonstrate expression of CA19.9 in the majority of MTC samples. Differences in CA19.9 expression levels were not associated with clinical or oncological features disease but it were significantly higher in hereditary MTC samples. Three distinct expression patterns were observed, and the diffuse pattern was associated with the presence of distant metastases at diagnosis. In conclusion, CA19.9 is widely expressed in MTC and presents distinct characteristics of other markers currently used. Additional studies may define the role of this marker in the management of MTC patients.

Parte I

ADVANCES AND CONTROVERSIES IN THE MANAGEMENT OF MEDULLARY THYROID CARCINOMA

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Advances and controversies in the management of medullary thyroid carcinoma

Ana Luiza Maia, Simone Magagnin Wajner e Carla Vaz Ferreira Vargas

Thyroid Section, Endocrine Division, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Rua Ramiro Barcelos 2350, 90035-003 Porto Alegre, Rio Grande do Sul, Brazil;

Corresponding author: Ana Luiza Maia, M.D., Ph.D.

Serviço de Endocrinologia

Hospital de Clínicas de Porto Alegre

Rua Ramiro Barcelos 2350

90035 –003 Porto Alegre, RS, Brazil

Phone: 55-51-33310207

E-mail: almaia@ufrgs.br

Purpose of review

Medullary thyroid carcinoma (MTC) comprises approximately 4% of all malignant thyroid neoplasias. While the majority of patients have good prognosis, a subgroup will have progressive disease and require systemic therapy. Here, we focus on the current MTC therapeutic approach and discuss the advantages and disadvantages of molecular targeted therapies based on data reported so far.

Recent findings

Targeted molecular therapies that inhibit RET and other tyrosine-kinase receptors involved in angiogenesis have showed improvement in progression-free survival in patients with advanced MTC. Two drugs, vandetanib and cabozantinib, were approved and several others demonstrated variable efficacy in progressive or symptomatic MTC. No compound has been shown to produce improve survival. Although no definitive recommendation can be currently drawn, cumulative data indicate that the tumor mutational profile may refine the use of targeted therapy in MTC.

Summary

Tyrosine-kinase inhibitors represent an effective therapeutic approach in progressive MTC. Nevertheless, it is not clear which patients will benefit most, and the decision regarding when and how to initiate tyrosine-kinase inhibitor therapy should be based on the medical history and tumor behavior. Hopefully, in the near future, molecular testing of MTC can be used to determine the best molecular target therapeutic.

Keywords: medullary thyroid carcinoma, RET proto-oncogene, tyrosine-kinase inhibitors

Introduction

Medullary thyroid carcinoma (MTC) is a rare type of tumor that originates from the parafollicular C-cells and accounts for 3–4% of all malignant thyroid neoplasias. Calcitonin, the main secretory product of MTC, is a specific and highly sensitive biomarker of C-cell disease. Carcinoembryonic antigen (CEA) is also produced by neoplastic C cells. These molecules are widely used as prognostic markers during follow-up in MTC patient. The reported 10-year mortality rate for MTC varies from 13.5% to 38%, accounting for approximately 15% of all thyroid cancer-related deaths[1,2].

MTC presents as sporadic (75–80%) or inherited tumors (20–25%). Hereditary MTC is part of an autosomal dominant disorder known as multiple endocrine neoplasia type 2 (MEN2). The most common form of this syndrome is MEN2A, characterized by MTC, pheochromocytoma, and/or hyperparathyroidism, whereas MEN2B includes MTC, pheochromocytoma, ganglioneuromatosis and marfanoid habitus. MEN2A is rarely associated with cutaneous lichen amyloidosis or Hirschsprung's disease. Germline activating mutations in the RET proto-oncogene are responsible for hereditary MTC. The majority of MEN2A kindred have point mutations in the RET extracellular domain (exon 10 and 11) and less commonly in exons 5, 8, 13, 14, and 15. Approximately 95% of the MEN2B cases occur through the specific M918T mutation[3-5].

