Advances in thyroid cancer treatment: latest evidence and clinical potential

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***Abstract*:** Advanced thyroid carcinoma is an infrequent tumor entity with limited treatment possibilities until recently. The extraordinary improvement in the comprehension of genetic and molecular alterations involving the RAS/RAF/mitogen-activated protein kinase and phosphatidylinositide 3-kinase/Akt/mammalian target of rapamycin signaling and interacting pathways that are involved in tumor survival, proliferation, differentiation, motility and angiogenesis have been the rationale for the development of new effective targeted therapies. Data coming from phase II clinical trials have confirmed the efficacy of those targeted agents against receptors in cell membrane and cytoplasmic molecules. Moreover, four of those investigational drugs, vandetanib, cabozantinib, sorafenib and lenvatinib, have reached a phase III clinical trial with favorable results in progression-free survival and overall survival in medullary thyroid carcinoma and differentiated thyroid carcinoma. Further analysis for an optimal approach has been conducted according to mutational profile and tumor subtypes. However, consistent results are still awaited and the research for adequate prognostic and predictive biomarkers is ongoing. The following report offers a comprehensive review from the rationale to the basis of targeted agents in the treatment of thyroid carcinoma. In addition, current and future therapeutic developments by the inhibition of further molecular targets are discussed in this setting.

Keywords: angiogenesis, phosphatidylinositide 3-kinase/Akt/mammalian target of rapamycin, RAS/RAF/mitogen-activated protein kinase, targeted agents, thyroid carcinoma

Introduction

Thyroid carcinoma (TC) is a rare tumor entity representing 1% of all oncological diagnoses [Tuttle *et al.* 2013]. The most frequent subtype is the differentiated TC (DTC) derived from epithelial cells. This first group includes papillary (PTC, 80%), follicular (FTC, 11%) and other less frequent histologic subtypes, such as Hürthle cells, insular, poorly differentiated TC (PDTC) and follicular variant of PTC or tall cell carcinoma. The second group is represented by medullary TC (MTC) derived from the calcitonin-producing parafollicular cells (C cells) of the thyroid gland and accounts for 5–10% of all TCs [Pusztaszeri *et al.* 2014]. Finally, the anaplastic TC (ATC) is a highly aggressive tumor present in only 2% of patients, followed by other subtypes even less frequently, such as lymphomas or sarcomas from the thyroid gland.

For the last 5–10 years, major serious efforts have been made to improve investigation into the molecular pathways and critical alterations involved in the tumorigenesis of TC and, in the latter, to increase the therapeutic possibilities for patients with TC based on targeted therapies [Xing *et al.* 2013] (Figure 1).

What have we learned recently about molecular processes of DTC and MTC?

Tumorigenesis of DTC

The main oncogenic pathways involved in initiation and progression of thyroid carcinogenesis are the RAS/RAF/mitogen-activated protein kinase (MAPK) and phosphatidylinositide 3-kinase (PI3K)/Akt pathways because of their relevance in survival, proliferation, differentiation and motility [Nikiforov and Nikiforova, 2011] (Figure 2). Progressive tumor dedifferentiation involves the sum of activated kinases or inactivated tumor suppressor genes. This tumor is, at last, less dependent on thyrotropin stimulation [Guerra *et al.* 2014]. Disorders such as RAS and BRAF

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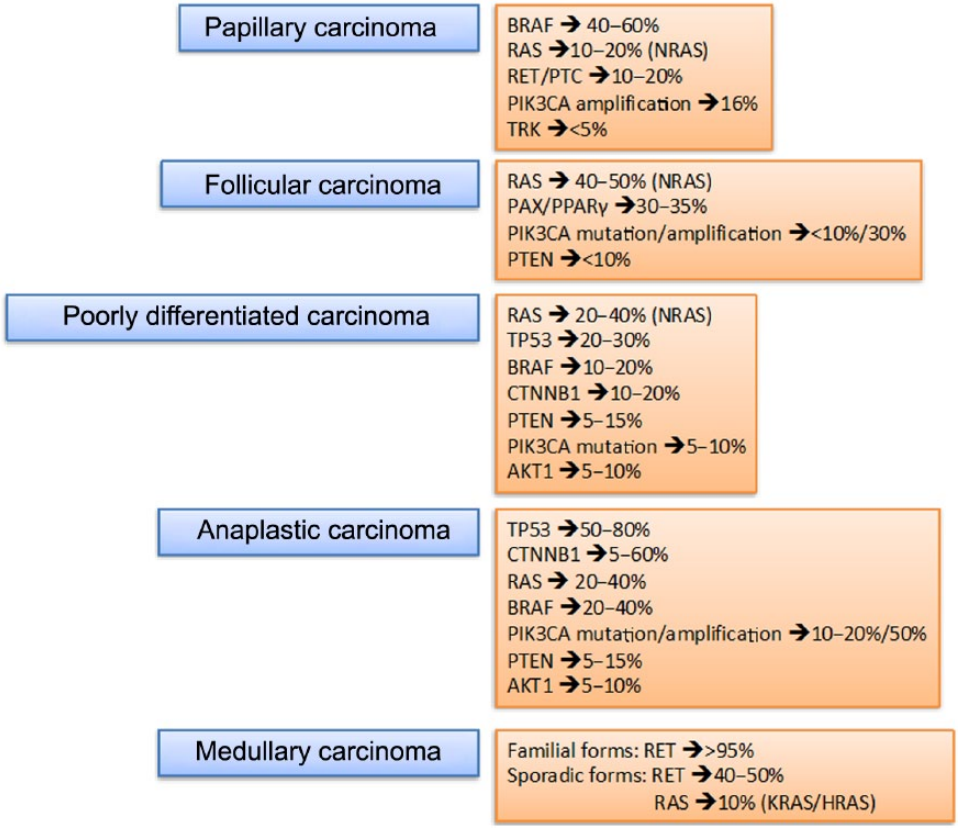

Figure 1. Thyroid tumors and mutational profile. PTEN, phosphatase and tensin homolog.
BRAF, b-type rapidly accelerated fibrosarcoma;RAS, rat sarcoma; RET/PTC, rearranged during transfection/papillary thyroid carcinoma; PI3KCA, phosphatidylinositol 3-kinase oncogene; TRK, receptor thyrosine kinase; PAX8/PPAR, paired box 8/peroxisome proliferator-activated receptor gamma; PTEN, phosphatase and tensin homolog; TP53, tumor protein p53; CTNNB1, catenin (cadherin-associated protein beta 1); AKT1, v-akt murine thymoma viral oncogene homolog 1;RET, rearranged during transfection.

point mutations or RET/PTC and AKP9/BRAF rearrangements have been identified in 70% of PTCs, in which overlapping mutations have been rarely described. In FTC, the most common alterations includes RAS mutations and PAX8/peroxisome proliferator-activated receptor (PPARγ) rearrangements [Nikiforova and Nikiforov, 2008].

