Sorafenib and thyroid cancer

Poupak Fallahi[1], Silvia Martina Ferrari[1], Francesca Santini[1], Alda Corrado[1], Gabriele Materazzi[2], Salvatore Ulisse[3], Paolo Miccoli[2], Alessandro Antonelli[1]

[1] Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy; [2] Department of Surgical, Medical, Molecular Pathology and Critical Area, University of Pisa, Pisa, Italy; [3] Department of Experimental Medicine, Sapienza University of Rome, Rome, Italy.

Running title: Sorafenib and thyroid cancer

Corresponding Author:

Alessandro Antonelli, MD

Department of Clinical and Experimental Medicine

University of Pisa

Via Savi, 10

56126 Pisa, Italy

telephone number: +39 050 992318

fax number: +39 050 553235

e-mail: alessandro.antonelli@med.unipi.it

Manuscript Word Count: 9932

Abstract

Sorafenib (Nexavar) is a multikinase inhibitor which has demonstrated both anti-proliferative and anti-angiogenic properties *in vitro* and *in vivo*, inhibiting the activity of targets present in the tumor cell [c-RAF (proto-oncogene serine/threonine-protein kinase), BRAF, ^{V600E}BRAF, c-KIT, and FMS-like tyrosine kinase 3] and in the tumor vessels (c-RAF, vascular endothelial growth factor receptor-2, vascular endothelial growth factor receptor-3, and platelet-derived growth factor receptor β). Since several years, Sorafenib has been approved for the treatment of hepatocellular carcinoma and advanced renal cell carcinoma. After previous studies showing that Sorafenib was able to inhibit oncogenic RET mutants, ^{V600E}BRAF, and angiogenesis and growth of orthotopic anaplastic thyroid cancer xenografts in nude mice, some clinical trials proved the effectiveness of Sorafenib in advanced thyroid cancer. Currently, the evaluation of the clinical safety and efficacy for the treatment of advanced thyroid cancer is ongoing. This paper reviews the antineoplastic effect of Sorafenib in thyroid cancer.

Several studies have evaluated (or are ongoing) the long term efficacy and tolerability of Sorafenib in patients with papillary and medullary aggressive thyroid cancer. The results suggest Sorafenib is a promising therapeutic option in patients with advanced thyroid cancer not responsive to traditional therapeutic strategies.

1. Introduction

Thyroid cancer is the most prevalent type of endocrine malignancy and it represents the 7th most common cause of any new malignancy in the US for women, (25480 cases in 2007, 3.8% of new cases), while causing only about 1% of new cases in men (8070 cases in 2007; incidence rank: 14th) [1].

While in the last fourty years the incidence of thyroid cancer has been increasing, the percentage of cancer deaths per year, relative to the number of new cases, has decreased from 15% to 5% [1].

Differentiated thyroid cancer (DTC) is the most common histological subtype and it is primarily responsible for the increased overall incidence of thyroid cancer [2]. After surgery, patients with papillary (PTC) and follicular (FTC) thyroid cancer are followed by basal and thyroid-stimulating hormone (TSH)-stimulated thyroglobulin (Tg) determination, and by neck ultrasonography [3-6].

However, the improvement of survival leads to an increased cancer related morbidity and to a higher number of advanced diseases that are less responsive to the traditional therapeutic modalities (i.e., combination of surgery radioiodine ablation and TSH suppressive therapy). Cellular dedifferentiation occurs in up to 5% of cases during tumor progression and it is characterized by more aggressive growth, metastatic spread and loss of iodide uptake ability. Also radiotherapy and conventional chemotherapy are of modest importance in the treatment of dedifferentiated thyroid cancer [7]. The only systemic therapy approved in most countries is doxorubicin, which has traditionally been a limited option because complete or partial responses (PR) are rare and toxicity profile is unfavourable. Therefore, the management of dedifferentiated thyroid cancer requires new therapeutic options.

Some molecular pathways are involved in the development of thyroid cancer. In PTC, rearranged during transfection/PTC (RET/PTC) rearrangements are found in 30-40%, RAS mutations in about 10%, and BRAF mutations in approximately 40-50%, with no overlap among these mutations, whereas a higher prevalence of BRAF mutations (up to 70%) has been observed in dedifferentiated papillary thyroid cancer (DePTC) [8,9].

In FTC, RAS mutations are found in 40-50% of tumors [10] and may also correlate with tumor dedifferentiation and less favorable prognosis [11].