The molecular mechanisms involved in sporadic MTC (sMTC) have not yet been clarified. Somatic RET or RAS mutations seem to represent alternative genetic events in sMTC tumorigenesis. Somatic RET M918T mutation occurs in approximately 23–66% of the cases. Mutations in codons 618, 603, 634, 768, 804, and 883 and a partial deletion of the RET gene have been described in a few tumors[4-6]. However, the mutations are not uniform among the various tumor cell subpopulations, suggesting that sMTC might be of polyclonal origin or that these mutations are not the initial events of MTC tumorigenesis[7].

RET polymorphisms have been associated with susceptibility to the development or progression of MTC[8,9]. The presence of multiple RET variants (G691S, L769L, S836S, or S904S) seems to increase the risk[10]. Nevertheless, the mechanism by which these variants modulate the MTC pathogenesis is still unclear. Recently, linkage disequilibrium between RET S836S and 3'UTR variants was demonstrated. The RET mRNA sequence carrying the S836S/3'UTR haplotype had higher structural and thermodynamic stability, suggesting a functional involvement of the 3'UTR variant allele in the posttranscriptional control of RET transcripts[11].

RAS mutations, mainly H- and K-subtypes have been described in RET-negative sMTC. The prevalence of RAS mutations varies between 0–41.2 and 0–40.9% for HRAS and KRAS, respectively, and between 0–1.8% for NRAS, depending on the reported series[12]. Remarkably, approximately 40–60% of sMTC cases are still negative for all known genetic abnormalities[13]. Recent studies using next-generation sequencing (NGS) have involved a comprehensive search for new genes involved in the MTC pathogenesis. However, to date, no new genes have been identified[14,15].

Prognostic Markers in MTC

The likelihood of attaining cure for MTC depends on the tumor stage at diagnosis. The main factors associated with poor prognosis include older age, tumor size, local and distant metastases, somatic M918T mutation, and the calcitonin and CEA doubling-times[16].

The calcitonin and CEA levels in persistent disease might remain steadily high for years or might exhibit rapid increases. Thus, serial calcitonin and CEA measurements allow a more accurate assessment of disease progression. The calcitonin doubling-time correlates with the survival and tumor recurrence rates. The 5- and 10-year survival rates are 25% and 8%, respectively, when the doubling-time is <6 months, and 92% and 37%, respectively, when the doubling-time ranges from 6 months to 2 years. The calcitonin doubling-times display a better

performance as a predictor of survival, whereas the CEA doubling-times had a greater impact on prognosis[16].

Higher levels of the carbohydrate antigen (CA19.9), classically used as a marker for pancreatic neoplasms, have been reported in patients with very aggressive MTC disease, low calcitonin levels and increased CEA levels[17,18]. Elisei et al. (2015) recently evaluated the serum CA19.9 levels in patients with advanced structural recurrent/persistent MTC. In the group of patients with high CA19.9 levels, 68.7% died from the disease, contrasting with only 23.8% in the group of patients with normal CA19.9 levels ($P<0.001$)[19]. CA19.9 was also associated with advanced disease stages in a recent small pilot study[20]. All specimens from patients with stage IV disease were positive for CA19.9 compared to only 40% of stage I-III cases ($P=0.03$).

Signaling Pathways of Medullary Thyroid Carcinoma

The RET encodes a transmembrane receptor, and activating mutations promote continuous autophosphorylation of tyrosine-kinase residues, thus triggering signaling pathways responsible for cell survival, differentiation and proliferation. Four glial-derived neurotrophic factor (GDNF) family ligands, bind RET with one of four glycosylphosphatidylinositol-anchored co-receptors. RET mutations lead to the activation of major intracellular oncogenic pathways, including RAS/ERK, PI3K/AKT, nuclear factor kB (NFkB) and JUN kinase pathways[21].

