Personalization of Targeted Therapy in Advanced Thyroid Cancer

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Abstract: Although generally the prognosis of differentiated thyroid carcinoma (DTC) is good, approximately 5% of people are likely to develop metastases which fail to respond to radioactive iodine, and other traditional therapies, exhibiting a more aggressive behavior. Nowadays, therapy is chosen and implemented on a watch-and-wait basis for most DTC patients. Which regimen is likely to work best is decided on the basis of an individual's clinical information, but only data referring to outcomes of groups of patients are employed. To predict the best course of therapy, an individual patient's biologic data is rarely employed in a systematic way. Anyway, the use of not expensive individual genomic analysis could lead us to a new era of patient-specific and personalized care. Recently, key targets that are now being evaluated in the clinical setting have been evidenced in the pathogenesis of these diseases. Some of the known genetic alterations playing a crucial role in the development of thyroid cancer include B-Raf gene mutations, rearranged during transfection/ papillary thyroid carcinoma gene rearrangements, and vascular endothelial growth factor receptor-2 angiogenesis pathways. The development of targeted novel compounds able to induce clinical responses and stabilization of disease has overcome the lack of effective therapies for DTC, which are resistant to radioiodine and thyroid stimulating hormone-suppressive therapy. Interestingly, the best responses have been demonstrated in patients treated with anti-angiogenic inhibitors such as vandetanib and XL184 in medullary thyroid cancer, and sorafenib in papillary and follicular DTC.

INTRODUCTION

The most common endocrine malignancies include thyroid carcinoma [1], and papillary (PTC) and follicular (FTC) thyroid cancer, which belong to the differentiated thyroid cancers (DTC), representing about 94% of these cases. Primary surgery, thyroid-stimulating hormone (TSH) suppressive therapy, and ablation of the thyroid remnant with radioactive iodine (RAI) constitute the standard treatments. After surgery, patients with PTC and FTC are followed by basal and TSH-stimulated thyroglobulin determination, and by neck ultrasonography [2-4]. Recurrent disease is present in about 10-15 % of patients with thyroid cancer. Although generally the prognosis of thyroid carcinoma is good, approximately 5% of patients are likely to develop metastatic disease which fails to respond to RAI, and exhibits a more aggressive behavior [1, 5-7].

Currently, therapy is chosen and implemented on a watch-and-wait basis for most thyroid cancer patients. Which regimen is likely to work best is decided on an individual's clinical information, but only data referring to outcomes of groups of patients are employed. To predict the best course of therapy, an individual patient's biologic data is rarely employed in a systematic way. Anyway, the advent of low-cost individual genomic analysis could lead to a new era of patient-specific and personalized care.

In the last 2 decades, a number of somatic mutations in various pathways of thyroid carcinomas have been shown and associated with the development and progression of these malignancies [8], and clinical research directed to these pathways has been evaluated. The significance of disease stabilization in patients with thyroid cancer is far from appreciation, as stable disease (SD) in the absence of active treatment is common and partial responses (PRs) have been reported with many of these agents. Here, we review the molecular target pathways and the drugs developed against them in dedifferentiated thyroid cancer (DeDTC) (Table **1**).

MOLECULAR PATHWAYS INVOLVED IN THYROID CANCER

RET

RET encodes a transmembrane receptor and its gene is located on chromosome 10q11.2 [9, 10]. The RET receptor has an intracellular domain which contains two tyrosine kinase (TK) regions able to activate intracellular signal transduction pathways. Once activated, RET triggers autophosphorylation of tyrosine residues that are docking sites for adaptor proteins, which coordinate cellular signal transduction pathways [e.g., phosphatidylinositol 3-kinase, mitogen-activated protein kinase (MAPK), etc.], important in the regulation of cell growth [11].

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Table 1.   Drugs and relative molecular targets, used in clinical trials in thyroid cancer.

Agent	Molecular Targets	Authors (Year)	Study Phase	Cancer Subtype/s	No. of pts with TC	No. of pts with PR (%)	No. of pts with SD (%)	Median PFS, Weeks
Sorafenib	RAF, VEGFR-1 and -2, RET, PDGFR, c-KIT	Gupta-Abramson *et al.*, 2008	II	DTC, MTC	30	7 (23)	16 (53)	79
		Kloos *et al.*, 2009	II	DTC (of which 41 PTC)	56	6 (15)	23 (56)	60
		Hoftijzer *et al.*, 2009	II	DTC	31	8 (25)	11 (34)	58
		Cabanillas *et al.*, 2010	II	DTC	15	3 (20)	9 (60)	79
		Ahmed *et al.*, 2011	II	DTC(19), MTC (15)	34	3 (15)	14 (74)	Not reported
		Brose *et al.*, 2009	II	DTC (47), MTC (3), ATC (5)	55	18 (38)	22 (47)	93,6 overall, 96 for DTC
		Marotta *et al.*, 2013	II	DTC	17	5 (30)	7 (41)	36
		Schneider *et al.*, 2012	II	DTC	31	8 (31)	11 (42)	72
		Savvides *et al.*, 2013	II	ATC	20	2 (10)	5 (25)	7.6
Sunitinib	VEGFR-2, PDGFR, c-KIT, RET, CSF-1R, FLT3	Cohen *et al.*, 2008	II	DTC (37), MTC (6)	43	4 (11) DTC	21 (57) DTC 2 (33) MTC	Not reported
		Goulart *et al.*, 2008	II	DTC, MTC	18	7 (38)		Not reported
		Ravaud *et al.*, 2008	II	DTC 8, MTC 4, ATC 1	17	1 (6)	12 (70)	Not reported
		Carr *et al.*, 2010	II	DTC 28, MTC 7	35	10 (29)	16 (46)	Not reported
Imatinib	Bcr-abl, RET, PDGFR, c-KIT	de Groot *et al.*, 2007	II	MTC	15	0 (0)	4 (27)	Not reported
		Frank-Raue *et al.*, 2007	II	MTC	9	0	7 (77) (12 weeks)	Not reported
Vandetanib	VEGFR-2, VEGFR-3, RET, EGFR	Wells *et al.*, 2010	II	MTC	30	6 (20)	9 (30)	Not reported
		Robinson *et al.*, 2010	II	MTC	19	3 (16)	10 (53)	Not reported
		Fox *et al.*, 2013	I/II	MTC	15 with M918T RET germline mutations	7 (47)	1 (7)	Not reported
		Leboulleux *et al.*, 2012	II	DTC	72+ 73 placebo	6 (8)	41 (57)	44