The RAS/RAF/MEK/MAPK/ERK pathway

RAS is activated through different membrane receptors and it recruits RAF for a subsequent phosphorylation of MAPK-ERK kinases. Once ERK is translocated to the nucleus, transcription factors, such as c-myc, c-jun, ets or c-fos are activated. Indeed, Erk may activate cytosolic apoptotic proteins, such as Bad, MCL-1, caspase 9 and cytoskeletal proteins, such as paxillin, calnexin and vinexin [Caronia *et al.* 2011]. Oncogenic modifications in RAS have been hypothesized to be one of the first steps in TC development because of the presence in well differentiated TCs (WDTCs) and PDTC or ATC and MTC. An initial analysis of hotspot mutations at codons 12, 13 and 61 of the three forms of RAS (HRAS, KRAS, NRAS) in 125 TC samples from 107 patients at different stages of disease demonstrated an overall incidence of a RAS mutation of 32.7% [Garcia-Rostan *et al.* 2003]. The most frequent mutation was KRAS (24.3%), followed by NRAS (8.4%) and HRAS (4.7%). PDTC and ATC were the histologic subtypes with the greater incidence of RAS mutations (55.2% and 51.7%, respectively). A significant association between the presence of an activating RAS mutation and poor survival was identified in patients with DTC ($p < 0.001$). A recent investigation in 58 resected FTC tumor samples also showed a significant association between the NRAS codon 61 mutation and the presence of distant metastasis ($p = 0.020$) and between the presence of any RAS mutation and worse prognosis ($p = 0.042$)

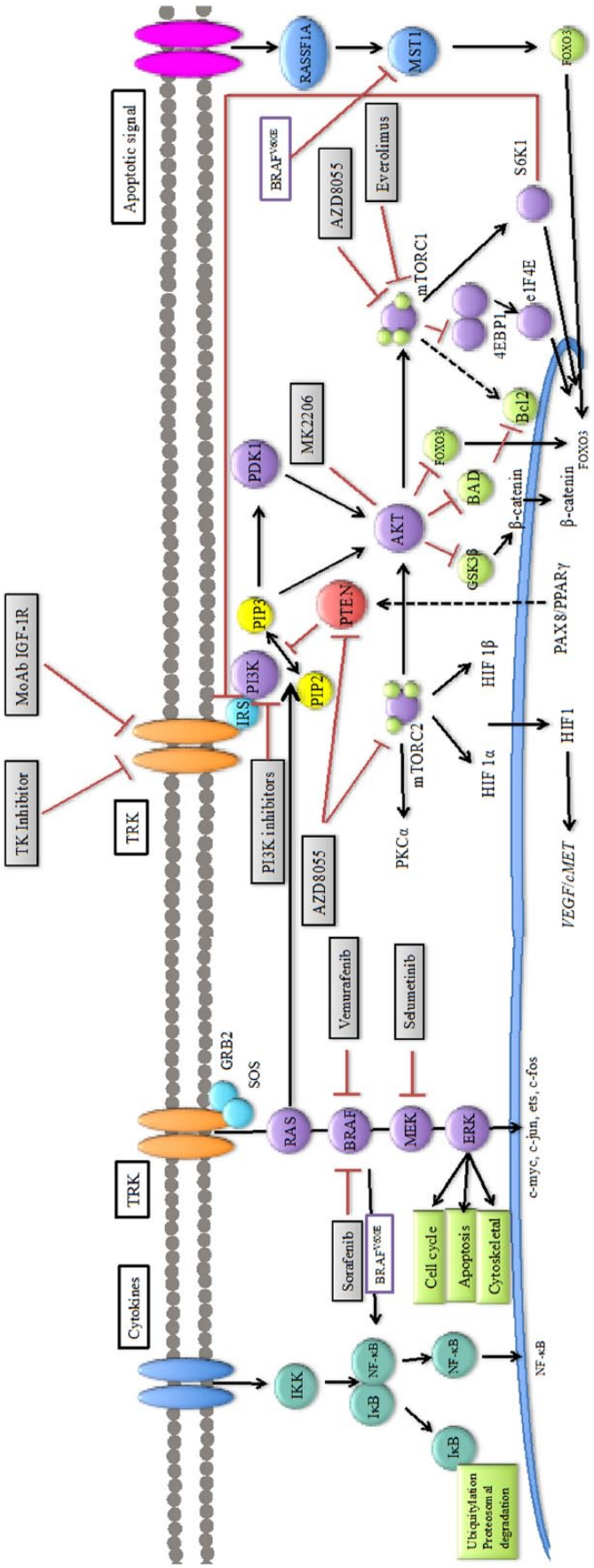

Figure 2. Main oncogenic pathways and targeted therapies in thyroid carcinoma. HIF1, hypoxia-inducible factor 1; mTOR, mammalian target of rapamycin; NF-κB, nuclear factor κB; PI3K, phosphatidylinositide 3-kinase; PKC, protein kinase C; PPAR, peroxisome proliferator-activated receptor; PTEN, phosphatase and tensin homolog; VEGF, vascular endothelial growth factor; IKK, IB kinase; IB, inhibitor of kappa; B NF-KB, nuclear factor KB; TRK, Receptor thyrosine kinase; Grb2, Growth factor receptor-bound protein 2; SOS, son of sevenless; RAS, Rat sarcoma; BRAF, B-type rapidly accelerated fibrosarcoma; ERK, extracellular signal-regulated kinases; IGF-1R, insulin-like growth factor 1 receptor; IRS, Insulin receptor substrate; PI3K, phosphatidylinositide 3-kinasen; PKC, protein kinase C; mTOR, mammalian target of rapamycin;
HIF1, hypoxia-inducible factor; PTEN, phosphatase and tensin homolog; VEGF, vascular endothelial growth factor; MET, mesenchymal epithelial transition; PPAR, peroxisome proliferator-activated receptorn; PDK1, Phosphoinositide dependent kinase-1; FOXO3, forkhead box O3; BAD, Bcl-xL/Bcl-2-Associated Death Promoter Bcl2, B-cell lymphoma 2; GSK3, glycogen synthase kinase 3 beta 4EBP1: 4E-binding protein 1 EIF4E, eukaryotic translation initiation factor 4E; S6K1, S6 kinase 1; RASSF1A, Ras association domain family 1 isoform A; MST1, macrophage stimulating protein.

[Fukahori *et al.* 2012]. In a different report with 65 PDTC tumor samples, the most common molecular alteration was RAS mutation identified in 25% of carcinomas. The most frequent RAS mutation was the point mutation at codon 61 of NRAS [Volante *et al.* 2009]. Once again, a strong relationship between the presence of a RAS mutation and poorer survival was detected ($p = 0.004$). However, a definitive conclusion about the prognostic value requires larger studies.

In MTC, a wide range of somatic RAS mutations have been reported from different investigations (7.9–68%), particularly in patients without a RET mutation [Agrawal *et al.* 2013; Moura *et al.* 2011]. Results from a meta-analysis including trials with complete screening showed an overall incidence of RAS mutations of 8.8% (HRAS 8.1%, KRAS 6.5% and NRAS 0.5%) [Ciampi *et al.* 2013].

From this particular pathway, BRAF mutation status has been the most common and established prognostic biomarker, particularly in PTC or in dedifferentiated tumors, probably developed from the first one [Xing *et al.* 2013]. Valine to glutamate amino acid substitution at residue 600 (V600E) is the most frequent point mutation in the BRAF gene (98–99%). Other alterations less frequently described have been the lysine to glutamine amino acid substitution (L601E), deletions or insertions around codon 600 or AKAP9/BRAF rearrangements. Mutations in BRAF have been associated with tumor recurrence and loss of response to radioiodine treatment, in part influenced by the secondary overexpression of vascular endothelial growth factor receptor (VEGFR) and MET [Elisei *et al.* 2008]. To overcome initial controversial results about its prognostic value, a meta-analysis conducted by Li and colleagues included 32 studies (only two were prospective) with 6372 patients with PTC (3244 patients with BRAF mutation) [Li *et al.* 2012]. A significant association between the presence of a $BRAF^{V600E}$ mutation and tumor size [odds ratio (OR) 1.57; 95% confidence interval (CI) 1.29–1.92], lymph node metastasis (OR 1.72; 95% CI 1.53–1.94), extrathyroid extension (OR 2.60; 95% CI 2.27–2.99), multifocality (OR 1.30; 95% CI 1.13–1.49), vascular invasion (OR 1.23; 95% CI 0.76–2.01), absence or infiltration of the tumoral capsule (OR 2.07; 95% CI 1.64–2.61) and advanced clinical stage (OR 1.82; 95% CI 1.58–2.10) were identified. Those results were also consistent with the results obtained from initial PTC stages (pT1/T2-N0) [Elisei *et al.* 2012]. Indeed, as a prognostic factor, only a $BRAF^{V600E}$ mutation was found to be significantly associated with disease-free survival.