Although the inhibition of the RET is actually one of the most studied, other signal transduction pathways have been recognized to contribute to MTC pathogenesis and may constitute attractive therapeutic targets. The mammalian target of the rapamycin (mTOR) pathway is activated in hereditary and sMTC through RET mutations. Functional studies indicated a crosstalk between miR-183, mTOR and RET, leading to activation of

RAS/MAPKK/ERK and the phosphoinositide 3-kinase (PI3K)/AKT/mTOR pathways that control cell proliferation and invasion[22].

Recent experimental data supports a crosstalk between RET and hypoxia-inducible factor-1 (HIF) in MTC, leading to the activation of hypoxia-responsive genes. Indeed, the expression pattern of carbonic anhydrase IX, a direct HIF target implicated in tumor progression, suggested contribution of both hypoxic and oncogenic signaling in MTC[23]. Sonic Hedgehog (SHh) pathway has also been evaluated in TT/MZ-CRC-1 cell lines. Interestingly, SHh activation increased the expression of Smoothened (Smo) and Gli2, key signaling components. Conversely, inhibition of the SHh pathway decreased expression of these genes, leading to decreases in cellular growth and increased apoptosis [24].

Although not fully understood, a tumor loss of ER (heterodimers that bind DNA-specific estrogen-responsive elements) function was described in MTC cell lines, resulting in an ERα-driven ESR2 c.948delT frame shift mutation. ESR2 (Estrogen Receptor 2) inhibits cell proliferation in vitro and can lead to RET upregulation and increased cell proliferation[25]. Dysregulation in miRNA expression has also been implicated in the pathogenesis of MTC[12].

Update of Current Surgical Therapeutic Strategies

Total thyroidectomy is the only curative MTC treatment. Patients without evidence of lymph node or distant metastases should undergo a total thyroidectomy and central lymph node dissection[5,26]. Prophylactic dissection of the lateral compartments might be considered when the tumor is > 1 cm, if metastases are found in the central compartment, or with elevated calcitonin levels[27]. Recently, Tuttle and Ganly[28] proposed a novel dynamic risk stratification of postoperative MTC. The 5- and 10-year recurrence rates vary from <1–8.5% in patients who achieve an excellent response, defined as undetectable calcitonin levels after surgery.

Patients with persistent or recurrent MTC localized to the neck are candidates for repeat neck operations. However, in the presence of extensive regional or metastatic disease, extensive surgery is not associated with a higher cure rate, and less aggressive procedures should be considered[5,21].

As a rule, the surgical approach should be implemented at referral centers with large volume of thyroid surgeries.

General therapeutic approach in metastatic MTC

Several patients with distant metastases have an indolent course that may not require treatment for years. Chemotherapy and external beam radiation therapy for cervical recurrent or distant disease have limited response rates[5,26]. Localized therapy with external beam radiation (EBRT) and/or antiresorptive agents should be considered to palliate painful bone metastases or to prevent other skeletal-related events[29,30]. Embolization or cryoablation of liver metastatic disease may be of benefit in selected cases to decrease tumor burden, pain or refractory diarrhea[31]. Interestingly, MTC-related Cushing syndrome, a rare condition observed in MTC patients, has been successful controlled using vandetanib or sorafenib treatment[32,33].

Systemic Therapy for Advanced MTC: Tyrosine kinase inhibitors

Uncontrolled tyrosine-kinase receptor activation is one of the main mechanisms of cancer development and progression. The role of RET tyrosine-kinase receptor is well-documented in MTC pathogenesis. Vascular endothelial growth factor (VEGF), and hepatocyte growth factor (c-MET), as well as their tyrosine-kinase receptors, are overexpressed in MTCs and play an important role in the pathogenesis, progression, and disease recurrence[34,35]. Thus, the identification of compounds that inhibit the catalytic activity of tyrosine-kinase receptors has opened up an era of targeted MTC therapy. Tyrosine-kinase inhibitors (TKIs) are

orally administered agents that compete with adenosine-triphosphate for its binding site, leading to inhibition of phosphorylation of the proteins involved in signal transduction.

