Cabozantinib in Thyroid Cancer

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Abstract
Cabozantinib is an oral once-daily multitarget tyrosine kinase inhibitor of MET, VEGFR2, RET, acting against KIT, AXL, FLT3 and Tie-2. Cabozantinib has shown anti-cancer effects in preclinical and clinical models of cancers derived from both epithelial and mesenchymal origins [prostate cancer, non small lung cancer, medullary thyroid cancer (MTC) and differentiated thyroid cancer (DTC), renal cell carcinoma, *etc.*]. In a phase III clinical study, cabozantinib improved PFS (11.2 months *versus* 4.0 months in the placebo group) of patients with MTC (independently of age, bone metastases, RET status and prior treatment). Cabozantinib was approved in 2012 by FDA for metastatic MTC and in 2013 by EMA. Cabozantinib has been also evaluated in metastatic DTC patients, because they have activation on tyrosine kinases, including MET, VEGFR2 and RET, suggesting the possible use of cabozantinib in metastatic DTC. Actually, two phase II trials of cabozantinib in DTC patients resistant to RAI are ongoing. To increase the antineoplastic effect of cabozantinib, and to overcome the occurence of drug resistance, combination studies with other anticancer agents are ongoing. In conclusion cabozantinib has shown to exert an important therapeutic effect in patients with MTC improving PFS. In DTC patients cabozantinib has shown promising results.

Keywords: Cabozantinib; differentiated thyroid carcinoma; epidermal growth factor receptor; medullary thyroid cancer; papillary thyroid cancer; RET; tyrosine kinase inhibitors; vascular endothelial growth factor.

Introduction

Thyroid cancer (TC) is the most frequent endocrine tumor and its incidence increased in the last decades, especially for papillary carcinoma (PTC). The causes of increased incidence of TC are a topic still debated [1]. Certainly, the widespread use of ultrasound and fine-needle aspiration (FNA) resulted in increased number of TC diagnoses, also recognizing small TC [2]. Exposure to ionizing radiation is a well-documented risk factor for TC; particularly, the incidence of TC is increased after exposure to nuclear explosions (Chernobyl) especially in children [3]. Iodine deficiency, autoimmune thyroiditis and also dietary factors and environmental pollutants are known risk factors [4-8], and new ones are emerging in the last decades [9,10]. While the incidence of PTC is increasing, the mortality rate has not changed [11,12].
The main histologic types of TC are: a) differentiated TC of follicular origin (DTC): papillary (PTC, 80%), follicular (FTC, 11%) and Hürthle cells TC; b) medullary TC (MTC) [derived from C cells (2-5% of all TCs)], that can be sporadic (75%), or hereditary (25%). Hereditary MTC includes Familial MTC (FMTC), MEN 2A, MEN 2B [13]; c) anaplastic TC (ATC) (2% of all TCs) [14].

Differentiated thyroid carcinoma

Oncogenic pathways in DTC

Several oncogenic pathways are involved in the development of TC. The main are RAS/RAF/mitogen-activated protein kinase (MAPK) and phosphatidylinositide 3-Kinase (PI3K)/Akt pathways [15].
RAS ("Rat Sarcoma") genes (H-RAS, K-RAS, N-RAS) encode for intracellular G-proteins involved in activation of several intracellular signaling pathways, responsible for the cell growth, differentiation and survival. RAS mutations are found especially in FTCs (40-50%), but also in ~10% of PTCs and 50% of ATCs. It has been shown that RAS mutations are related to more aggressive tumor pathways [16, 17].

Activated RAS recruits BRAF, member of RAF family proteins which phosphorylating MEK, activates MAPK cascade. BRAF V600E (substitution of valine to glutamate at residue 600) is the most common point mutation [45% of PTC, 10-20% of poorly differentiated TC (PDTC), 20% of ATC, infrequently in FTC], and it is associated with tumor recurrence, loss of radioiodine (RAI) uptake ability and worse prognosis [18-20]. Other BRAF mutations or rearrangements (as AKP9/BRAF) are less frequent.

Rearrangement of PAX8/peroxisome proliferator-activated receptor (PPAR)γ leads to the overexpression of a protein that inhibits tumor suppressor activity of PPARγ. This mutation is found in 30-40% of FTC, 1-5% of follicular variants of PTC and 2-13% of follicular adenomas [21].