In addition to upstream molecular alterations, secondary dysregulations in MAPK activation, such as hypometilation or genome-wide hypermetilation of many tumor suppressor genes (DAPK1, RARB, TIMP3, SLC5A8), and upregulation of oncogenic proteins may enhance the carcinogenic process [Hu *et al.* 2006; Xing, 2007]. Those alterations have an important role in cell metabolism and cell functions.

The PI3K/AKT/mammalian target of rapamycin pathway

The PI3K/AKT pathway is related to cell growth, proliferation, survival, motility and regulation of iodide uptake. In an oncological context, it also enhances angiogenesis, metastasis development and resistance to chemotherapy [de Souza *et al.* 2011]. This pathway is related to a progressive dedifferentiation (losing thyroid-stimulating hormone [TSH] signaling and increasing PI3K signaling) and acquisition of new oncogenic alterations. *In vivo* investigations have shown that persistent stimulation of TSH leads to an overactivation of mammalian target of rapamycin complex1/S6 kinase 1/S6 [Brewer *et al.* 2007]. A comprehensive analysis of a large panel of genes from FTC ($n = 64$) and ATC ($n = 51$) samples was carried out to establish the rationale for the development of targeted therapies in TC [Liu *et al.* 2008]. Frequent overexpression of VEGFR1, platelet-derived growth factor receptor 1 (PDGFR1), PDGFRβ or epidermal growth factor receptor (EGFR) was observed. The most frequent mutated genes were RAS (20.3% in FTC), PIK3Ca (12% in ATC) and phosphatase and tensin homolog (PTEN) (16.7% in ATC) and RET/PTC rearrangements were identified in 15% of ATC samples. A high percentage, 81% of ATC, had a genetic alteration involved in both the MAPK and PI3K/Akt pathways. In fact, genetic alterations in the second pathway are seen, predominantly in progressive dedifferentiated tumors, such as ATC and PDTC.

The PI3K/Akt deregulation may come from different genetic alterations as described below, such as the presence of thyroid hormone β receptor (TRβPV) mutant that binds with a greater affinity than the wild type TRβ to the p85 regulatory subunit of PI3K and may lead to Akt activation. Other deregulations involve RET/PTC rearrangements, RET mutations, overexpression of RTK (EGFR, VEGFR, FGFR, insulin-like growth factor 1

receptor [IGF-1R], KIT, MET), PIK3CA amplification or mutations (mainly in the catalytic domain region) [Ricarte-Filho *et al.* 2009], Akt activation in nuclear and cytoplasmic membrane, increased levels of pAkt or Akt mutations (AKT1E17K) [Ricarte-Filho *et al.* 2009] and loss of phosphatase and tensin homolog (PTEN) by mutations, gene methylation or reduced expression levels. In addition, phosphoinositide-dependent kinase-1 (PDK-1) gene amplification has been identified because it is recruited by activated PI3K and phosphorylates Akt at the cell membrane. However, its role in tumorigenesis of TC has not been clearly established [Liu *et al.* 2008]. Finally, RAS can also interact and activate PI3K downstream cascade. Initial results in cell lines have identified partial resistance to MEK inhibitors in cells with RAS mutations compared with cell lines harboring $BRAF^{V600E}$ mutation [Leboeuf *et al.* 2008].

RET point mutations and RET rearrangements

In thyroid tumors, RET can be activated by fusion to other genes in tumors derived from follicular cells or by point mutations in tumors arising from parafollicular cells.

RET/PTC rearrangements are suggested to be an initial step in TC related to childhood PTC and to radiation exposure [Hamatani *et al.* 2008]. There are more rearrangements identified, but RET/PTC1 (partner gene is a coiled-coil domain containing gene 6, CCDC6) and RET/PTC3 (partner gene is a nuclear receptor coactivator gene 4, NcoA4), which are intrachromosomal paracentric inversions, are the most common [Nikiforov, 2002]. RET/PTC is ligand independent, dimerized by autophosphorylation of thyrosine residues and binding to other adaptor proteins (GRB2, SOS, Shc, FRS2) for RAS/MAPK and PI3K downstream activation and interaction with different cytoplasmic substrates [Antonelli *et al.* 2012]. In most reported series, RET/PTC1 was the most frequent (60–70%), followed by RET/PTC3 (20–30%) and RET/PTC2 (<10%) [Nikiforov, 2002]. The presence of nonclonal RET/PTC has also been reported in benign lesions. The association to aggressiveness and tumor recurrence has not been well established.

Despite what was previously considered, dual mutation in $BRAF^{V600E}$ and RET/PTC can coexist in patients with well differentiated PTC. In an analysis of 72 tumor samples, 19.3% presented both alterations [Guerra *et al.* 2014]. Moreover, rearrangements of a different RTK, NTRK1 gene, have been reported from less than 2–15% of patients with PTC [Nikiforov and Nikiforova, 2011].

Germline point mutations have been identified in almost all patients with hereditary MTC and somatic point mutations in 40–50% of patients with sporadic MTC. The most frequent mutation in sporadic MTC is the substitution of a methionine to a threonine amino acid in the codon 918 that corresponds to the tyrosine kinase 2 domain. This mutation is present in 85% of patients [Frank-Raue *et al.* 2010]. In hereditary MTC, 95% of patients with MEN 2B present a germline mutation in codon 918, whereas 5% present in codon 883 (A883F). In contrast, 85% of patients with MEN 2A harbor a germline mutation in codon 634 (mostly C634R) corresponding to the extracellular cysteine-rich domain. In addition, patients with familiar MTC present more varied mutations involving codons belonging to the extracellular and intracellular domains [de Groot *et al.* 2006].

The oncogenic activation of RET depends on the location of the amino acid change leading to a ligand-independent activation through aberrant intermolecular disulfide bond formation, constitutive dimerization of the oncoprotein, activation of the tyrosine kinase domain or modifications of the substrate specificity and residue phosphorylation. Therefore, the binding of adaptor and effector proteins to docking sites enhances the activation of several pathways involved in embryogenesis, cell survival, proliferation, differentiation, motility, calcium release and intracellular transport. Consequently, it can induce oncogenic transformation through activation of the RAS/MAPK, PI3K/AKT and JAK/STAT pathways, protein kinase C (PKC) and direct signaling through SRC kinases, protein kinase A (PKA), focal adhesion kinase (FAK) and β-catenin domains [Mulligan, 2014] (Figure 3).