Several TKIs, such as motesanib[36], sorafenib[37,38], sunitinib[39], axitinib[40] and imatinib[41], have been studied in MTC. Overall, the response rate is ~30%, whereas stable disease is the most commonly achieved outcome. Two TKIs have been approved to treat advanced MTC. The first approved compound, vandetanib, selectively targets the RET, VEGF, and the epidermal growth factor (EGF) receptors[42]. The efficacy of vandetanib was evaluated in 331 individuals with metastatic MTC who were randomized to receive vandetanib (300 mg) or a placebo[43]. The results showed a significant increase in progression free survival (PFS) in the vandetanib-treated group (30.2 vs. 19.2 months; hazard risk (HR)=0.46, 95% confidence interval (CI)=0.31–0.69). Vandetanib has also been successfully used in children with MEN2B[44]. The second compound, cabozantinib, is a c-MET, VEGFR2, and RET multikinase inhibitor. A randomized study of 330 individuals with documented MTC progression found a significant increase in PFS in the cabozantinib-treated group (11.2 vs. 4.0 months; HR=0.28, 95% CI=0.19-0.40, p <0.0001)[45]. The effect of vandetanib or cabozantinib on the survival rate of MTC patients remains unknown, but interim analyses of the overall survival (OS) did not show a difference between the two drug-treated and placebo groups[43,45].

More recently, lenvatinib, a multi-targeted TKI of the VEGFRs 1, 2, and 3, FGFRs 1- 4, PDGFRα, RET, and KIT signaling networks, was evaluated in a phase 2 trial. Fifty-nine patients with unresectable progressive MTC were included. The disease-control rate was 80% (95% CI: 67–89%), the highest reported to date. Of interest, the objective response rate (ORR) was similar between patients with (35%) or without (36%) prior anti-VEGFR therapy, confirming the lack of cross resistance between TKIs in patients with prior VEGFR-targeted treatment. The 6-month PFS rate was 67% (95% CI: 52–78%) and the 12-month PFS rate was 46% (95% CI: 31–

60%). In this study, tumor response did not correlate with RET mutation status[46]. The results of TKI trials are summarized in Table 1.

A limitation of TKI therapy is the development of an escape mechanism, allowing the tumor start to grow again after a variable period of treatment. This phenomenon is independent of the type of TKI used or tumor treated[47]. In such cases, a second TKI might be considered.

Tumor mutational profile and response to TKI therapy

In vitro studies have shown specific effects of TKIs on cell proliferation according to the different RET mutations; cabozantinib was the most potent inhibitor in 634 codon mutations, and vandetanib was the most effective in cells harboring M918T mutations. Most interestingly, no compound displayed superiority for all of the cell lines tested, indicating that mutation-specific therapies could be beneficial in treating MTC[48].

In a phase 3 trial, MTC patients harboring somatic RET M918T mutations exhibited a better response rate to vandetanib compared with mutation-negative patients (54.5 vs. 30.9%). However, data was inconclusive due to the sample [43]. Interestingly, overexpression of miR-375 followed by SEC23A downregulation synergistically increased the sensitivity of transfected MTC cells to vandetanib, resulting in both a decrease in cell proliferation and augmented apoptosis. These findings raise the question whether the miR-375 and SEC23A expression levels may be used as indicator of eligibility for vandetanib use. [49]. The clinical relevance of identification of cooperating oncogenic driver alterations was recently illustrated. A patient harboring RET M918T mutation developed resistance to vandetanib. Everolimus (mTOR inhibitor), which alone has limited activity against MTC, was added to vandetanib treatment and a 25% reduction has occurred [50]. Of note, preclinical studies have indicated that RET codon 804 mutations induce resistance to vandetanib[51].