PAX8-peroxisome proliferator-activated receptor (PPAR)γ rearrangement (PAX8-PPARγ) occurs in ~ 35% of conventional follicular carcinomas, and with lower prevalence in oncocytic (Hurthle cell) carcinomas

[12]. Tumors harboring PAX8-PPARγ tend to present at a younger age, be smaller in size, and more frequently have vascular invasion.

Alteration of the RET proto-oncogene plays a casual role in the familial forms of medullary thyroid carcinoma (MTC) and has also been found in sporadic forms of the disease.

The knowledge of the molecular pathways involved in the pathogenesis of thyroid cancer has made possible the development of new therapeutic drugs able to block oncogenic kinases (V600EBRAF, RET/PTC) or signaling kinases [vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptors] involved in cellular growth and proliferation [13].

2. RET

The RET gene is located on chromosome 10q11.2 and it encodes a transmembrane receptor [14].

The natural alternative splicing of the RET gene results in the production of 3 different isoforms of the protein RET. RET51, RET43 and RET9 contain 51, 43 and 9 amino acids in their C-terminal tail respectively. The biological roles of isoforms RET51 and RET9 are the most well studied *in vivo* as these are the most common isoforms in which RET occurs [15].

RET is the receptor for members of the glial cell line-derived neurotrophic factor family of extracellular signalling molecules or ligands [15].

RET activation triggers autophosphorylation of tyrosine residues that serve as docking sites for adaptor proteins, which coordinate cellular signal transduction pathways [eg, mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase, etc.] which are important in the regulation of cell growth [15].

3. PTC

In PTC, RET can be activated by chromosomal rearrangement known as RET/PTC rearrangement [15]. In RET/PTC, the 3' portion of the RET gene is fused to the 5' portion of various unrelated genes. At least 13 types of RET/PTC have been reported to date, all formed by the fusion of RET to different partners [15]. The two most common rearrangement types, RET/PTC1 and RET/PTC3, account for the vast majority of all rearrangements found in PTCs. RET/PTC is tumorigenic in thyroid follicular cells, as it transforms thyroid cells in culture and gives rise to thyroid cancers in transgenic mice [15].

Papillary carcinomas with RET/PTC rearrangements typically present at younger age and have a high rate of lymph node metastases, classic papillary histology and possibly more favorable prognosis, particularly those harboring RET/PTC1 [16].

In tumors arising after radiation exposure, RET/PTC1 was found to be associated with classic papillary histology, whereas RET/PTC3 type was more common in the solid variants [17].

BRAF mutations are highly prevalent in PTCs with classical histology and in the tall cell variant [18]. In many studies, the presence of BRAF mutation has been found to correlate with aggressive tumor characteristics such as extrathyroidal extension, advanced tumor stage at presentation, and lymph node or distant metastases [19]. BRAF mutations have also been associated with the decreased ability of tumors to trap radioiodine and treatment failure of the recurrent disease, which may be due to the dysregulation of function of the sodium iodide symporter [19].

In PTCs, RAS mutations occur in ~10% of tumors. PTCs with RAS mutations almost always have the follicular variant histology [16]. Some studies have reported the association between RAS mutations and more aggressive behaviour of PTC and with higher frequency of distant metastases [20].

4. MTC

MTC originates from the calcitonin-producing neuroendocrine cells of the thyroid gland and accounts for 5% of all thyroid malignancies [21]. MTC is usually a slow-growing tumor, and patients with metastatic disease have 10-year overall survival (OS) rates of 40-50% [22]. Most cases of MTC are sporadic and present with metastatic disease at diagnosis; approximately 20% of MTC cases occur as a component of multiple endocrine neoplasia syndrome type 2 (MEN2). Surgery remains the only curative modality available [23]. However, there are no effective therapeutic options available for distant metastatic disease because the tumor does not respond to external radiotherapy, radionuclide therapy or chemotherapy.

Activating point mutations in RET can give rise to the hereditary cancer syndrome known as MEN2 [24]. There are three subtypes based on clinical presentation: MEN2A, MEN2B, and familial medullary thyroid carcinoma [25]. There is a high degree of correlation between the position of the point mutation and the phenotype of the disease.

Activating mutations of the tyrosine kinase receptor RET have been reported in nearly all hereditary cases

of MTC and in 30-50% of sporadic tumor cases [25]. Thus, RET has become an important therapeutic target for MTC.