More recently, an increased expression of vascular endothelial growth factor (VEGF) has been shown in DTC [22]. The VEGF family stimulates angiogenesis, endothelial cell proliferation, migration, survival, and vascular permeability via VEGF receptors (VEGFR): VEGFR-1, VEGFR-2, and VEGFR-3 [23]. VEGF/VEGFR pathway is often constitutively activated in tumors; it induces angiogenesis and it is associated with a worse prognosis (increased risk of recurrence and decreased survival) [24,25]. In fact, depending on the tumor's capability of inducing an imbalance between angiogenic stimulators and inhibitors, TC growth and metastasis formation occur, prevailing the first [26].
Epidermal growth factor (EGF) stimulates growth and metastasis ability of the tumor, binding to EGF receptor (EGFR). It has been shown that EGFR is overexpressed in ATC and PDTC [27] and in lymph node metastasis in PTC [28]. In TC, the incidence of EGFR mutations is about 30% [29].

RET (REarranged during Transfection) is a proto-oncogene, located on chromosome 10, which encodes for a tyrosine kinase transmembrane receptor, which once activated, stimulates cell motility, reproduction and survival. RET is not present in thyroid follicular cells but its mutations are found in TC [30, 31]. Particularly, RET/PTC rearrangements (also present in thyroid adenomas and benign lesions) are found in 20-40% of sporadic PTC [32, 33]. RET/PTC rearrangements stimulate uncontrolled cell proliferation [34]. RET/PTC1 and RET/PTC3 are the most common [35].

Cytokines and chemokines are molecules that influence activation, growth, and differentiation of several target cells, and are involved in the tumorigensis of TC, or can generate antitumor response [36,37].
Cytokines and chemokines (that are usually involved in autoimmune disorders [38,39]), are expressed in TC cells, and they can be targets of new drugs, or they can be used for evaluating the inhibitory effects of different therapies [37,40-43].

DTC therapy (*Table* ***1)***

Generally, patients with DTC have a good prognosis (5 year survival rate is 97.8%), when early treated [44]. Large primary tumor size, old age, extrathyroidal extension, nodal metastases, and distant metastases (present at diagnosis in 5% of cases) are poor prognostic factors for DTC [45]. For PTCs and FTCs, surgery (total thyroidectomy) is the first-line treatment, with subsequent RAI ablation in intermediate to high-risk TC patients, while levothyroxine therapy is indicated in all patients [46, 47]. After treatment, long-term follow-up is very important, dosing thyroglobulin (Tg) and neck ultrasonography [46-48].

Some patients with recurrent (10-15% of cases) or metastasic cancer cannot be treated with surgery and/or RAI. Indeed, during progression, tumor cells lose RAI uptake ability, becoming resistant [49-51].

In metastatic cancer, the NCCN Thyroid Carcinoma Panel recommends localized therapy depending on tumor location. For patients with symptomatic and/or progressive cancer (untreatable with RAI), systemic and/or locoregional therapy (external-beam radiation therapy, resection, stereotaxic radiotherapy) may be recommended (although not curative) [44].

Systemic therapy, such as chemotherapeutic agents and kinase inhibitors, can be used in unresectable, progressive or not responsive to RAI tumors [52].

Systemic chemotherapy using doxorubicin (but also radiotherapy) is poorly effective in patients with aggressive cancer and has a significant toxicity [53, 54].
Kinase inhibitors are an alternative therapy in progressive and aggressive cancers [55]. Actually, only sorafenib and lenvatinib are approved by US Food and Drug Administration (FDA) and European Medicines Agency (EMA) for the treatment of aggressive DTC.

Medullary thyroid cancer

Oncogenic pathways in MTC

Germline or somatic point mutations in RET are the main responsible for MTC [56]. Particularly, activating mutations of RET have been found in almost all cases of hereditary MTC and about 50% of sporadic MTC [57]. The substitution of methionine by threonin at codon 918 (M918T) is the most common mutation in sporadic MTC [58]; also somatic RET mutations are linked to advanced MTC at diagnosis and a worse prognosis [59]. Germline gain of function RET mutations are found in 98% MEN2A (often, mutation of RET is in cysteine 634), 85% FMTC and more than 98% MEN2B (the most common are M918T and A883F) [60-62]. For its key-role in the development of MTC, RET is an important therapeutic target for MTC [63] and novel compounds (inhibitors of RET) are used for therapy of cancers with increased RET kinase activity [64,65].
In sporadic MTC without RET mutations, other mutations can be responsible for the development of MTC, such as somatic H-RAS ones, which is present in 56% of cases, but also KRAS, or rarely NRAS [66-69].

Furthermore, an increased expression of VEGF-A, VEGF-C, VEGFR-1, -2, -3 are found in MTC [70], and particularly, an overexpression of VEGFR-2 in MTC correlate with metastasis [71]. Several antiangiogenic molecules which block VEGF are studied, but often antiangiogenic therapy alone does not induce durable remission [72].