A recent phase 3 trial evaluated the influence of RET and RAS (HRAS, KRAS, and NRAS) mutations on cabozantinib efficacy. The median PFS for the RET mutation-positive population was 60 weeks with cabozantinib and 20 weeks with the placebo (HR, 0.23; 95% CI, 0.14–0.38; P<.0001). Patients without RET mutation had a median PFS of 25 weeks with cabozantinib and 23 weeks with the placebo (HR, 0.53; 95% CI, 0.19-1.50). The best PFS benefit seems to occur in the RET M918T subgroup (PFS values of 61 weeks against 17 weeks with the placebo, HR, 0.15; 95% CI, 0.08-0.28; P<0001). Patients with RAS mutation had a median PFS of 47 weeks versus 8 weeks with placebo (HR, 0.15; 95% CI, 0.02-1.10). These data suggest that cabozantinib provides the best clinical benefit to patients with MTC who have RET M918T or RAS mutations[52]. Cabozantinib induces the HIF pathway in hypoxic MTC cells, which may contribute to drug-resistance by increasing the expression of the downstream factors[53].

Safety and tolerability of tyrosine-kinase inhibitor therapy

The vast majority of TKI-related adverse events (AEs) are common to the different drugs. The most common AEs associated with TKIs are diarrhea, rash, fatigue, and nausea. Hypothyroidism is also a frequent TKI side effect and increases in levothyroxine dose are often required. As a rule, these effects are tolerable (G1-G2), and the majority of AEs are managed with symptom-related treatment[54]. However, in 5–10% of cases AEs are severe or life threatening (G3–G4) and may require dose reduction, interruption, or discontinuation (Table 1). Of note, recent studies on the use of vandetanib and sorafenib to MTC treatment outside a trial observed a similar profile of AEs[38,55]. Although rare, TKI-related serious AEs leading to death have also been reported[36-41,43,45,46,56]. Interestingly, TKI toxicities have be proposed as a surrogate marker of the drug response[57].

Selecting patient and tyrosine-kinase Inhibitor

As a function of their chronic use and side effect profiles, caution is mandatory when identifying patients who might benefit from systemic TKI therapy. The criteria for initiating therapy include tumor burden and the rate of disease progression using sequential imaging and tumor markers (calcitonin and CEA doubling-times), tumor involvement that threatens vital structures that cannot be managed with localized therapy or symptomatic disease[58]. To date, it is not entirely clear which patients will benefit most from TKI therapy. To optimize therapeutic benefit, clinicians should select treatment based on patient's medical history, adverse-event tolerance, and risk factors (Table 2). If a patient is not a good candidate for vandetanib or cabozantinib, a clinical trial or other commercially available TKIs may be considered.

Conclusions and future directions

Advanced TKI therapy has changed significantly the management of MTC in the last years. However, improvement in PFS suggests potential significant clinical benefit but, to date, no compound has been shown to improve OS. Toxicities of these compounds are common and clinicians must be familiar with drug-related side effects. The low rate of partial response, absence of complete responses and the eventual tumor progression points to the need to develop of either more effective TKI or to identify synergistic combinations of therapeutic targets. Based on cumulative knowledge of TKI-associated signaling pathways, one can anticipate that a comprehensive genomic profiling of genetic alterations in MTC specimens may refine the use of these compounds.

Key Points

- Tyrosine kinase target therapy has changed the management of MTC over the last years.
- Two compounds, vandetanib and cabozantinib, has been approved as first-line treatment for metastatic MTC but several others TKIs have demonstrated variable efficacy on disease control.
- Recent experimental and clinical data indicate that the assessment of the tumor mutation status may be useful on planning the therapeutic strategies.
- To date, it is not entirely clear who will benefit most from systemic therapy, and patients should be selected taken into account the disease progression and tumor characteristics, as well as adverse-event tolerance, and risk factors.
- The use of comprehensive genomic profiling of genetic alterations to identify the oncogenic drivers involved in MTC pathogenesis will, hopefully, refine the targeted therapy in near future.

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Conflicts of interest

ALM has served as advisor/speaker for AstraZeneca and Sanofi-Genzyme within the past 2 years. ALM and CVFV have served as principal investigator and coordinator, respectively, in multicenter studies for AstraZeneca within the past 2 years. SMW has nothing to disclosure.

Table 1.Tyrosine kinase inhibitors and results of clinical trials with in thyroid cancer patients.