(Table 1) contd….

Agent	Molecular Targets	Authors (Year)	Study Phase	Cancer Subtype/s	No. of pts with TC	No. of pts with PR (%)	No. of pts with SD (%)	Median PFS, Weeks
Motesanib	VEGFR-1, -2, -3, PDGFR, c-KIT	Sherman *et al.*, 2008	II	DTC	93	13 (14)	33 (35) - ≥ 24 wks	40
		Schlumberger *et al.*, 2009	II	MTC	91	2 (2)	74 (81) - ≥ 24 wks	48
		Bass *et al.*, 2010	II	DTC, MTC	184	11/139 (8)	113/139 (81)	40 (DTC), 48 (MTC)
Axitinib	VEGFR-1, -2, and -3, PDGFR, c-KIT	Cohen *et al.*, 2008	II	DTC, MTC	60	18 (30)	23 (28) - ≥ 16 wks	72.4
Gefitinib	EGFR	Pennell *et al.*, 2008	II	DTC, MTC	27	0 (0)	5 (24) - ≥ 24 wks	14.8
Cabozantinib (XL184)	VEGFR-1 and -2, C-MET, RET, c-KIT, FLT3, and Tie-2	Kurzrock *et al.*, 2011	I	MTC	37	10/35 (29)	15/37 (41) - ≥ 24 wks	Not reported
		Zhang *et al.*, 2010	I	MTC	34	14 (41)	9 (26) - not reported	Not reported
		Cabanillas *et al.*, 2012	I	DTC	15	8 (53)	6 (40)	Not reported
Pazopanib	VEGFR-1, -2, -3, PDGFR, c-KIT	Bible *et al.*, 2010	II	DTC	37	18 (49)	Not reported	46.8
Lenvatinib (E7080)	VEGFR-1,-2,-3, PDGFRb, RET, c-KIT, FGFR-1,-2,-3,-4	Sherman *et al.*, 2011	II	DTC	58	29 (50)	Not reported	50.4

In PTC, RAS mutations are present in about 10%, RET/PTC rearrangements in 30-40%, and BRAF mutations in approximately 40-50% of cases, and no overlap has been shown among these mutations. A higher prevalence of BRAF mutations (up to 70%) has been observed in dedifferentiated PTC (DePTC) [7, 12]. To date, approximately 13 types of RET/PTC have been reported, in particular RET/PTC1 and RET/PTC3. RET/PTC is tumorigenic in thyroid follicular cells [13]. Medullary thyroid cancer (MTC) derives from the calcitonin-producing neuroendocrine cells of the thyroid gland and is related to about 5% of all thyroid malignancies [14, 15]. About 80% of cases of MTC are sporadic, while 20% constitute a component of multiple endocrine neoplasia syndrome type 2 (MEN2). Activating mutations of TK receptor RET are associated with the pathogenesis of MTC and have been demonstrated in nearly all hereditary and in 30-50% of sporadic MTC cases [16], making this receptor an excellent target for small-molecule inhibitors for this tumor.

Raf Kinase Pathway

The most common genetic alterations found in patients with thyroid cancer are B-Raf gene mutations, occurring in about 45% of sporadic PTCs [17, 18]. Among them, the V600E mutation (T1799A) in the exon 15, which is present in 77.8% of patients with recurrent disease, represents >90%

of B-Raf mutations [18]. The presence of B-Raf mutations in PTC has been independently associated with the tumor recurrence, the absence of tumor capsule and tumor iodine (I131) avidity, and treatment failure of recurrent disease [19]. The inhibitors of Raf kinase activity have been demonstrated to be able to effectively inhibit the growth of DTC cell lines that harbor mutations in RET or B-Raf, *in vitro* [20].

Vascular Endothelial Growth Factor (VEGF) Pathway

Growth factors that stimulate or inhibit the formation of new blood vessels control the complex process of angiogenesis. VEGF-A, VEGF-B, and VEGF-C belong to the VEGF family. Of these, VEGF-A is the major mediator of tumor angiogenesis, promotes the proliferation and survival of endothelial cells and increases vascular permeability [21]. High levels of both angiopoietin-2 and VEGF are expressed in DTC, because of up-regulation of its main receptor, VEGFR-2, in comparison with normal thyroid [22-24]. The increased expression of VEGF in thyroid cancer has been associated with the presence of distant metastasis, an increase in tumor size, and a poor prognosis [23, 25].