MET (Mesenchymal-epithelial transition) is a protoncogene coding for hepatocyte growth factor (HGF) receptor (HGFR or c-MET), having tyrosine kinase (TK) activity [73]. Mutations in c-MET or its overexpression are in several tumors, including MTC. Activated c-MET stimulates cell reproduction, migration and angiogenesis while inhibits apoptosis; in this way, it leads to tumor progression and metastatic ability [74]. Also, mutations in MET pathways have been correlated with poor clinical outcomes and drug resistance in patients with cancer [75]. An elevated coexpression of MET and HGF has been shown in a subgroup of MTCs [76,77]. MET has been recently investigated as a potential target in the treatment of TC and preliminary clinical benefits have been reported [78].

Furthermore, overexpression of EGFR in some MTC and activation of the mammalian target of rapamycin intracellular signaling pathway in hereditary MTC have been shown [79, 80].

MTC therapy (*Table* ***1)***

MTC prognosis depends on tumor size and RET mutations. In fact, it is good for patients with MTC confined to the thyroid; however, in 50% of cases, at diagnosis, MTC are already metastatic or locally advanced. In these patients there is a worse prognosis (10-year survival rate 40%) [81, 82].

The serum calcitonin (MTC marker) measurement has an important role for an early MTC diagnosis, because it has high diagnostic sensitivity and specificity [83].

In most cases, patients with sporadic MTC have a poorer prognosis because the diagnosis is made late; while MEN2A and FMTC are detected early. Three risk levels related RET mutations have been identified [32].

In MTC without neck lymph node or distant metastases, ATA Guidelines recommend a total thyroidectomy and dissection of the lymph nodes in the central compartment, while the dissection of lymph nodes in the lateral compartments is performed after considering serum calcitonin levels; while in patients with MTC confined to the neck and cervical lymph nodes, a total thyroidectomy, dissection of the central lymph node compartment and resection of the involved lateral neck compartments should be performed [84]. C cells do not concentrate iodine, then radiotherapy has no effect on MTC [52].

For locally advanced or metastatic progressive MTC, external beam radiotherapy, systemic chemotherapy (doxorubicin alone or in association with other agent such as 5FU and dacarbazine), and other nonsurgical therapies should be evaluated to reach tumor control. Chemotherapy treatment in MTC is poor effective. For these reasons, the development of new therapies was necessary [85].
For recurrent or persistent aggressive MTC, tyrosine kinase inhibitors (TKIs) are actually recommended. Recently, several TKIs have been tested in phase I, II, and III clinical trials of patients with advanced MTC but only vandetanib (2011) and cabozantinib (2012) have been approved by FDA and EMA [84].

TKIs
TKIs are molecules that compete with ATP on tyrosine kinase receptors (TKRs), blocking TK activation and then oncogenic pathways [86], like RAF, VEGFR, EGFR, MET, platelet-derived growth factor receptor (PDGFR), c-KIT, RET kinases. A TKI can block only one TK or several TKs (multikinase inhibitor), in fact TKIs have been tested on different cancers including TC [87].

In the last decade, several studies have evaluated a possible use of axitinib, lenvatinib, motesanib, pazopanib, sorafenib, sunitinib, cabozantinib and vandetanib alone or in association, in aggressive DTC or MTC, but only a few have been approved [50,88,89].

Cabozantinib

Cabozantinib is an oral once-daily multitarget TK inhibitor of MET, VEGFR2, RET and also acts against KIT (mast/stem cell growth factor), AXL, FLT3 (FMS-like tyrosine kinase 3) and Tie-2 (tunica interna endothelial cell kinase 2), involved in angiogenesis and cell proliferation [90,91]. Research about c-Met/VEGFR-2 dual inhibitor has made considerable progress, and there are several dual inhibitors in clinical research (CN103848838) [92].

All TKs are determinant in the development and progression of TC [93]; inhibiting TKs, cabozantinib avoids receptors phosphorylation and thus blocks cell proliferation, angiogenesis, growth and invasiveness of tumors [94,95].

Recently, an *in vitro* study showed that cabozantinib inhibited cell proliferation in a time-dependent and dose-dependent manner and had effect on signal transduction pathways in PTC cells harboring RET/PTC1 rearrangement. Then, it should be used to enhance the expression of iodide-handling genes and inhibit the expression of glucose transporter genes [96].