Novel pathways

Targeting the mesenchymal–epithelial transition (MET) has been recently investigated as a potential target in the treatment of TC. MET is involved in the disruption of cadherin-based cell to cell adhesion and cell motility that are decisive in embryogenesis and enhance molecular signaling for cell survival and proliferation, wound healing and organ homeostasis. The main downstream signaling activated by the ligand, HGF (hepatocyte growth factor), are the RAS/MAPK

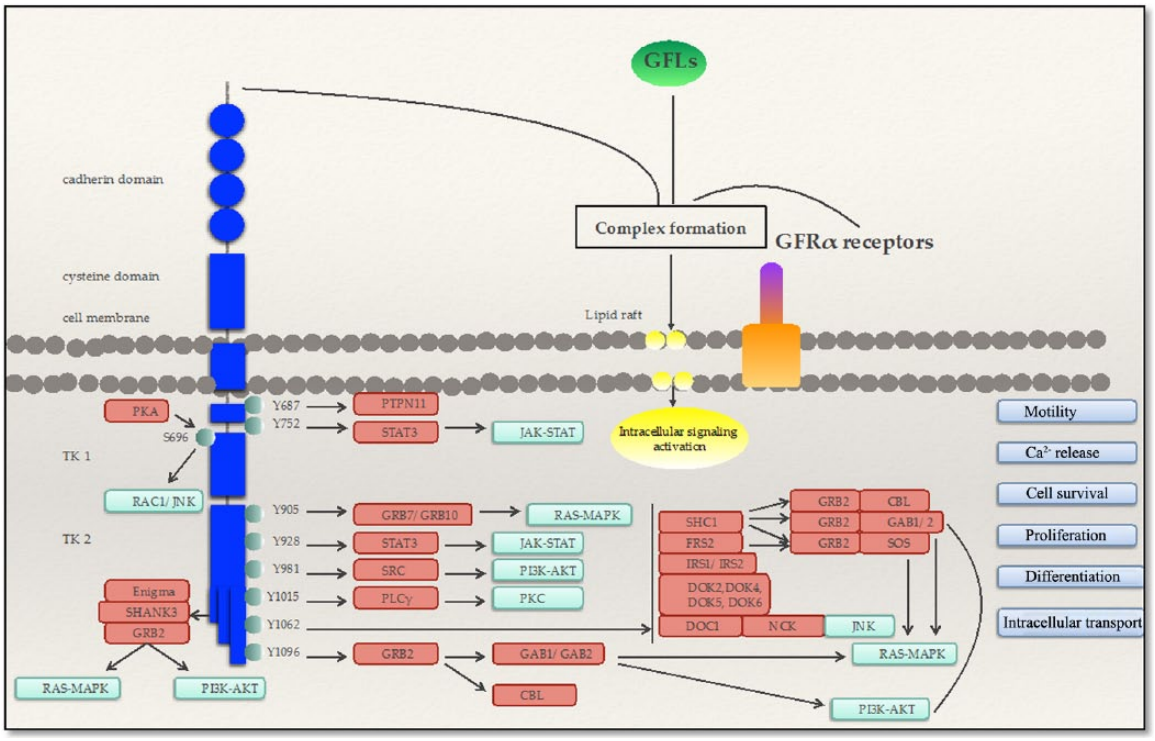

Figure 3. RET signaling in thyroid cells. MAPK, mitogen-activated protein kinase; JNK, Janus kinase; PI3K, phosphatidylinositide 3-kinase; PKA, protein kinase A; PKC, protein kinase C; PKA: protein kinase A; RAC1/JNK: Ras-related C3 botulinum toxin substrate 1/Janus kinase; SHANK3: SH3 and multiple ankyrin repeat domains 3; Grb2: Growth factor receptor-bound protein 2; MAPK: Mitogen-activated protein kinases; PI3K: phosphatidylinositide 3-kinase AKT/PKB: protein kinase B; PTPN11: Tyrosine-protein phosphatase non-receptor type 11; STAT3: Signal transducer and activator of transcription 3; PLC: Phospholipase C; PKC: protein kinase C; GAB1/GAB2; CBL: Casitas B-lineage Lymphoma; SHC1: Src homology 2 domain containing transforming protein 1; FRS2: fibroblast growth factor receptor substrate 2; IRS1/ IRS2: Insulin receptor substrate 1/ Insulin receptor substrate 2; DOK: downstream of tyrosine kinase; NCK: Non-Catalytic Region Of Tyrosine Kinase.

and PI3K/Akt pathways, FAK, Janus kinase 1 and PKC. The varied biological responses enhanced by MET will provide the ability to promote survival, angiogenesis, invasiveness and metastasis in tumor tissue. The overexpression of MET in TC may imply an aggressive phenotype favoring metastasis development [Peters and Adjei, 2012].

Rearrangement of paired box 8/peroxisome proliferator-activated receptor (PAX8/PPARγ) results in overexpression of a quimeric protein that downregulates the tumor suppressor activity of PPARγ and is detected in benign follicular adenomas (2–13%) and malignant FTC (30–35%) or PTC with follicular variants (1–5%) [Omur and Baran, 2014].

Loss of function of p53 is extremely rare in WDTC and correlates with tumor dedifferentiation. This alteration is seen in PDTC and ATC with a reported frequency of 15–30% and 60–80%, respectively [Volante *et al.* 2009]. Similarly, mutations in ALK are associated with PDTC.

The Wnt signaling pathway is involved in embryonic development, cell differentiation and proliferation and, in addition, in metastatic disease development due to its involvement in the migration process; the epithelial–mesenchymal transition. The canonical Wnt signaling is related to the cytoplasmic protein β catenin and, in the absence of Wnt, this protein is phosphorylated through a destruction complex [axin, adenomatous polyposis coli (APC) and glycogen synthase kinase 3 beta (GSK3β). The oncogenic mechanism in TC is related to the accumulation of β catenin in cytoplasmic cells because of the inability to be degraded through the ubiquitin-dependent pathway and the disassembly of the destruction complex. Consequently, transduction signaling is activated by frequent nuclear translocation of β catenin and binding to the lymphoid

enhancer-binding factor 1/transcription factor (LEF-1/TCF) complex for gene transduction, such as *c-myc* and *bcl-1* [Garcia-Rostan *et al.* 1999; Rezk *et al.* 2004]. In PDTC and in ATC, a cadherin-associaed protein beta 1 (*CTNNB1)* point mutation in exon 3 has been detected in 0–20% and 60% of patients respectively and are suggested to be associated with poor outcome [Garcia-Rostan *et al.* 2001]. Other mutations have been found in different proteins, such as APC and axin. Also, upregulation of this pathway is secondary to GSK3β inactivation due to PI3K/Akt downstream activation, which can be stimulated by RET/PTC [Xing, 2013].

Nuclear factor κB (NF-κB) is a transcription factor activated by the upstream MAPK signaling pathway and has an important role in inflammatory reactions during tumorigenesis. Particularly, $BRAF^{V600E}$ seems to stimulate IκB (inhibitor of NF-κB) degradation.

Hypoxia-inducible factor 1 α (HIF-1α) pathway regulates genes involved in angiogenesis by binding to HIF-1β for HIF-1 transcription factor formation, particularly influenced by VEGF-A. It is involved in tumor development in ATC, PTC and MTC by the downstream signaling enhanced by MAPK and the PI3K/mTOR pathway.

Alterations in micro-RNAs, involved in gene expression regulation, have been found in TC. A deregulation has been observed in miR-222, miR-221 and miR-146b in PTC [Pallante, 2006], possibly associated with a worse outcome and to p27kip1 and KIT. Furthermore, alterations in miR-197, miR-346, miR-155 and miR-224 in FTC and miR-30d, miR125b, miR26a and miR-30a-5p in ATC have also been described [Nikiforov and Nikiforova, 2011].

Mutational status of BRAF and RET: are they ready for primetime?