Epidermal Growth Factor (EGF) Receptor (EGFR)

The cell-surface receptor for members of the EGF-family of extracellular protein ligands is EGFR (ErbB-1; HER1 in

humans) [26]. The EGFR belongs to the ErbB receptors family, a subfamily of four closely related receptor TKs: EGFR (ErbB-1), HER2/c-neu (ErbB-2), Her 3 (ErbB-3) and Her 4 (ErbB-4). Different types of cancers, including lung cancer, anal cancers [27] and glioblastoma multiforme are associated with mutations that lead to EGFR overexpression, or overactivity. Amplifications, mutations, or misregulations of EGFR (or one of the other family members) are implicated in approximately 30% of all epithelial cancers. In anaplastic thyroid cancer (ATC), EGFR is overexpressed and implicated in invasion and tumor progression in thyroid cancer [28-30].

RAS

Codons 12, 13, and 61 of NRAS, HRAS and KRAS within RAS genes are involved in point mutations, and in particular mutations of NRAS and HRAS at codon 61 and mutations of KRAS at codon 12/13 are the most common. The PI3K/AKT and MAPK pathways are constitutively activated by mutant RAS proteins, but RAS mutations are not restricted to a particular histological subtype of thyroid tumor, differently from the other markers. RAS mutations are evidenced in about 10-15% PTCs (higher in follicular variant of PTC) and are more prevalent in FTC (40-50%). Approximately 35% of poorly differentiated carcinomas and ~50% of ATCs show the presence of RAS mutations, which seem to correlate with a more aggressive tumor behavior [31, 32]. Moreover, RAS mutations are present in 20-40% of follicular adenoma, but it is not clear whether these tumors represent pre-invasive follicular carcinomas.

PAX8/peroxisome Proliferator-activated Receptor (PPAR)γ Rearrangements

About 30-40% of conventional FTCs and ~5% of oncocytic carcinomas show PAX8/PPARγ rearrangements [33]. Tumors associated with PAX8/PPARγ usually show a good prognosis, and do not carry any RAS mutation, suggesting that the development of FTC involves two different independent pathways, either PAX8/PPARγ translocation or RAS mutation [33]. PAX8/PPARγ rearrangements are even evidenced in 2-10% of follicular adenomas, and in the follicular variant of PTC [34, 35], while have been reported in a very low percentage (0-1%) of PTC [34].

TARGETED THERAPY FOR THYROID CANCER

RET Pathway

The TK inhibitor (TKI) imatinib has been approved by the US Food and Drug Administration (FDA) and European Agency for the Evaluation of Medicinal Products (EMEA) for the treatment of gastrointestinal stromal tumor and chronic myelogenous leukemia [36-40]. In cells expressing the bcr-abl translocation, platelet-derived growth factor (PDGF) receptor (PDGFR), stem cell factor, and c-Kit, imatinib inhibits proliferation and induces apoptosis [41].

As c-Kit and RET belong to the same subfamily of TK receptors, imatinib has been tested for its capacity to achieve growth inhibition of MTC. It is not clear whether imatinib can inhibit RET *in vitro* [41-43].

A phase II study enrolled 15 patients with confirmed diagnosis of MTC, who were treated with imatinib 600

mg/daily and in the case of objective response the dose was escalated to 800 mg/daily [44]. The median duration of treatment was 4 months and no objective responses were evidenced [44]. Subsequently, another study enrolled 9 patients who received imatinib at 600 mg/daily with a median duration of treatment of 13 months. Seven patients had SD after 3 months of treatment, and only 1 of these patients remained in SD at 12 months. The median progression free-survival (PFS) was 6 months and no clinical response was shown [45].

The pyrazolo[3,4-d]pyrimidines PP1 [46], PP2 [47], and Si34 [48] have been investigated in thyroid cancer. The PP1 pyrazolopyrimidine exerted potent inhibitory effects on RET kinase [46], while PP2 pyrazolopyrimidine, reduced RET/PTC1-mediated MAPK signaling. Moreover, PP2 pyrazolopyrimidine inhibited the invasive phenotype and the proliferation of human thyroid carcinoma cells sustaining RET/PTC1 rearrangements [47]. However, PP2 acted as a good inhibitor of c-Src and related kinases too, it was not selective for RET [49]; for this reason, it was not possible to exclude additional indirect effects of PP2, mediated by the inhibition of other kinases *in vivo*. The same was also true for Si34 [48], whose inhibitory effects on two human tumor cell lines derived from MTC, namely TT and MZ-CRC-1, were due to the inhibition of the TK c-Src.

More recently, two novel pyrazolo[3,4-d]pyrimidine derivatives have been reported (CLM3 and CLM29), which were able to inhibit the proliferation of primary cells of DePTC *in vitro* by increasing apoptosis. Furthermore, CLM3 and CLM29 inhibited the migration of DePTC cells. Interestingly, in the primary DePTC cells the anti-proliferative action of CLM3 and CLM29 observed was independent from the presence or absence of RET/PTC or BRAF mutation. These results concur well with the concept that CLM3 and CLM29 are proposed for a multiple signal transduction inhibition (including the RET, TK, EGFR, VEGFR) and they have an anti-angiogenic effect [50, 51].