Cabozantinib (Cometriq, XL184, Exelixis), known variously as N-(4-{[6,7-bis(methyloxy)quinolin-4-yl]oxy}phenyl)-N'-(4- fluorophenyl)cyclopropane-l,l-dicarboxamide (WO2014165786), was approved in November 2012 by FDA for metastatic MTC and in December 2013 by EMA for progressive, unresectable locally advanced or metastatic MTC [97,98].

Oral formulations exist: capsules (CN103830203) and dispersible tablets. These latter, compared with common tablets, have high solubility, bioavailability, rapid in *in vivo* distribution, are stable in quality, good in taste (CN103751140) [99,100].

The recommended dose is 140 mg once daily (one 80 mg and three 20 mg capsules) and patients should not eat 2 hours before and at least 1 hour after intake of cabozantinib. The therapy may be continued until disease progression or unacceptable toxicity occurs [101].

Pharmacokinetic

A phase I study has shown that, after oral administration of 175 mg of cabozantinib (corresponding to 140 mg free base), a peak plasma concentration has reached, after 5 hours. The half-life was 91.3±33.3 hours and steady state plasma levels were reached by day 15. XL184 displayed a linear pharmacokinetic profile; 175 mg is the maximum tolerated dose [102,103]. Cabozantinib binds to ≥ 99.7% of plasma protein [104].
In vitro, cabozantinib is a substrate of cytochrome P450 (CYP) 3A4, and CYP2C8 is the CYP isoenzyme most potently inhibited by cabozantinib. For the key-role of CYP3A4 in cabozantinib metabolism, use of strong CYP3A4 inducers (*e.g.*, phenytoin, carbamazepine, rifampicin, rifabutin, rifapentine, phenobarbital) and inhibitors (*e.g.*, ketoconazole, itraconazole, clarithromycin, atazanavir, nefazodone, saquinavir, telithromycin, ritonavir, indinavir, nelfinavir, voriconazole) should be avoided when cabozantinib is administered. For patients to have therapy with CYP3A4 inhibitor or inducer, the daily dose of cabozantinib

may be, respectively, reduced or increased by 40 mg. For the same reason, the patients should no take foods or nutritional supplements inducing CYP450 activity [101].

Cabozantinib is an inhibitor, but not a substrate, of P-glycoprotein (P-gp) and then, if co-administered substrates of P-gp, its plasma concentrations may increase [105,106].
Cabozantinib is not adviced in patients with moderate-to-severe liver impairment while there are no data to support its use in patients with a creatinine clearance below 30 mL/min [107].

Preclinical studies

Cabozantinib has shown anti-cancer effects in preclinical models of cancers derived from both epithelial and mesenchymal origins (brain, breast, lung, pancreatic and thyroid cancers) [108,109].

A study, conducted on non-small-cell lung cancer (NSCLC) xenografts has demonstrated MET inhibition, after administration of oral cabozantinib, and also reduction tumor size with a dose-dependent mechanism [110].

In vitro, a study showed that cabozantinib at low doses (0.1-0.5μM), after four hours incubation, was able to inhibit MET phosphorylation and thus to block growth of malignant peripheral nerve sheath tumors and metastasis in severe combined immunodeficient (SCID) mice [111].

Yakes *et al.* evaluated the action of cabozantinib against a panel of about 270 human kinases [90]. The results have shown a potent inhibition of MET and VEGFR2, as well as KIT, RET, AXL, TIE2, FLT3. Also, cabozantinib inhibits, *in vitro*, endothelial cell tubule formation (with an antiangiogenetic effect rather than cytotoxic). *In vivo,* XL184 inhibits MET and VEGFR2 phosphorylation and is able to induce hypoxia and apoptosis by tumor and endothelial cell death and disruption of tumor vessels. Inhibition of tumor growth (in several human tumor models) realizes in a dose dependent manner. Furthermore, unlike other VEGFR2-targeting drugs, cabozantinib inhibits metastasis [90].

Another study, conducted in a model of pancreatic carcinoma on RIP-Tag2 transgenic mice, has shown that cabozantinib was able to reduce tumor size after 3 weeks ($p < 0.05$), more than vehicle-treated tumors or anti-VEGF antibody. Also, tumors treated with cabozantinib had regular borders, few projections into the acinar pancreas; they were less invasive resulting in poor development of liver metastases and a prolongated suvival. This study demonstrated that the simultaneous inhibition of VEGFR and c-MET, in contrast to the inhibition of VEGFR alone, reduces the metastasis and improves survival [112].