Classical cytotoxic drugs have demonstrated limited activity in TC, urging the need for new treatment options. The extensive improvement in the recognition of the primordial pathways and subsequent alterations in most TCs have led to the development of new treatment agents that have changed the landscape in such an orphan disease.

Sorafenib

Sorafenib is a multikinase inhibitor of RET, VEGFR1–3, Flt-3, KIT and CRAF/BRAF (wild type and V600E mutated). Based on the overexpression of VEGFR/PDGFR in TC and the key value of constitutive activation of RAS/BRAF in TC oncogenesis [Gupta-Abramson *et al.* 2008], several retrospective and phase II clinical trials have investigated the role of sorafenib in all types of thyroid tumors and have showed promising results that are presented in Table 1. The data observed support the efficacy of sorafenib in DTC and MTC. With regard to ATC, it is suggested that those tumors coming from a dedifferentiation of WDTC or those tumors harboring areas of differentiated PTC should obtain a better response with sorafenib [Savvides *et al.* 2013]. However, the low number of patients included in the trials does not allow any definitive conclusion.

The predictive value of several biomarkers could not be well established. Controversial results concerning the decrease of tumor markers [carcinoembryonic antigen (CEA), calcitonin and thyroglobulin (Tg)] and radiological response were observed [Lam *et al.* 2010; Ahmed *et al.* 2011; Capdevila *et al.* 2012]. In addition, the role of BRAF overactivation on tumor response could not be established. However, a significant decrease in pVEGFR, pERK and increase in pVEGF were observed in a subgroup analysis harboring a $BRAF^{V600E}$ mutation [Kloos *et al.* 2009].

Adverse events associated with sorafenib treatment were hand–foot syndrome (80%; 95% CI 68–91), diarrhea (68%; 95% CI 59–77), fatigue (67%; 95% CI 57–78), rash (66%; 95% CI 50–82), weight loss (52%; 95% CI 33–72) and hypertension (31%; 95% CI 21–42) [Shen *et al.* 2014].

To address definitive conclusions about the activity of sorafenib in DTC, a phase III randomized, double-blind, placebo-controlled trial was conducted [Brose *et al.* 2014]. The DECISION trial included 417 patients (57% PTC, 25% FTC, 10% PDTC) who were randomized to sorafenib 400 mg/12 h (N = 207) or placebo (N = 210) until disease progression. At that time, patients were offered to crossover to sorafenib according to the investigator's decision. The primary endpoint of progression-free survival (PFS) was met, showing a

Table 1. Initial trials of sorafenib in thyroid carcinoma: phase II and retrospective studies.

	Study design	Inclusion criteria	Number of patients	BRAF mutation	Response rate	PFS (months)	OS (months)
Gupta-Abramson *et al.* [2008]	Phase II prospective	PTC (18) FTC (9) MTC (1) PD/ATC (2)	30	–	PR = 7 (23%) SD = 16 (53%)	21	–
Kloos *et al.* [2009]	Phase II prospective	Arm A: PTC (19) Arm B: PTC (22) FTC (11) ATC (4)	46	PTC = 17/22 nPTC = 0/6	PR_{PTC} = 6 (14%) PR_{nPTC} = 0 (0%) SD_{PTC} = 25 (61%) SD_{nPTC} = 10 (67%)	10–16 (nPTC = 4.5)	23–37 (nPTC = 24.2)
Cabanillas *et al.* [2010]	Retrospective	PTC (8) FTC (7)	15	PTC = 4/7	12 (80%) PR = 3 (20%) SD = 9 (60%)	19	67% at 2 years
Lam *et al.* [2010]	Phase II prospective	sMTC (16) hMTC (5)	21	RET mutation: sMTC = 10/12 hMTC = 5/5	PR = 1 + 1 (9.5%) SD = 8 + 1 (43%)	17.9	–
Ahmed *et al.* [2011]	Phase II prospective	PTC (8) FTC (9) PDTC (2) MTC (15)	34	DTC = 1/3	PR = 5 (15%) SD = 25 (73%)	71% at 2 years	79% at 2 years
Schneider *et al.* [2012]	Phase II prospective	DTC	31	BRAF: 10/32 K/N-RAS: 3/9 PIK3CA: 2/6	PR=8 (31%) SD=11 (42%)	18	34.5
Capdevila *et al.* [2012]	Retrospective	PTC (7) FTC (9) MTC (15) ATC (3)	34	–	PR_{DTC} = 3 (19%) PR_{MTC} = 7 (47%) PR_{ATC} = 1 (33%) SD_{DTC} = 8 (50%) SD_{MTC} = 6 (40%) SD_{ATC} = 0 (0%)	10.5 DTC = 13.3 MTC = 10.5 ATC = 4.4	23.6 DTC = 23.6 MTC = NR ATC = 5
Savvides *et al.* [2013]	Phase II prospective	ATC	20	–	PR = 2 (10%) SD = 5 (25%)	1.9	3.9

ATC, anaplastic thyroid carcinoma; DTC, differentiated thyroid carcinoma; FTC, follicular thyroid carcinoma; hMTC, hereditary medullary thyroid carcinoma; MTC, medullary thyroid carcinoma; nPTC, nonpapillary thyroid carcinoma; OS, overall survival; PDTC, poorly differentiated thyroid carcinoma; PFS, progression-free survival; PR, partial response; PTC, papillary thyroid carcinoma; SD, stable disease; sMTC, sporadic medullary thyroid carcinoma.

significant benefit in the experimental group [10.8 months in the sorafenib group *versus* 5.8 months in the placebo group; hazard ratio (HR) 0.59; 95% CI 0.45–0.76, $p < 0.0001$]. This benefit was observed in all subgroups analyzed (age, sex, histologic subtypes, metastasis location, fludeoxyglucose uptake, tumor size, total I^{131} dose received and mutational status). Median PFS in patients with a BRAF mutation was 20.5 months *versus* 9.4 months in the sorafenib and placebo group, respectively (HR 0.46; 95% CI 0.24–0.90; $p = 0.02$). In patients without a BRAF mutation, median PFS was 8.9 months *versus* 3.8 months in the sorafenib and placebo group, respectively (HR 0.55; 95% CI 0.38–0.79; $p < 0.001$). Patients harboring a RAS mutation showed a median PFS of 5.5 months *versus* 3.5 months in the sorafenib and placebo group, respectively (HR 0.49; 95% CI 0.24–1.0; $p = 0.045$). In addition, in patients without a RAS mutation, median PFS was 10.8 months *versus* 5.8 months in the sorafenib and placebo group, respectively (HR 0.60; 95% CI 0.42–0.85; $p = 0.004$). The BRAF mutation was more frequently identified in patients with PTC (46.2%) and the RAS mutation in PDTC (32.3%), suggesting that differences in PFS were associated with the tumor subtype because the magnitude of effect of sorafenib was similar in all groups demonstrated by a similar HR.

Table 2. Phase III clinical trials of vandetanib and cabozantinib in MTC.