Raf Kinase Pathway

The orally active multi-kinase inhibitor (mKI) sorafenib targets VEGFR-1 and -2, B-Raf, RET, and c-Kit. Its effects on RET, the B-Raf pathway (previously described), and angiogenesis render it a potentially effective agent for patients with thyroid cancer. Two phase II clinical trials have been published about the use of sorafenib in patients with metastatic iodine refractory thyroid carcinoma. The first trial enrolled 56 patients and was conducted by Kloos *et al.* [52] (PR was shown in 6/41 PTC patients, and SD>6 months in 23 patients). The median PFS was 15 months and median duration of PR was 7.5 months. The second phase II trial, in which 30 patients were treated with sorafenib 400 mg orally twice daily (b.i.d.), was conducted by Gupta-Abramson *et al.* [53]. Sixteen patients had SD lasting 14 to 89 weeks and 7 patients had PR lasting 18 to 84 weeks; median PFS was 79 weeks. Among the patients in whom serial thyroglobulin levels were present, 95% showed a decrease in thyroglobulin levels (mean decrease of 70%). Considering toxicity, a single patient died of liver failure [53].

An open-label phase II study of sorafenib, presented in the 2009 American Society of Clinical Oncology (ASCO)

annual meeting by Brose *et al.*, enrolled 55 patients with metastatic, iodine-refractory thyroid carcinoma and reported an increased PFS for patients with B-RafV600E, with respect to patients with wild-type B-Raf (84 vs. 54 weeks; p 1⁄4 .028) [54]. Final results of this trial using sorafenib are awaited.

In the same year, a phase II study was published; the re-induction of RAI uptake was the primary endpoint. Among 31 patients with progressive metastatic or locally advanced radioiodine refractory DTC who received sorafenib at 800 mg/daily, no re-induction of RAI uptake at metastatic site was evidenced after 26 weeks of therapy, but 25% of patients showed a PR, 34% SD and PFS was 58 weeks [55].

Another study evaluated sorafenib in the treatment of 15 DTC (8 PTC, 7 FTC) patients refractory to radioiodine; PR was observed in 3 (20%), SD in 9 (60%), and median PFS was 79 weeks. Interestingly, lung metastases responded better than lymph node metastases to the sorafenib treatment [56].

A phase II trial, conducted in UK, enrolled 34 patients (15 MTC, and 19 with DTC) treated with sorafenib 400 mg b.i.d.. The radiological response rate (RR) was more significant at 12 than at 6 months (21% and 15% respectively), SD was observed in 74% at 6 months and most of the patients required a dose reduction for adverse events (AEs). A dramatic response was reported after three months of therapy in one patient with mutation of BRAF V600E (however, BRAF was determined in only 3 patients) [57].

In the 2011 ASCO annual meeting, Keefe *et al.* presented another phase II study, evaluating anti-tumor activity of sorafenib in advanced thyroid cancer (47 iodine-refractory DTC or poorly DTC, 5 ATC, 3 MTC). A rate of 38% and 47% for PR and SD respectively was observed in DTC patients, while the PFS was 96 weeks. In 66% tissues from these patients at least 1 mutation was evidenced (45% BRAF, 19% RAS, 11% RET, 9% PIK3CA), while in 17% multiple mutations [58].

On the basis of previous data, the International, double-blind, multicenter, randomized phase III trial DECISION (stuDy of sorafEnib in loCally advanced or metastatIc patientS with RaIrefractory thyrOid caNcer) has been designed. In order to test if sorafenib improves PFS in these patients, 380 patients with locally advanced or metastatic RAI-refractory DTC (PTC, FTC, Hürthle cell, or poorly differentiated carcinoma), have been randomized 1:1 to receive sorafenib 800 mg/daily or placebo. The trial is ongoing and results are being awaited [59, 60].

In September 2012, a retrospective, longitudinal study evaluating the activity of oral sorafenib in patients with progressive RAI-refractory DTC was published. The drug was administrated at 400 mg twice daily to 17 patients and was generally well tolerated, but 3 fatal events for bleeding were reported. However, PR was observed in 30% of patients, progressive disease (PD) in 18%, and SD in 41%, median PFS was 9 months, and median overall survival (OS) 10 months. These results were associated with worse baseline clinical condition of patients with respect to other studies. The radiological response was more pronounced in lymph nodes than lung metastases. All patients needed dose reduction for AEs [61].

Finally, a phase II study was done to determine the long-term effects of sorafenib in patients with advanced RAI-refractory DTC. In this trial, 31 patients were treated at conventional dose of sorafenib; PR was seen in 31% and SD in 42% after a median follow-up of 25 months [62].

A multi-institutional phase II trial of sorafenib was conducted in patients with ATC who had failed up to previous therapies. Among them, 20 patients were treated with sorafenib 400 mg twice daily. Two/20 (10%) patients had a PR and 5/20 (25%) had SD. The overall median PFS was 1.9 months, the median and a 1 year survival being 3.9 months and 20%, respectively. The Authors conclude that sorafenib is active in ATC even if at a low frequency [63].

VEGF PATHWAY

Vandetanib

The orally bioavailable mKI vandetanib targets EGFR, VEGFR-2 and -3, and RET kinases, and is a promising agent for MTC treatment because of its effects on both RET activation and angiogenesis [64]. Two phase II trials on vandetanib have been conducted in patients with MTC. The first one was conducted by Wells *et al.* [65] in patients with RET germline mutation and locally advanced or metastatic hereditary MTC, treated with vandetanib 300 mg daily. Thirty patients were enrolled, and PR was reported in 6 patients (20%) and SD in other 9 subjects (30%). The observed AEs (presented in >50% of the patients) were diarrhea, rash, nausea, fatigue, and asymptomatic QTc prolongation (17%) [65]. Robinson *et al.* [66] conducted the second trial on vandetanib 100 mg in 19 patients (79% with confirmed RET germline mutation). In eligible patients, post-progression dose increment to vandetanib 300 mg was evaluated. Three patients showed confirmed objective PRs, yielding an objective RR of 16% (95% confidence interval 3.4-39.6). In other 10 patients (53%), SD lasting 24 weeks or longer was reported; therefore, the disease control rate was 68% (95% confidence interval 43.4-87.4) [66].