In another study, carried out *in vitro* in biochemical and cell-based assays, cabozantinib inhibited several forms of oncogenic RET kinase, known to be involved in MTC development, and blocked the proliferation of TT cells presenting a C634W activating mutation of RET frequently associated with MEN2A and familial MTC. *In vivo*, in MTC tumor model in nude mice, after oral administration of cabozantinib, the tumor was reduced dose-dependently, associated with reduced circulating plasma calcitonin levels. Furthermore, *in vivo*, cabozantinib decreased the levels of phosphorylated MET and RET, and tumor cellularity, proliferation, and vascularization [113]. In a study on brain lysates of non-tumor-bearing mice, central nervous system penetration of XL184 has been evidenced [114].

More recently, a study showed that cabozantinib resolves bone scans in tumor-naïve mice harboring skeletal injuries, unlike axitinib or crizotinib [115].

Clinical Trials

Several clinical trials have tested cabozantinib on different tumor types, including prostate cancer, NSCLC, MTC and DTC, renal cell carcinoma, gliomas, hepatocellular carcinoma, gastric or gastroesophageal junction cancer, melanoma, ovarian cancer and primary peritoneal or fallopian tube carcinoma [116].

In a prospective phase II trial conducted on patients with RET fusion-positive NSCLCs treated with cabozantinib (NCT01639508), preliminary data showed partial response (PR) in 2 patients out of 3, and all 3 patients remained progression-free on treatment [117].

Several studies of cabozantinib in prostate cancer were carried out and drugs combination of cabozantinib and abiraterone have been developed (WO2014165779) [118].

A phase II study showed improvement in progression-free survival (PFS) in patients with castration-resistant prostate cancer (mCRPC) treated with cabozantinib (100mg daily oral dosing). Also, in these patients a reduction of soft tissue tumors and bone turnover markers was observed [119]. Recently, results of two trials (COMET-1 and COMET-2), in mCRPC patients, were published. COMET-1 analyzed cabozantinib *versus* prednisone in 1028 patients previously treated with docetaxel and abiraterone and/or enzalutamide and concluded that cabozantinib improved bone scan response (BSR) and PFS, but did not significantly increase overall survival (OS) [120].

COMET-2 study compared cabozantinib *versus* mitoxantrone/prednisone on pain palliation (primary endpoint) in mCRPC patients previously treated with docetaxel and abiraterone and/or enzalutamide. Final results have shown the failure to achieve the primary endpoint of improving pain response, while an improvement in BSR and OS was observed in cabozantinib-treated patients [121].
Also, several association studies among cabozantinib and other drugs are ongoing. Among these: a) a phase I trial of cabozantinib and docetaxel and prednisone in patients with metastatic castration-resistant prostate cancer (NCT01683994); b) another study with cabozantinib and panitumumab is ongoing to identify the tolerability and maximum-tolerated dose (MTD) to treat KRAS Wild-Type Metastatic Colorectal Cancer (NCT02008383); c) also a trial of cabozantinib and gemcitabine in advanced pancreatic cancer (NCT01663272) is ongoing.

Clinical trials in MTC

Phase I trial

In a phase I cohort dose escalation study, 25 patients with several advanced tumors were treated across 7 dose levels of cabozantinib. The study concluded that cabozantinib has a good tolerability profile and antitumor activity was evidenced (not associated with toxicity), in patients with cancers including those with RET mutations (medullary thyroid) or MET (papillary renal) mutations. In three patients with MTC (one with RET mutation), the study showed a substantial reduction in plasma calcitonin [122].

In another important phase I non-randomized, open-label, uncontrolled, single-group, dose-escalation clinical trial, Kurzrock *et al.* [102] evaluated the safety, pharmacokineticks and MTD of cabozantinib in 85 patients with metastatic or unresectable solid tumors or lymphomas not responding to conventional therapy. A cohort of 37 metastatic, recurrent and/or local advanced MTC patients was included in the study, of which 22 with a somatic RET mutation and 3 with inherited MTC. Also, 16 MTC patients had received previous therapy with TKIs. Among patients there were one PTC and one FTC. The patients were administered with 13 dose levels with two different plans of administration (intermittent and daily) and formulations of cabozantinib. The MTC cohort received fixed daily dose of 175 mg (MTD), with capsules. Evaluation of response, using Response Evaluation Criteria in Solid Tumors (RECIST), was carried out in 77 patients, including 35 MTC. Among these last, 10 patients obtained a PR, and 3 of these 10 responses occurred in patients previously treated with TKIs, including vandetanib and sorafenib. Stable disease (SD) for at least 6 months was observed in 41% of 37 MTC patients, and SD of at least 3 months in 38% of non-MTC patients. Also, decreased levels of calcitonin (from 3 to 99%, below baseline) were observed in 28/30 patients with tumor shrinkage (however no correlation has been displayed between size of tumor reduction and decrease of calcitonin levels); reduced phosphorylation of MET and RET was observed in skin biopsies from one patient with MTC. In one MTC patient, carrier of functioning BRAF mutation but not RET mutation, tumor progression was observed. Adverse events (AEs) were observed in 90% of patients; the most frequent grade 1 or 2 AEs were diarrhea, fatigue, lack of appetite, nausea, palmar plantar erytrodysesthesia, rash, increased aspartate transaminase, vomiting, mucositis, hypertension (14%); also, two cases of hypertension grade 3 and one case of pulmonary embolism grade 4 severity were observed. This trial showed effectiveness of treatment with cabozantinib in MTC patients, including those who arbor somatic RET mutations, with an acceptable safety profile and AEs similar to other TKIs. The reduction of tumor size was evidenced in patients with/without RET mutations, and SD and durable tumor shrinkage were observed in 12/15 MTC patients presenting a somatic M918T mutation in RET. The results of this trial suggested that the antitumor activity of cabozantinib was due to its action against MET and VEGFR2, in addition to RET [102].