Clinical trial	ZETA	EXAM
Study design	Randomized (2:1), double-blind, placebo-controlled, phase III trial	Randomized (2:1), double-blind, placebo-controlled, phase III trial
Treatment	Vandetanib 300 mg/24 h *versus* placebo	Cabozantinib 140 mg/24 h *versus* placebo
Number of patients	331 (231 vandetanib + 100 placebo)	330 (219 cabozantinib + 111 placebo)
Tumor stage	Locally unresectable/metastatic	Locally unresectable/metastatic Documented RECIST progression
Previous treatment lines	132 (40%) patients previously treated	128 (40%) patients previously treated: Previous TKI: 44 (cabozantinib), 24 (placebo) ⩾ 2 previous systemic therapies: 52 (cabozantinib), 31 (placebo)
RET mutational status: RET+/RET–/RET unknown	56% (187)/2.4% (8)/41% (136)	48% (159) / 12% (41) / 39% (130)
Primary endpoint	PFS	PFS
Progressive disease	Not mandatory	Yes
Overall response rate	45% *versus* 13%	28% *versus* 0%
Disease control rate	87% *versus* 71%	55.3% *versus* 13.5%
Calcitonin response	69% *versus* 3% ($p < 0.001$)	–45.2% *versus* +57.2% ($p < 0.001$)
CEA response	52% *versus* 2% (p<0.001)	–23.7% *versus* +88.7% ($p < 0.001$)
Progression-free survival (PFS)	NR (30.5 months) *versus* 19.3 months (HR 0.27)	11.2 *versus* 4.0 months (HR 0.28)
Overall survival	HR 0.83 (95% CI 0.60–1.14)*	HR 0.83 (95% CI 0.60–1.14)
Adverse events ⩾ G3	Diarrhea, hypertension, QTc prolongation, fatigue	Diarrhea, palmo-plantar erithrodermia, hypertension, fatigue
Treatment dose reductions	35% *versus* 3%	79% *versus* 9%
Treatment discontinuations due to adverse event	12% *versus* 3%	16% *versus* 8%

*Less than 50% of events had occurred.
CEA, carcinoembryonic antigen; CI, confidence interval; HR, hazard ratio; NR, not reached; TKI, tyrosine kinase inhibitor.

However, neither BRAF nor RAS mutational status were associated with prognosis. Radiological objective response rate (ORR) was 12.2% *versus* 0.5% and stable disease (SD) at 6 months was 42% *versus* 33% in the sorafenib *versus* placebo group, respectively. Changes in serum Tg were observed according to radiological tumor response and treatment designation. However, they were not enough for an individual recommendation as a definitive predictive value. At the time of analysis, median overall survival (OS) was not reached in both groups (HR 0.802; 95% CI 0.54–1.2, $p = 0.14$). Despite a longer follow up, a difference in OS will be difficult to achieve due to crossover: 71% of patients in the placebo group and 27% of patients in the sorafenib group received off-label sorafenib. Based on these results, sorafenib was approved in November 2013 by the US Food and Drug Administration for the treatment of late-stage (metastatic) DTC.

Vemurafenib

Searching for more potent and directed inhibitors of BRAF for effective tumor control growth in patients with DTC and a BRAF mutation, vemurafenib has been investigated in this context.

Vemurafenib is a potent kinase inhibitor of $BRAF^{V600E}$ and CRAF and less potent for BRAF wild type. Initial results came from preclinical investigations in human TC cell lines with and without the $BRAF^{V600E}$ mutation [Nucera *et al.* 2011]. Vemurafenib was able to inhibit downstream phosphorylation of ERK1/ERK2 involved in cell proliferation, as well as migration and invasion in 8505c cells harboring the $BRAF^{V600E}$ mutation and in PTC1 cells with wild type BRAF. Those results were confirmed in *in vivo* models of ATC with a $BRAF^{V600E}$ mutation. Considering the clinical relevance of inhibiting BRAF in PTC, vemurafenib was given to three patients with metastatic PTC

Table 3. Phase II clinical trials of other targeted agents with activity over RAS/RAF/MAPK and PI3K/AKT pathways.

	Molecular target	Study	Tumor type	*N*	Treatment doses	Mutational profile	Response (PR/SD)	PFS (months)	Adverse events
Axitinib	VEGFR1–3, PDGFRβ, KIT	Phase II [Cohen *et al*. 2008]	DTC (60) MTC (11)	71	5 mg/12 h	–	2/3 (45%)	18	Hypertension, fatigue, GI, anorexia, proteinuria
Lenvatinib	VEGFR1–3, FGFR1, PDGFR	Phase II [Schlumberger *et al*. 2012]	MTC	59	24 mg/24 h	–	21/– (36%)	9	
Motesanib	VEGFR1–3, PDGFR, KIT, RET	Phase II [Schlumberger *et al*. 2009]	MTC	91	125 mg/24 h × 48 weeks/ DP/toxicity	RET 33/47	2/44 (50.5%)	12	Diarrhea, hypertension, fatigue, weight loss, hypothyroidism
		Phase II [Sherman *et al*. 2008]	DTC	93	125 mg/24 h × 48 weeks/ DP/toxicity	$BRAF^{V600E}$ 10/33 RAS 6/33 RET 0/33	13/33 (49%)	10	
Pazopanib	VEGFR1–3, PDGFRα-β, KIT	Phase II [Bible *et al*. 2010]	DTC	39	800 mg/24 h		18/– (49%)	11.7	Hypertension, fatigue, diarrhea, anorexia, nausea, mielotoxicity, hepatotoxicity
Sunitinib	VEGFR1–3, PDGFR, KIT, FLT3, CSF-1R, RET	Phase II [De Souza *et al*. 2010]	MTC	25	50 mg/24 h 4/2	RET 11/13 (85%)	8/13 (92%)	–	Asthenia, diarrhea, hand foot syndrome, neutropenia, leukopenia
		Phase II [Carr *et al*. 2010]	DTC (28) MTC (7)	35	37.5 mg/24 h continuously	–	11/13 (80%)	12.8	
		Phase II [Ravaud *et al*. 2008]	PTC (8) ATC (1) MTC (4) Miscellaneous	17	50 mg/24 h 4/2	–	1/12 (76.4%)	–	
Everolimus	mTOR	Phase II	DTC (25) ATC (6) MTC (9)	40	10 mg/24 h	–	2/17 (50%)	11.7	Mucositis, anorexia, ALT/ AST elevations
Selumetinib	MEK1/2	Phase II [Ho *et al*. 2013]	DTC	20	75 mg/12 h	$BRAF^{V600E}$ 9 RAS 5 RET/PTC 3	5/3 (40%)	–	Fatigue, maculopapular rash, AST elevation diarrhea, peripheral edema
		Phase II [Hayes *et al*. 2012]	PTC	39	100 mg/12 h	$BRAF^{V600E}$ 12/26 NRAS Q61R 1/26	1/12 (40.6%)	8	

ALT, alanine transaminase; AST, aspartate transaminase; ATC, anaplastic thyroid carcinoma; CSF-1R, colony-stimulating factor 1 receptor; DTC, differentiated thyroid carcinoma; FGFR, fibroblast growth factor receptor; GI, gastrointestinal; MAPK, mitogen-activated protein kinase; MTC, medullary thyroid carcinoma; mTOR, mammalian target of rapamycin; PDGFR, platelet-derived growth factor receptor; PI3K, phosphatidylinositide 3-kinase; PFS, progression-free survival; PR, partial response; PTC, papillary thyroid carcinoma; SD, stable disease; VEGFR, vascular endothelial growth factor receptor.

Table 4. Ongoing clinical trials in thyroid carcinoma.