In the 2010 ASCO meeting, the results of the ZETA trial, a double-blind, randomized, phase III trial in patients with locally advanced or metastatic MTC using 300 mg/daily of vandetanib vs. placebo, were shown. The trial included 331 patients with mean age of 52 years. A positive RET mutation status was shown in 56% of the patients. A statistically significant PFS, overall RR, biochemical response and disease control rate, were observed for vandetanib vs. placebo after a median follow-up of 24 months [67]. An improved PFS using mKI in patients with MTC was first shown by this phase III trial; for this indication, the US FDA and EMA approved vandetanib [68].

The improved PFS has been reported in a double-blind phase II study recently published. One hundred and forty-five patients with metastatic or locally advanced DTC (papillary, follicular, or poorly DTC) were enrolled and randomized 1:1 to receive 300 mg vandetanib (72 patients) or placebo (73 patients). The results obtained at the end of the study showed a PFS longer in the vandetanib group (11.1 months) with respect to the placebo group (5.9 months). The PR and the SD were 8% and 57% respectively for the patients treated with TKI in the vandetanib group, while for the

other group were 5% and 42% respectively. The safety and tolerability in this study were consistent with previous studies of vandetanib [69].

Recently, a phase I/II trial of vandetanib for adolescents (13-18 years) and children (5-12 years) with MTC has been conducted. In this trial, 16 patients with metastatic or locally advanced MTC were treated. Vandetanib 100 mg/m(2)/d was demonstrated to be a well-tolerated, and highly active treatment for adolescents and children with locally advanced or metastatic MTC and MEN2B [70].

Motesanib

The orally bioavailable mKI motesanib diphosphate is a highly selective inhibitor of PDGFR, VEGFRs (-1, -2 and -3), and c-Kit and inhibits cellular proliferation and angiogenesis. In patients with metastatic or advanced radioiodine-resistant thyroid cancer, 2 phase II trials on motesanib diphosphate (125 mg orally once daily) have been performed. Sherman *et al.* [71] treated 93 patients with DTC, of whom 57 (61%) had PTC. The overall RR was 14%, 8% had PD, and SD was achieved in 67% of the patients and persisted for 24 weeks or longer in 35% of them. The median PFS was 40 weeks. The most common treatment-related AEs were hypertension (56%), diarrhea (59%), fatigue (46%), and weight loss (40%) [71]. The second phase II trial by Schlumberger *et al.* [72] was conducted in 91 patients with MTC treated with motesanib 125 mg/daily. PR was evidenced in 2 patients (2%), SD in 81%, and median PFS was 48 weeks. In 83% and 75% of patients, respectively, a decrease in carcinoembryonic antigen (CEA) and serum calcitonin during treatment was observed. Diarrhea, hypertension, fatigue, hypothyroidism, and anorexia were the most common treatment-related AEs in 29% of the patients, similarly to other trials with motesanib [72]. Bass *et al.* [73] enrolled 184 patients (DTC=93, MTC=91) in a recent phase II trial, and treated them with motesanib 125 mg/day orally for up to 48 weeks. About half of the patients (48%) with MTC achieved SD for at least 24 weeks, and more than 2/3 (76%) of patients had a reduction of the tumor size compared to baseline.

Axitinib

The mKI axitinib targets PDGFR, VEGFR-1, -2, and -3, and c-Kit. Axitinib has great selectivity against VEGFR-2, and is considered the most potent VEGFR-2 inhibitor available. A strong activity of axitinib against thyroid cancer was demonstrated by a phase I clinical trial [74], and by another phase II trial on 60 patients with advanced, iodine-refractory thyroid cancer using axitinib 5 mg b.i.d [75]. PR was shown in 18 patients (30%; 8 patients with PTC, 6 FTC, 2 MTC, and 1 ATC). Moreover, SD was also observed in other 23 patients (38%) and median PFS was found to be 18.1 months (72.4 weeks). Hypertension was the most common toxicity, 3 patients showed grade 4 toxicity (including hypertension, stroke, and reversible posterior leukoencephalopathy), and 8 patients (13%) discontinued the therapy because of AEs.

Sunitinib

The mKI sunitinib targets c-Kit, VEGFR-2, PDGFR, RET, FLT-3, and CSF-1R, that have an important role in the

development of thyroid cancer [22-24, 64]. Sunitinib is a potent inhibitor of RET/PTC onco-proteins, as demonstrated by preclinical studies, decreasing STAT3 activation, RET/PTC autophosphorylation, and blocking the transforming capacity of RET/PTC [76]. It also inhibits cell growth on a thyroid carcinoma cell line (TPC-1) that spontaneously harbors RET/PTC rearrangement [76]. In the 2008 ASCO annual meeting, the preliminary results of two phase II trials with sunitinib were presented. The first one included 43 subjects with MTC and DTC, treated with sunitinib (50 mg/daily on a 4-week-on/2-week-off schedule) [77]. In the 31 DTC patients, the overall RR was 13%, with SD in 68% of them, while no responses were evidenced in patients with MTC, even though an SD of 83% was observed [77]. Thrombocytopenia, neutropenia, fatigue, hypertension, palmar-plantar erythrodysesthesia, and gastrointestinal symptoms were the observed AEs. Sunitinib was given at 37.5 mg/daily to 2-deoxy-2-[18F]fluoro-D-glucose-positron emission tomography (FDG–PET) avid advanced thyroid cancers, in the second trial [78]. Fifteen patients had DTC and 3 patients had MTC; 7 patients showed the FDG-PET RR, all of them with DTC histology; the RECIST RR was still being evaluated at the time of report [78].