Phase III trial

An international, double-blind, randomized trial called EXAM, was conducted in 330 patients who had shown radiographic progression of metastatic MTC in the previous 14 months [123]. This study compared 140 mg/day of cabozantinib with placebo, in a 2:1 ratio; patients were stratified by age (>65 years or ≤ 65 years) and prior TKIs therapy. In about 50% of patients, RET mutations were present, mainly M918T (74%), while mutation status was unknown in 39% of patients. PFS (primary endpoint) was 11.2 months in the

cabozantinib group *versus* 4.0 months in the placebo group (hazard ratio, 0.28; 95% CI, 0.19 to 0.40; p <0.001) with an improvement of PFS independent of age, bone metastases, RET status and prior treatment. Kaplan-Meier estimates of patients alive and progression-free at 1 year were 47.3% for cabozantinib, and 7.2% for placebo. In the cabozantinib group of patients, response rate (partial) was 28% (duration of response of 14.6 months), and it was similar in RET + and RET- patients. A reduction of lesion size was observed in 94% of patients in the cabozantinib arm, *versus* 27% in the placebo arm. Moreover, a significant decrease of calcitonin and CEA levels were observed in the cabozantinib treated patients, from baseline to week 12, compared with an increase in the placebo group. This reduction was correlated to the reduction of the size of target lesions [123].

At the American Society of Clinical Oncology (ASCO) 2013 meeting, data relating to RET mutation, were presented: PFS was significantly better in patients with RET mutation (60 weeks *versus* 20 weeks), with an improvement in OS in M918T patients [14]. In 69% of the cabozantinib group (33% of placebo), AEs grade 3 or 4 severity were observed, such as diarrhea (15.9%), palmar plantar erythrodysesthesia (12.6%), fatigue (9.3%) and hyperthension (8.4%). Also, increase of thyroid-stimulating hormone level was shown in 57% of cabozantinib patients *versus* 19% of placebo patients. Grade 5 AEs (fistula, respiratory failure, hemorrhage, multiorgan failure and sepsis, hepatic failure, sudden death, cardiopulmonary failure, pneumonia and others) were reported in 7.9% of cabozantinib-treated patients and 7.3% of placebo-treated patients. The appearance of AEs has led to the need of reduction dose in 79% (9% in placebo) and interruption of cabozantinib therapy in 65% (*versus* 17%) of patients [123].

Clinical trials in DTC

Phase I trial
More recently, the studies on cabozantinib were extended to patients with metastatic DTC, because often, they have activation on TKs, including MET (overexpressed in about 90% of PTC), VEGFR2 and RET. This suggests the possible use of cabozantinib in metastatic DTC [124,125].