5. Tyrosine Kinase Receptor Inhibitors

Tyrosine kinase inhibitors (TKIs) are small organic compounds that affect tyrosine kinase-dependent oncogenic pathways, competing with the ATP-binding site of the catalytic domain of the tyrosine kinase [26]. Occupation of this site inhibits autophosphorylation and activation of the tyrosine kinase and prevents further activation of intracellular signalling pathways. TKIs can be specific to one or several homologous tyrosine kinases. Thus, a single TKI may target multiple tyrosine kinases [27]. Several small-molecule TKIs directed toward RET kinase have been tested *in vitro*, and in pre-clinical, and clinical studies (**Table 1** [28-40] and **Table 2** [41-51]). ZD6474, also known as Vandetanib, an orally active low-molecular-weight receptor TKI, is a potent inhibitor of the VEGFR-2 and effectively blocks RET tyrosine kinase [41]. ZD6474 has been shown to block phosphorylation and signaling from RET/PTC3 and RET carrying the most common MEN2A and MEN2B mutations *in vitro*, to induce growth arrest of human papillary cancer cell lines carrying RET/PTC1, and to prevent tumor growth in nude mice after injection of RET/PTC3-transformed fibroblasts or RET mutation-positive medullary cancer [52].

Various classes of small TKIs have shown inhibition of RET activity in pre-clinical studies, including pyrazolopyrimidine inhibitors PP1 and PP2, 2-indolinone derivative RPI-1, and indolocarbazole derivatives CEP-701 and -751 [42-44]. PP1 and PP2 have been tested in pre-clinical studies and found to be effective in therapeutic concentrations in blocking RET/PTC signaling *in vivo* and abolishing its tumorigenic effects in experimental animals. A multikinase inhibitor SU12248 (Sunitinib) has been shown to effectively inhibit signaling from RET/PTC kinase in the experimental models and has been progressed to phase II clinical trial in radioiodine-refractory, unresectable DTC and MTC [53].

In a recent study [54] it has been compared the effect of four TKIs (Axitinib, Sunitinib, Vandetanib, and XL184) on cell proliferation, RET expression and autophosphorylation, and ERK activation in cell lines expressing a MEN2A (MTC-TT), a MEN2B (MZ-CRC-1) mutation, and a RET/PTC (TPC-1) rearrangement. XL184 and Vandetanib most effectively inhibited cell proliferation and RET autophosphorylation. XL184 being the most potent inhibitor in MEN2A and PTC and Vandetanib the most

effective in MEN2B *in vitro*.

A phase I dose-escalation study of oral Cabozantinib was conducted in patients with advanced solid tumors [55]. Eighty-five patients were enrolled, including 37 with MTC. Eighteen patients experienced tumor shrinkage of 30% or more, including 17 (49%) of 35 patients with MTC with measurable disease. Additionally, 15 (41%) of 37 patients with MTC had stable disease (SD) for at least 6 months, resulting in SD for 6 months or longer or confirmed PR in 68% of patients with MTC. These results suggest that Cabozantinib has an acceptable safety profile and is active in MTC.

6. Sorafenib

Sorafenib is a small molecular (a bi-aryl urea), multi-targeted TKI, approved for the treatment of primary kidney cancer [advanced renal cell carcinoma (RCC)] and advanced primary liver cancer [hepatocellular carcinoma (HCC)].

It demonstrated inhibitor activity against VEGFR-2 and 3, c-Kit, platelet-derived growth factor receptors, RET/PTC, and Raf kinases (more avidly C-Raf than B-Raf) [56,57], and it is the only TKI able to target the Raf/Mek/Erk pathway (MAPK pathway). In addition, Sorafenib has been also shown to induce apoptosis through down-regulation of Mcl-1 in many cancer types [58].

Recently, Matsuse et al. [59] tested the efficacy of Sorafenib in a novel complex BRAF mutation (BRAF p.V600delinsYM) identified in 4/492 Japanese PTC cases (0.81%). *In vitro* kinase assay and western blotting revealed that this mutation conferred high kinase activity on the BRAF protein, leading to constitutive activation of the MAPK signaling pathway. The degree of all the functional characteristics was comparable to that of ^{V600E}BRAF, and treatment with a BRAF inhibitor Sorafenib was also equally effective in this mutant [59].

Sorafenib was approved by the U.S. Food and Drug Administration in December 2005 [60], and received European Commission marketing authorization in July 2006 [61], for the treatment of advanced renal cancer.