In the same meeting, the results for first period of a phase II study (known as THYSU study) were presented [79]. Primary endpoint was objective response and secondary endpoints were safety, OS and time to progression in patients with advanced thyroid cancer refractory to RAI (1 anaplastic, 4 medullary, 8 papillary and 4 other thyroid cancer) treated with sunitinib (50 mg/daily). Twelve patients had SD, with 1 patient with >90% decrease of thyroglobulin and 1 patient with a dramatic decrease of symptoms, and 1 patient had a PR [79].

Carr *et al.* [80] have presented the results from the largest open-label phase II trial, which included 28 patients with progressive DTC and 7 patients with MTC. They showed complete response in 1 patient, PR in 28%, and SD in 46% of patients. Further analysis suggested that reduction in FDG–PET was a predictor of PR or SD [80].

Cabozantinib (XL184)

Another promising therapeutic agent against thyroid cancer is the oral multiple-receptor kinase inhibitor cabozantinib (XL184). VEGFR-1 and -2, C-MET, RET, c-Kit, FLT3, and Tie-2 are its targets [81, 82]. Kurzrock *et al.* [83] conducted a phase I trial in 37 MTC patients. One hundred and seventy-five mg/daily was the maximum-tolerated dose found. Ten/35 (29%) patients with measurable disease had PR, while 15/37 (41%) had SD for 6 months or longer. Diarrhea, nausea, mucosal inflammation, anorexia, fatigue, increased AST, hypertension and vomiting were the frequent treatment-related AEs. Substantial reductions in plasma calcitonin and CEA were observed in most MTC patients. Zhang *et al.* enrolled 34 patients with advanced MTC, 14 of which (41%) experienced a PR [84]. In a recent phase I study by Cabanillas *et al.*, 15 DTC patients were treated with cabozantinib; 8/15 (53%) had a PR, while 6/15 (40%) had an SD [85]. In November 2012, FDA has approved cabozantinib for the treatment of progressive, metastatic MTC [86]. The approval was based on the demonstration of substantial PFS

prolongation with cabozantinib compared to placebo (median PFS was 11.2 and 4 months respectively) in 330 patients with metastatic MTC [87]. Subsequently, the EMA has accepted for review the Marketing Authorization Application for the same indication [88].

Recently, the *in vitro* biochemical and cellular inhibitory profile of cabozantinib against RET was evaluated, as well as its anti-tumor efficacy *in vivo* (using a xenograft model of MTC). Multiple forms of oncogenic RET kinase activity were inhibited by cabozantinib, in biochemical assays. Additionally, the proliferation of TT tumor cells that harbor a C634W activating mutation of RET was inhibited. The oral administration of cabozantinib in these same cells grown as xenograft tumors in nude mice caused a dose-dependent tumor growth inhibition that correlated with a reduction of the levels of circulating plasma calcitonin. These results demonstrated that cabozantinib effectively inhibits the growth of MTC *in vivo* and *in vitro* [89].

Pazopanib

PDGFR, VEGFR-1, -2, and -3, and c-Kit are inhibited by pazopanib, a small-molecule inhibitor, that has been approved for the treatment of renal cell carcinoma [90]. Thirty-nine DTC patients (37 were assessed) were enrolled in a phase II trial using pazopanib 800 mg/daily; 18/37 (49%) patients confirmed PR [91]; 88% of the patients had a significant reduction ($\geq 30\%$) in baseline thyroglobulin levels; twenty-two patients (59%) had a progression of disease; and a median PFS of 11.7 months (46.8 weeks) was observed [91]. AEs included skin and hair hypopigmentation, fatigue, alopecia, nausea, diarrhea, vomiting, altered tasted, anemia, and leucopenia.

Lenvatinib (E7080)

Lenvatinib (E7080) is an oral inhibitor of PDGFRb, VEGFR-1, -2, -3, RET, fibroblast growth factor receptors-1, -2, -3, -4, and c-KIT. It inhibits tumor cell invasion and migration, but it does not significantly affect tumor cell proliferation [92].

Sherman *et al.* enrolled 58 patients with advanced DTC and administered them with 24 mg/day of lenvatinib. Twenty-nine (50%) patients had a PR, while median PFS was 12.6 months (50.4 weeks). Hypertension, fatigue and diarrhea were the most frequent AEs [93].

CLM94, CLM3

Recently, Antonelli *et al.* have shown the anti-tumoral activity of a novel cyclic amide, CLM94, with VEGFR-2 and anti-angiogenic activity, in primary ATC cells (ANA) both *in vitro* and *in vivo* [94].

Another mKI, CLM3 can inhibit *in vitro* the proliferation of ANA, inducing also apoptosis. CLM3, significantly decreased the *VEGF-A* expression and microvessel density in ANA, and furthermore inhibited EGFR, AKT and ERK1/2 phosphorylation, and down-regulated cyclin D1, in these cells. These results showed that the anti-tumor and anti-angiogenic activity of CLM3 is very promising in ATC, opening a future avenue to clinical evaluation [95].