Cabanillas *et al.* have studied cabozantinib tolerability, safety, and antitumor activity in DTC patients, in a single-arm open-label drug-drug interaction phase I trial [126]. The study involved 15 patients with progressive DTC resistant to RAI (7 with PTC, 5 with FTC and 3 with Hurthle cell carcinoma). Among patients with DTC, 11 were administered with prior VEGF pathway inhibitor therapy and one patient **underwent** prior chemotherapy. Patients received daily 140 mg free base (equivalent to 175 mg salt) of cabozantinib. PR occurred in 53% of patients (5 patients with PTC and 3 patients with FTC) with a duration from 2.0 to 14.5 months and SD occurred in 40% (in 4 patients SD for 6 months or more). Thirteen patients have reported at least one AE grade 3 or higher; among these the most frequent were diarrhea (20%) and hypertension (13%), but also lack of appetite, fatigue, vomiting, weigth decreased. Two patients had grade 4 AEs (myocardial infarction and aspiration pneumonia) and one patient died for aorto-tracheal fistula. For the development of AEs, a dose reduction (3 patients reduced one dose level to 100 mg and 11 patients two dose levels to 60 mg) was necessary in all 14 evaluable patients. All patients with PR achieved their response after reduction dose of cabozantinib to 100 mg or 60 mg, maintaining the response with lower doses. Tumor regression, by magnetic resonance imaging or computed tomography scan, was observed in all 14 evaluable patients. Improvement of median PFS and median OS were not obtained, with a median follow-up, respectively of 12 and 26 months. This trial showed that cabozantinib was well tolerated also in advanced DTC showing in DTC a safety profile like the one of other VEGFR-TKIs [126].

Actually, two phase II trials of cabozantinib in DTC patients resistant to RAI are ongoing (NCT01811212 and NCT02041260).

AEs

Gastrointestinal AEs of cabozantinib include serious gastrointestinal perforations and fistulas, observed respectively in 3% and 1% of patients. For this reason, patients with perforations or fistulas should not take cabozantinib.

Cabozantinib may cause serious and sometimes deadly hemorrhage, and increases risk of thrombotic events rather than placebo. Thromboembolic risk is greater in the venous district than in arterial (6% *versus* 2%); in case of occurrence of thromboembolic arterial events or acute myocardial infarction, cabozantinib should be discontinued. Treatment should be interrupted at least 28 days before scheduled surgery. It's also recommended blood pressure monitoring before and during treatment in patients treated with cabozantinib because an increased incidence of hypertension was observed in 61% of patients [101].

Other reported AEs are osteonecrosis of the jaw (1%) [127], hand–foot syndrome (50%) [128], proteinuria (2%), reversible posterior leukoencephalopathy syndrome; while, subcortical vasogenic edema occurred in one patient [129].
Published data showed that cabozantinib may cause thyroid dysfunctions (93.1%), from subclinical hypothyroidism (predominant disease), to symptomatic thyrotoxicosis. Then, an assessment of thyroid function before therapy with cabozantinib and during follow-up is needed [130].

Recently, a study in patients with urothelial carcinoma concluded that cabozantinib monotherapy is associated with 1 or more cutaneous AE in most patients (73%). In this study, AEs included hand-foot syndrome, hair depigmentation, xerosis, scrotal erythema/ulceration and nail splinter hemorrhages [131].

Drug resistance and limits

The efficacy of cabozantinib in patients with DTC, even if promising, is limited by the occurrence of drug resistance, that might depend on the activation of different mitogenic signals [132].
Inhibition of a single TKR can cause compensatory signaling that maintains cell growth; for example, targeting the VEGFR alone could promote tumor growth due to compensatory upregulation of MET. By targeting VEGF and MET, cabozantinib blocks the MET-driven resistance to agents that inhibit either target independently. These therapies cannot be administered to some patients, owing to specific RET mutations confering resistance (RET V804 confers resistance to vandetanib) that has not yet been shown with cabozantinib [133].

The combination with other drugs [docetaxel and prednisone (NCT01683994); panitumumab (NCT02008383); gemcitabine (NCT01663272)] has been recently proposed to overcome this resistance (see above).

To personalize the cabozantinib therapy in each patient with MTC or DTC, attempts have been made on the basis of the genetic characterization of the tumor [134].

More recently it has been suggested the possibility to evaluate the sensitivity of primary DTC or MTC cells from each subject to different TKIs to ameliorate the effectiveness of the treatment [135,136].
In vitro drug screening, using primary human neoplastic cells, could predict the activity of clinical responses [137,138], preventing the administration of inactive drugs, potentially dangerous, to the patients. In fact, a positive (*in vitro*) chemosensitivity test (using primary human neoplastic cells) predicts *in vivo* effectiveness (in the same patient) in 60% of cases, while a negative (*in vitro*) is associated with a 90% of ineffectiveness of the therapy *in vivo* [137,138].

Primary human TC cell cultures can be obtained, from dedifferentiated PTC [139], from ATC [140], and also fom MTC [141,142], from surgical samples. However, more recently, it has been shown the possibility to establish primary cell cultures from FNA samples of TC opening the way to the use of FNA-cells to evaluate the preclinical sensitivity to different therapies in each patient [143-146].

Cabozantinib in other cancers

Cabozantinib is currently undergoing in clinical trials in a broad panel of other solid epithelial malignancies, including brain, ovary, bladder, melanoma, pancreas, prostate, and NSCLC tumors [130, 147-152].