Investigational Drugs	Molecular Targets	Partial response / Stable disease (%)	Serious Adverse Events (Grade ≥ 3)	References
Phase I and II clinical trials				
Motesanib	VEGFR-1-3, c-Kit, RET, PDGFR	2 / 48	Diarrhea (13%), fatigue (8%), hypertension (10%)	[36]
Sorafenib	VEGFR-2-3, c-Kit, RET	6 / 50	Diarrhea (10%), hand-foot-skin reactions (14%), hypertension (10%), neurologic infection (10%)	[37,38]
Sunitinib	VEGFR-1-3, RET, c-Kit	28 / 46	Fatigue (11%), diarrhea (17%), hand/foot syndrome (17%), cytopenias (46%)	[39]
Axitinib	VEGFR-1-3, c-Kit	18 / 27	Hypertension (12%)	[40]
Imatinib	RET, c-Kit, PDGFR	0 / 27	Hypothyroidism, rash, malaise, laryngeal mucosal swelling	[41]
Lenvatinib	VEGFR-1-3, FGFRs 1- 4, PDGFRα, RET,c- KIT, SCFR	50 / 43	Weight loss (12%), hypertension (10%), proteinuria (10%), diarrhea (10%), fatigue (9%), dehydration (9%)	[46]

Drugs approved	Molecular Targets	PFS drug vs. Placebo (months)	Hazard Ratio	Serious Adverse Events (Grade ≥ 3)	References
Phase III Clinicaltrials					
Vandetanib	VEGFR-1-3, RET, EGFR	30.5 *vs*. 19.3	0.46	Diarrhea (11%), hypertension (9%), ECG QT prolonged (8%)	[43]
Cabozantinib	VEGFR-2, RET, c-MET	11.2 *vs*. 4.0	0.28	Diarrhea (15,9%), hand/foot syndrome (12,6%), fatigue (9,3%)	[45]

Table 2. Clinical and laboratorial data that may favor a particular tyrosine-kinase inhibitor as first-choice therapy for MTC

	Drug	Rationale
Medical History/ comorbities		
Long QT syndrome / arrhythmias or heart conduction defects	Cabozantinib	Vandetanib carries a higher risk for prolongation of the QT interval [43]
Hemorrhage	Vandetanib	Carbozantinib should be avoided due to higher risk of perforation or fistula [59]
Peptic ulcer disease	Vandetanib	
Diverticulitis	Vandetanib	
Laboratorial findings		
Hypocalcemia	Cabozantinib	These electrolyte abnormalities can augment the risk for vandetanib-associated arrhythmias or heart conduction defects [59]
Hypokalemia	Cabozantinib	
Hypomagnesemia	Cabozantinib	
Patient characteristics		
Low body mass index	Vandetanib	Vandetanib administration restore muscle and adipose tissues [60]
No willing/able to protect from sum exposure	Cabozantinib	Photosensitivity is a common adverse effect of vandetanib [43]
Jobs or hobbies with the use of hands (musicians) or foots (athletes)	Vandetanib	Hand/foot syndrome is a relative common side effect of cabozantinib [45]
Medication review		
Drugs causing QT prolongation	Cabozantinib	Vandetanib carries a high risk for prolongation of the QT interval and arrhythmias [43]
CYP3A4 inhibitor	Vandetanib	Concomitant use of CYP3A4 inhibitor drugs may increase serum concentration of cabozantinib [59]
CYP3A4 inducer	Cabozantinib	Concomitant use of CYP3A4 inducers may decrease serum concentration of vandetanib [59]
Tumoral characteristics		
Invasion of trachea, esophagus, or major blood vessels	Vandetanib	Carbozantinib carries a higher risk of perforation or fistula [45]
Rapid tumor progression that threatens vital structures	Cabozantinib	Cabozantinib is the only drug tested in patients with progressive MTC [45]
Mutation profile		
804 codon mutations	Cabozantinib	Pre-clinical studies have shown resistance to vandetanib[51]

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