VASCULAR DISRUPTING MECHANISM

Combretastatin

Combretastatin A4 phosphate, a microtubule depolymerizing agent, exhibits selectively the activity against established tumor vascular networks, producing grave interruption of tumor blood flow and the necrosis of the tumor mass [96]. In a phase I trial conducted on ATC, one patient treated with combretastatin reported a complete response, and was alive 30 months after treatment [97]. Combretastatin was given intravenously at 45 mg/m^2 in a phase II trial, that was conducted in 18 metastatic ATC patients (and no prior therapy) [98]; no objective responses were seen (33% of the patients had SD, PFS was 7.4 weeks). Common AEs included vomiting, mild to moderate nausea, tumor pain and headache.

EGFR PATHWAY

Gefitinib

The small molecule EGFR-TK inhibitor gefitinib has been shown to be effective in the treatment of patients with non-small cell lung cancer, in the presence of activating mutations of the EGFR gene [99]. RET/PTC1 and RET/PTC3 up-regulate EGFR expression, with a magnitude of induction similar to that seen with TSH [100]. Moreover, gefitinib inhibits cell growth in thyroid cancer lines and in RET-transfected cell lines at submicromolar concentrations [100]. The EGFR kinase inactivation induced by gefitinib potentiates the ionizing radiation-induced inhibition of cell proliferation on FTC and anaplastic cell lines [101].

In a patient with ATC treated with an intermittent high-dose gefitinib, and fixed-dose docetaxel, a PR was shown while being on dose level 2 (1500 mg) of gefitinib [102]. In a phase II trial, patients with advanced or metastatic thyroid cancer (DTC=18, MTC=4, etc.) were treated with gefitinib (250 mg/daily), and reported tumor volume reductions in 32% of cases (none of them met criteria for PR); 48% of them attained SD at 3 months, while 12% and 24% at 12 and 6 months, respectively. The OS and median PFS were 17.5 months (70 weeks) and 3.7 months (14.8 weeks), respectively, suggesting that gefitinib could not have clinically significant activity as monotherapy. The most common AEs were rash, diarrhea, anorexia, and nausea [103].

TARGETING PPARγ

PPARγ belongs to a superfamily of nuclear hormone receptors [104], and its activation elicits anti-neoplastic [104] and anti-inflammatory effects [105] in different types of mammalian cells. Ligands for PPARγ induce apoptosis and exert anti-proliferative effects on human PTC cells [106], and in nude mice *in vivo* prevent distant metastasis of BHP18–21 tumors [106], and induce redifferentiation in thyroid cancer [107-110]. The expressions of the PPARγ gene and protein were evaluated in 5 human anaplastic cancer cell lines [111]: a higher level of the PPARγ gene and protein expression was shown in the 5 ATC cell lines than that in PTC. PPARγ ligands inhibited cell proliferation inducing apoptosis and down regulated the invasive potential of these cell lines [111]. Other studies have confirmed these results [109, 112]. The role of PPARγ in ATC cell lines (OCUT-1, ACT-1) was evaluated also by Chung *et al.* [112]: PPARγ

was expressed and functional in both cell lines. The activation of PPARγ by its specific ligands (troglitazone and 15-deoxy-delta 12,14-prostaglandin J2) induced growth suppression in ATC cells via a p21- and p27-dependent cytostatic and a p53-independent pathway. The biological effects of the two PPARγ agonists, ciglitazone and rosiglitazone, were investigated by Aiello *et al.* [113] in ATC cell lines. Ciglitazone and rosiglitazone showed complex biological effects in ATC cells, as inhibition of migration and growth, and increased apoptosis rate. Since rosiglitazone increased the expression of thyroid-specific differentiation markers, it was suggested that in ATC cells PPARγ agonists induce a partial reversion of the epithelial mesenchymal transition. More recently, it has been shown that the high-affinity PPARγ agonist, RS5444, inhibits proliferation of ATC cells, inducing the cyclin-dependent kinase inhibitor p21; the reactivation of suppressed RhoB is a critical step for the inhibition of ATC growth [114].

Furthermore, it has been recently shown that PPARγ agonists, rosiglitazone and pioglitazone, are able to inhibit cell growth, and proliferation in primary ATC cells obtained by fine needle aspiration (FNA) in these patients [7, 115]. The results of *in vitro* chemosensitivity tests with PPARγ agonists, in primary ATC cells obtained directly from FNA, were similar to those obtained from surgical biopsies [116, 117].

A phase I study was initiated to determine the potential effectiveness of efatutazone (an oral PPARγ agonist), and paclitaxel, in 15 ATC patients. Six patients received 0.3 mg of efatutazone, 7 patients received 0.15 mg, and 2 patients 0.5 mg. One of the patients treated with 0.3 mg of efatutazone had a PR from day 69 to day 175; 7 patients attained SD. In patients receiving 0.15 mg of efatutazone and 0.3 mg of efatutazone, median times to progression were 48 and 68 days, respectively; corresponding median survival was 98 vs. 138 days. Ten patients had grade 3 or greater AEs, with 2 of these (edema and anemia) related to efatutazone. This study suggests that efatutazone and paclitaxel in combination were well tolerated and safe, and had biologic activity in ATC [118].

RESISTANCE TO TARGETED THERAPIES

Despite some impressive progresses, clinical experience suggests that most of treatment-responsive patients could experience relapse as a result of acquired drug resistance [119].