Treatment	Study design	Inclusion criteria	Primary endpoint	ClinicalTrials.gov identifier
Vemurafenib	Phase II Nonrandomized	PTC + $BRAF^{V600E}$	Best overall response rate	NCT01286753
Dabrafenib ± trametinib	Phase II randomized	TC + $BRAF^{V600E}$	ORR	NCT01723202
Vandetanib	Phase III randomized	DTC	PFS	NCT01876784
Vandetanib 300 *versus* 150 mg/day	Phase IV randomized	MTC	ORR	NCT01496313
Vandetanib + bortezomib	Phase I/II nonrandomized	Solid tumors (MTC)	ORR + biomarker response	NCT00923247
Cabozantinib	Phase II nonrandomized	DTC	Efficacy + safety	NCT02041260
Decitabine	Phase II nonrandomized	PTC + FTC	Restoration radioiodine uptake	NCT00085293
Sunitinib	Phase II nonrandomized	PTC + FTC + MTC	ORR	NCT00381641
Sorafenib + everolimus	Phase II nonrandomized	TC	ORR	NCT01141309

DTC, differentiated thyroid carcinoma; FTC, follicular thyroid carcinoma; MTC, medullary thyroid carcinoma; ORR, objective response rate; PFS, progression-free survival; TC, thyroid carcinoma.

harboring a $BRAF^{V600E}$ mutation in a phase I trial [Kim *et al.* 2013]. One patient achieved a partial response (PR) and two patients had disease stabilization. Clinical outcomes showed a median PFS of 11.4–13.2 months and median OS of 15–31.7 months. Investigation is ongoing, further studying the role of vemurafenib in selected patients, as well as the combination of an irreversible inhibitor of $BRAF^{V600E/K/D}$ and CRAF, dabrafenib, with a selective inhibitor of MEK1/MEK2, trametinib (Table 4).

Vandetanib

Vandetanib is a potent tyrosine kinase inhibitor (TKI) that competes with the adenosine triphosphate (ATP) binding site in the catalytic domain of RET, VEGFR2–3 and EGFR, which are important targets in TC [Deshpande *et al.* 2011]. It was the first targeted drug approved for the treatment of unresectable or metastatic MTC.

Its activity was initially demonstrated in NIH3T3 RET/PTC3, RET/MEN2A (C634R) mutant, RET/MEN2B (M918T) mutant, EGFR/RET and v-Ha-Ras transfected cells [Carlomagno *et al.* 2002]. NIH3T3 cells are mouse embryonic fibroblast cells whose characteristics make them suitable for the transfection host. In those cell lines, vandetanib demonstrated a potent inhibition of the downstream phosphorylation and colony formation activated by RET and EGFR, as well as *in vivo* tumor formation in nude mice.

Two dose-escalation phase I trials assessed the security of vandetanib in solid tumors. The first one included 77 patients and assessed the percentage of dose-limiting toxicities (DLTs) of diarrhea and rash at a dose of at least 500 mg per day. Steady-state concentrations were achieved after 28 days [Holden *et al.* 2005]. The other phase I trial conducted in 18 Japanese patients established the maximum tolerated dose at 400 mg per day. The authors recommended a dose of 300 mg per day in further clinical trials [Tamura *et al.* 2006].

The activity of vandetanib over cell lines harboring RET/PTC rearrangements or RET point mutations motivated its development in DTC and MTC. The efficacy and safety in 30 patients with hereditary MTC were assessed in a phase II open-label, single-arm study [Wells *et al.* 2010]. A confirmed PR was achieved in six (20%) patients and SD at 6 months

in 16 (53%) patients. The important decrease in CEA (53%) and calcitonin (80%) was not correlated with radiological response. Recent data suggest independent changes in calcitonin levels and changes in tumor growth during RET inhibition [Akeno-Stuart *et al.* 2007].

Finally, a pivotal phase III trial was conducted including 331 patients with unresectable locally advanced or metastatic MTC receiving vandetanib 300 mg per day until disease progression [Wells *et al.* 2012] (Table 2). At that time, patients were offered inclusion in an open-label phase with vandetanib. Excluding data from the open-label phase, the median PFS was 19.3 months in the placebo arm and not reached in the vandetanib arm (Weibull model predicted median of 30.5 months). HR for PFS was 0.27 (95% CI 0.18–0.41; $p < 0.001$). ORR was 13% in the placebo arm *versus* 45% in the vandetanib arm ($p < 0.001$; 12 patients in placebo arm responded during the open-label phase). Grade 3 and over QTc prolongation was observed in 19 patients. Consequently, regulatory agencies considered it mandatory for stringent electrocardiogram and electrolyte monitoring by expert physicians treating patients with vandetanib. Patients with sporadic MTC harboring a RET mutation, particularly the M918T mutation, significantly benefited from vandetanib. Responses to vandetanib were also observed in patients with RET unknown tumors and in patients with M918T negative tumors, suggesting that other RET mutations may also be susceptible to vandetanib inhibition.

The role of vandetanib in DTC was evaluated in a randomized phase II trial including 145 patients [Leboulleux *et al.* 2012]. Median PFS was 11.1 months in the vandetanib arm and 5.9 months in the placebo arm (HR 0.63, 95% CI 0.43–0.92, $p = 0.017$). The benefit was greater in the PTC subgroup than in the FTC/PDTC subgroup (16.2 and 7.7 months with vandetanib *versus* 5.9 and 5.6 months with placebo, respectively). Only one patient in the vandetanib group achieved a PR. However, SD was achieved in 56% of patients in the vandetanib group and 36% of patients in the placebo group ($p = 0.017$). A correlation between Tg decrease and radiologic response was not reported. These data supported the development of a phase III trial, currently ongoing (Table 4).

Cabozantinib

Cabozantinib is a potent ATP competitive inhibitor of VEGFR2, MET, KIT and RET followed by AXL and Flt3 [Viola *et al.* 2013] and is approved by the regulatory agencies for the treatment of progressive advanced MTC. Preclinical studies (*in vitro* and *in vivo*) demonstrated the activity of cabozantinib on key receptors in angiogenesis, invasiveness and cell growth [Yakes *et al.* 2011]. In cultured cells including human umbilical vein endothelial cells that are used for the investigation of endothelial cell pathophysiology, with human diploid fibroblasts and VEGF (60 ng/ml), cabozantinib (4.6 nmol/liter) was able to inhibit tubule formation. In addition, in thyroid tumor tissue, cabozantinib (100 mg/kg) was able to inhibit MET phosphorylation by its ligand, hepatocyte growth factor (HGF).

Thirty-seven patients with MTC were included in the expanded cohort of a phase I trial with a capsule dose of 175 mg daily [Kurzrock *et al.* 2011]. Sixteen (43.2%) patients had received previous TKI therapy, 17 (46%) overexpressed MET and 25 (67.5%) had RET mutation. DLT was observed at three level doses (intermittent suspension 11.52 mg/kg, daily suspension 265 mg and daily capsule 250 mg): hand–foot syndrome, mucositis and alanine transaminase and lipase elevations. Steady-state concentrations were achieved after 15 days. ORR was identified in 10 patients (29%), three of them were previously treated. SD at 6 months was seen in 15 patients (41%) and median duration of response was not reached after 17 months of follow up.