Current & Future Developments

Cabozantinib is an oral once-daily multitarget tyrosine kinase inhibitor (TKI) of MET, VEGFR2, RET, acting against KIT, AXL, FLT3 and Tie-2. Cabozantinib inhibits TKs, avoids receptors phosphorylation and blocks cell proliferation, angiogenesis, growth and invasiveness of tumors. Cabozantinib has shown anti-cancer effects in preclinical models of cancers derived from both epithelial and mesenchymal origins (brain, breast, lung, pancreatic and thyroid cancers).

Several clinical trials tested cabozantinib on different tumor types [prostate cancer, NSCLC, MTC and DTC, renal cell carcinoma, *etc*.].

In a phase III clinical study, cabozantinib improved PFS (11.2 months *versus* 4.0 months in the placebo group) of patients with MTC (independently of age, bone metastases, RET status and prior treatment). Cabozantinib was approved in 2012 by FDA for metastatic MTC and in 2013 by EMA. Cabozantinib has been also evaluated in metastatic DTC patients, because they have activation on TKs, including MET,

VEGFR2 and RET, suggesting the possible use of cabozantinib in metastatic DTC. Actually, two phase II trials of cabozantinib in DTC patients resistant to RAI are ongoing.

To increase the antineoplastic effect of cabozantinib, and to overcome the occurence of drug resistance, combination studies with other anticancer agents are ongoing. To personalize the cabozantinib therapy in each patient with MTC or DTC, attempts have been made on the basis of the molecular characterization of the tumor. More recently, it has been suggested the possibility to evaluate the *in vitro* sensitivity of primary DTC or MTC cell cultures from each subject to different TKIs to ameliorate the effectiveness of the treatment, preventing the administration of inactive drugs, potentially dangerous, to the patients.

In conclusion cabozantinib has shown to exert an important therapeutic effect in patients with MTC improving PFS. In DTC patients, cabozantinib has shown promising results, suggesting that in the next future it will be an alternative to sorafenib in these patients.

Conflict of Interest

The authors have nothing to declare.

Acknowledgements

A. Antonelli, P. Fallahi, S.M. Ferrari and F. Di Bari made substantial contribution in the conception and design of the paper, in the acquisition and interpretation of data, and in drafting of the manuscript. G. Materazzi, S. Benvenga and P. Miccoli made contribution in the drafting of the paper.

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Table **1.** The different types of thyroid cancers and the classical treatments applied for each of them.

Types of thyroid cancers	Applied treatments
Differentiated thyroid cancer PTC and FTC	Surgery (total thyroidectomy) is the first-line treatment, with subsequent RAI ablation in intermediate to high-risk TC patients, while levothyroxine therapy is indicated in all patients. After treatment, long-term follow-up is very important, dosing thyroglobulin (Tg) and neck ultrasonography
Progressive and aggressive cancers (untreatable with RAI)	Systemic (as chemotherapeutic agents and kinase inhibitors - sorafenib and lenvatinib are approved by FDA and EMA for the treatment of aggressive DTC) and/or locoregional therapy (external-beam radiation therapy, resection, stereotaxic radiotherapy) may be recommended (although not curative)
Medullary thyroid cancer MTC without neck lymph node or distant metastases	ATA Guidelines recommend a total thyroidectomy and dissection of the lymph nodes in the central compartment, while the dissection of lymph nodes in the lateral compartments is performed after considering serum calcitonin levels
MTC confined to the neck and cervical lymph nodes	A total thyroidectomy, dissection of the central lymph node compartment and resection of the involved lateral neck compartments should be performed
Progressive, unresectable locally advanced or metastatic MTC	External beam radiotherapy, systemic chemotherapy (doxorubicin alone or in association with other agent such as 5FU and dacarbazine), and other nonsurgical therapies should be evaluated to reach tumor control. Chemotherapy treatment in MTC is poor effective and the development of new therapies was necessary. TKIs are actually recommended: vandetanib (2011) and cabozantinib have been approved by FDA (2012) and EMA (2013)

Anaplastic thyroid cancer	Surgery (total thyroidectomy), external beam radiotherapy, systemic chemotherapy and other nonsurgical therapies should be evaluated (TKIs)

DTC differentiated thyroid cancer; EMA European Medicines Agency; FDA US Food and Drug Administration; FTC follicular thyroid cancer; MTC medullary thyroid cancer; PTC papillary thyroid cancer; RAI radioiodine; TC thyroid cancer; TKIs tyrosine kinase inhibitors.