For this reason, the study of the mechanisms by which drug resistance develops and the production of second-generation drugs to combat resistance is important [119-122].

The acquired resistance to targeted therapies is often associated with genomic changes originally present in minimal sub-clones of cancer cells; these range, from the amplification of a completely different cancer gene, to an additional point mutation within the gene that encodes the protein to which the drug is targeted [123].

For example, at least 50% of chronic myeloid leukemia (CML) patients who show relapse, while on imatinib therapy, harbor secondary mutations (at least 40) within the Abl

kinase domain. Reduced drug sensitivity seems to be conferred by all of these mutations [123]. For these reasons, second-generation Abl inhibitors have been developed (such as nilotinib and dasatinib) [121, 122], that can bypass the resistance of most of the imatinib-refractory Abl mutations, showing significant clinical activity in relapsed CML patients.

Other combination strategies involving targeting the protein products of the primary mutated gene and of other mutated genes have been evaluated. If in the primary tumor there are resistant clones at low frequency, their detection at an early stage could suggest the use of combination strategies that could minimize the possibilities of the resistant clones ever expanding.

For the above mentioned reasons, the identification of new active compounds against aggressive DTC is needed [94, 95].

OTHER TARGETED THERAPIES LIMITS

Some limitations in the selective use of novel compounds still exist, but the possibility of testing the sensitivity to different TKIs of primary thyroid cancer cells obtained from each subject could increase the effectiveness of the treatment [7, 94, 115].

Disease orientated *in vitro* drug screening in human tumor cell lines [124] can lead to a negative predictive value of 90%, and a positive predictive value of 60% [125] for the activity of clinical responses, allowing to avoid the administration of inactive chemotherapeutics to patients. The discrepancy between *in vitro* and *in vivo* results could be explained by several reasons: 1) the drug may be inactivated and/or metabolized in the tumor or in the body by different organs (liver, kidney, etc.); 2) cells could become resistant to the drug; 3) the growth curve of certain tumors is associated to a response to chemotherapeutics, as well. To date, primary thyroid cancer cell cultures have been obtained from surgical biopsies after therapeutic or diagnostic procedures. Bravo *et al.* [126] reported the establishment of primary cultures by FNA biopsy (FNAB) in 1 patient. However, in patients with thyroid cancer, reports of cutaneous needle track seeding after FNAB have been published [127, 128]; FNA cytology by-passes this problem. The possibility to obtain "primary cell culture directly from FNA cytology samples of ATC" (FNA-ANA) paves the way to the use of FNA-ANA to test the sensitivity in each patient to different drugs. This could avoid unnecessary surgical procedures, and the administration of inactive therapeutics [7, 115-117].

CONCLUSION

Among the different molecular pathways evaluated, RET, B-Raf, and VEGFR-2 seem to be the targets with the highest clinical significance in the progression of thyroid cancer [129]. The development of novel compounds that target genetic alterations playing a crucial role in the development of thyroid cancer has led to the introduction of new drugs able to induce clinical responses, that overcome the lack of effective therapies for DTC resistant to radioiodine and TSH-suppressive therapy [130]. The best responses have been demonstrated in patients treated with anti-angiogenic

inhibitors such as vandetanib and XL184 in medullary thyroid cancer, and sorafenib in papillary and follicular DTC. However, the effects on survival of TKI therapies are modest, and furthermore resistance and "escape" to TKIs treatments have been described. To reach the goals to extend life duration assuring a good quality of life, the identification of new compounds is needed. Furthermore, the advent of not expensive individual genomic analysis could lead to a new era of patient-specific, personalized care [131]. Moreover, the possibility to test these novel drugs in primary thyroid cancer cells (obtained from each patient) *in vitro,* could help improve the personalization of the treatment, avoiding the administration of inactive therapeutics.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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ABBREVIATIONS

DTC	=	Differentiated thyroid cancer
PTC	=	Papillary thyroid cancer
FTC	=	Follicular thyroid cancer
TSH	=	Thyroid-stimulating hormone
RAI	=	Radioactive iodine
PRs	=	Partial responses
SD	=	Stable disease
DeDTC	=	Dedifferentiated thyroid cancer
TK	=	Tyrosine kinase
MAPK	=	Mitogen-activated protein kinase
DePTC	=	Dedifferentiated PTC
MTC	=	Medullary thyroid cancer
MEN2	=	Multiple endocrine neoplasia syndrome type 2
VEGF	=	Vascular Endothelial Growth Factor
EGFR	=	Epidermal growth factor receptor
ATC	=	Anaplastic thyroid cancer
(PPAR)γ	=	Peroxisome proliferator-activated receptor
TKI	=	TK inhibitor
FDA	=	Food and Drugs Administration
EMEA	=	European Agency for the Evaluation of Medicinal Products
PDGF	=	Platelet-derived growth factor
PDGFR	=	PDGF receptor
PFS	=	Progression free-survival
mKI	=	Multi-kinase inhibitor
ASCO	=	American Society of Clinical Oncology

RR	=	Response rate
AEs	=	Adverse events
PD	=	Progressive disease
OS	=	Overall survival
CEA	=	Carcinoembryonic antigen
FDG–PET	=	2-deoxy-2-[18F]fluoro-D-glucose-positron emission tomography
ANA	=	Primary ATC cells
FNA	=	Fine needle aspiration
CML	=	Chronic myeloid leukemia
FNAB	=	FNA biopsy
FNA-ANA	=	FNA samples of ATC

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