Based on those promising results, a phase III clinical study was conducted in 330 patients with locally advanced or metastatic MTC with documented RECIST progression. Patients were randomly assigned to cabozantinib 140 mg per day or placebo until disease progression or unacceptable toxicity, but crossover was not allowed [Elisei *et al.* 2013] (Table 2). The study met its primary endpoint showing a longer PFS in the cabozantinib group compared with placebo (11.2 months *versus* 4.0 months; HR 0.28, 95% CI 0.19–0.40, $p < 0.001$). In contrast to previous analysis, significant correlation was detected between individual changes in calcitonin at week 12 and radiological response of target lesions at week 12, only in patients treated with cabozantinib ($p < 0.0001$). At the American Society of Clinical Oncology (ASCO) 2013 meeting, the results from the subgroup mutational analysis were presented. Patients harboring a RET mutation had a significant benefit in PFS ($N = 169$; 60 weeks *versus* 20 weeks; HR 0.23; 95% CI 0.14–0.38, $p < 0.0001$). Moreover, PFS results in patients with an M918T mutation also correlated with an improvement in OS (HR 0.53; $p = 0.0179$) in an interim analysis with 75% of

total events achieved [EMA, 2014]. In addition, in patients with unknown RET mutation status, a benefit in PFS was shown with cabozantinib (N = 115; 48 weeks *versus* 13 weeks; HR 0.30; 95% CI 0.16–0.57, p = 0.0001) [Sherman *et al.* 2013]. Definitive conclusions about the RET mutation negative group were difficult to draw due to the small and heterogeneous sample size (N = 46; 25 weeks *versus* 23 weeks; HR 0.53, p = 0.21), but an ORR of 22% was reported. Interestingly, but limited by the small sample size, patients who were RET mutation negative and RAS mutation positive seemed to benefit from cabozantinib in PFS (N = 16; 47 weeks *versus* 8 weeks; HR 0.15, 95% CI 0.02–1.10, p = 0.0317) with an ORR of 31%. The role of cabozantinib as first-line treatment in radioiodine-refractory DTC is currently being evaluated in a phase II trial (Table 4).

Other targeted agents

VEGFR inhibitors. Based on the relevance of angiogenesis in TC progression, additional TKIs to the ones already discussed have been investigated in different TC subtypes, demonstrating activity in phase II clinical trials (Table 3). Further clinical and investigational experience is improving with those agents. However, until now, lenvatinib is the only one that has achieved a randomized, double-blind, placebo-controlled phase III trial at the moment. Lenvatinib is a FGFR1 inhibitor that is upregulated in follicular thyroid cells and is involved in tumor progression through MAPK signaling pathway activation [Kondo *et al.* 2007]. The SELECT trial included 392 patients with progressive radioiodine-refractory DTC randomized to lenvatinib 24 mg daily (N = 261) or placebo (N = 131). Patients were allowed to receive one prior VEGF or VEGFR targeted agent (N = 93). The results were presented at the ASCO 2014 Meeting, showing a significant benefit in PFS for patients treated with lenvatinib compared with placebo (18.3 months *versus* 3.6 months; HR 0.21, $p < 0.0001$). ORR was 65% in the lenvatinib group and 2% in the placebo arm ($p < 0.0001$). The most frequent grade 3 and over adverse events related to lenvatinib treatment were hypertension, proteinuria, loss of weight, fatigue and diarrhea [Schlumberger *et al.* 2014].

PI3K/AKT/mTOR inhibitors. Investigations in cultured cells and animal models with FTC have shown modest activity of everolimus on tumor growth control, but not over metastasis development. However, a phase II clinical trial including all TC histologic subtypes showed a low response rate, but moderate disease stabilization and significant clinical benefit in half of patients [Lim *et al.* 2013]. These results suggest a better role of mTOR inhibitors in a combination strategy or in more advanced or aggressive tumors, considering the role of the PI3K/Akt pathway in TC dedifferentiation. In MTC, the activity of this drug over hyperactivation of PI3K was more effective by inhibiting downstream phosphorylation (mTOR and S6K1). However, inhibition of the negative feedback of S6K1 on Insulin receptor substrate 1 and the resistance to mTORC2 inhibition may limit the activity of the rapamycin analogs. Other targeted agents to overcome those limitations are currently being investigated: dual PI3K/mTOR inhibitors (BEZ 235, BGT 226, XL-765, GDC0980) alone or in combination with Raf inhibitors (RAF265) [Jin *et al.* 2011], PI3K inhibitors, Akt inhibitors, mTOR complex catalytic site inhibitors (AZD8055 that demonstrated greater activity by inhibiting the phosphorylation of p70S6K and 4E-BP1, substrate of mTORC1 and Akt, substrate of mTORC2) and molecules that reduce protein stability by interfering in protein interactions (heat shock protein 90) or by proteosomal degradation [Garcia-Echeverria and Sellers, 2008].

MEK inhibitors. A number of molecular alterations in the RAS/RAF/MAPK pathway harbor a common downstream effector, MEK1/2. Therefore, the inhibition of these molecules represents a relevant target in inhibiting tumor progression. Selumetinib is a non-ATP competitive MAPK kinase inhibitor (MEK1/2) whose activity has been demonstrated in preclinical trials [Leboeuf *et al.* 2008]. In a phase I trial, the pharmacokinetic results showed a median half life of 8 h. DLT was rash and the recommended dose for clinical safety was 100 mg/12 h [Adjei *et al.* 2008].

Two recent phase II trials have been conducted. Hayes and colleagues demonstrated a modest activity of selumetinib in unselected patients, but with greater results in the $BRAF^{V600E}$ subpopulation [Hayes *et al.* 2012]. Ho and colleagues investigated the role of selumetinib in the inhibition of the constitutive activation of MAPK signaling involved in thyroid hormone expression genes for a recovery in the ability of radioiodine uptake [Ho *et al.* 2013]. Interestingly, all patients harboring a NRAS mutation showed an increase in iodine uptake and some grade of tumor reduction. These results were not observed in patients with a $BRAF^{V600E}$ mutation. Recent findings suggest an additional upregulation of NF-κB independently of MEK-ERK activation

[Xing, 2013]. Dual inhibition of MEK-ERK and NF-κB may be effective in the patients who were $BRAF^{V600E}$ mutation positive. Further investigation with optimal patient selection may determine the best therapeutic role for selumetinib in TC.

PPARγ inhibition. The possible influence of the quimeric oncoproteins PPARγ/PAX8 in tumorigenesis has been the basis for the investigation of oral PPARγ regulators currently administered in patients with diabetes mellitus. Rosiglitazone has been studied in a phase II trial with patients with radioiodine-refractory DTC showing a RR of 25% [Kebebew *et al.* 2009].

Epigenetic modulating agents. Targeted agents targeting epigenetic changes such as histone deacetylase inhibitors or hypomethylating agents have been investigated in TC [Harris and Bible 2011]. The histone deacetylase inhibitors vorinostat, depsipeptide and romidepsin were studied in phase I and II trials. Limited activity with hardly any tumor responses and moderate rates of disease stabilization (46–71%) with considerable adverse events (fatigue, ataxia, cardiac toxicity, thrombosis) have restricted its investigation in TC. Hypomethylating agents have been studied for recovery of radioiodine uptake. Decitabine, a better tolerated agent compared with 5-azacytidine, is currently being investigated in a phase II trial (Table 4).

Conclusion

The identification of the components of downstream signaling from the RAS/RAF/MAPK and PI3K/Akt activated pathways involved in tumorigenesis have helped to identify novel effective targeted agents in TC that was previously without active treatments. The exploration of different therapies according to their mechanism of action has demonstrated not only efficacy in phase II trials, but also a significant benefit in survival in phase III clinical trials. In addition, this knowledge allows the investigation of potential prognostic or predictive biomarkers, such as $BRAF^{V600E}$, that will help for therapy optimization and patient selection.

However, a relatively high percentage of patients, approximately 30–45% including all histologic subtypes of TC, suffer the development of a TC with unknown genetic alterations, so further investigation for underlying aberrations is warranted.

From now on, several unresolved questions require further data from consistent trials: the sequential order of the demonstrated effective treatments, the benefit of combination therapies, the development of defined subgroups of patients according to histology or mutational profile that may benefit from directed agents, and the investigation of consistent predictive or prognostic biomarkers.

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