In October 2007, the European Commission granted marketing authorization to the drug for the treatment of patients with HCC. U.S. Food and Drug Administration approval for this indication followed in November 2007 [62].

7. Pharmacokinetic (PK) Profile

7.1 Absorption and distribution

After administration of Nexavar tablets, the mean relative bioavailability is 38–49% when compared to an oral solution. The absolute bioavailability is not known. Following oral administration Sorafenib reaches peak plasma concentrations in approximately 3 hours. When given with a high-fat meal Sorafenib absorption was reduced by 30% compared to administration in the fasted state. Mean C-max and area under curve increased less than proportionally beyond doses of 400 mg administered twice daily. *In vitro* binding of Sorafenib to human plasma proteins is 99.5%.

Multiple dosing of Nexavar for 7 days resulted in a 2.5- to 7-fold accumulation compared to single dose administration. Steady state plasma Sorafenib concentrations are achieved within 7 days, with a peak to trough ratio of mean concentrations of less than 2.

7.2 Biotransformation and elimination

The elimination half-life of Sorafenib is approximately 25–48 hours. Sorafenib is metabolized primarily in the liver and undergoes oxidative metabolism, mediated by cytochrome P450 3A4 (CYP3A4), as well as glucuronidation mediated by UGT1A9. Sorafenib conjugates may be cleaved in the gastrointestinal tract by bacterial glucuronidase activity, allowing reabsorption of unconjugated drug. Following oral administration of a 100 mg dose of a solution formulation of Sorafenib, 96% of the dose was recovered within 14 days, with 77% of the dose excreted in faeces, and 19% of the dose excreted in urine as glucuronidated metabolites. Unchanged Sorafenib, accounting for 51% of the dose, was found in faeces but not in urine, indicating that biliary excretion of unchanged drug might contribute to the elimination of Sorafenib.

Sorafenib accounts for approximately 70–85% of the circulating analytes in plasma at steady state. Eight metabolites of Sorafenib have been identified, of which five have been detected in plasma. The main circulating metabolite of Sorafenib in plasma, the pyridine N-oxide, shows *in vitro* potency similar to that of Sorafenib. This metabolite comprises approximately 9-16% of circulating analytes at steady state.

Neither age nor gender and race influence Sorafenib PK.

No relationship was observed between Sorafenib exposure and renal function in subjects with normal renal function, mild, moderate or severe renal impairment.

The PK of Sorafenib in Child-Pugh A and B non-HCC patients were similar to the PK in healthy volunteers. There are no data for patients with Child-Pugh C (severe) hepatic impairment. Sorafenib is mainly eliminated via the liver, and exposure might be increased in this patient population.

7.3 Drugs interactions

Sorafenib is metabolized by CYP3A4 such as Sunitinib, Pazopanib and Vandetanib and it seems to be the more susceptible to CYP3A4 inducers or inhibitors [63].

Sorafenib inhibited CYP2B6, CYP2C8 and CYP2C9 *in vitro* with similar potency. However, in clinical PK studies, concomitant administration of Sorafenib 400 mg twice daily with Cyclophosphamide, a CYP2B6 substrate, or Paclitaxel, a CYP2C8 substrate, did not result in a clinically meaningful inhibition. These data suggest that Sorafenib at the recommended dose of 400 mg twice daily may not be an *in vivo* inhibitor of CYP2B6 or CYP2C8.

Additionally, concomitant treatment with Sorafenib and Warfarin, a CYP2C9 substrate, did not result in changes in mean prothrombin time-international normalized ratio compared to placebo. Thus, also the risk for a clinically relevant *in vivo* inhibition of CYP2C9 by Sorafenib may be expected to be low. However, patients taking Warfarin or Phenprocoumon should have their international normalized ratio checked regularly.

Concomitant administration of Sorafenib and Midazolam, Dextromethorphan or Omeprazole, which are substrates for cytochromes CYP3A4, CYP2D6 and CYP2C19 respectively, did not alter the exposure of these agents. This indicates that Sorafenib is neither an inhibitor nor an inducer of these cytochrome P450 isoenzymes. Therefore, clinical PK interactions of Sorafenib with substrates of these enzymes are unlikely.

As already described above, although Sorafenib is metabolized by CYP3A4, which can be inhibited or induced by various other drugs and environmental chemicals, biliary excretion of the unchanged parent drug accounts for more than half of the elimination of Sorafenib, and this route of elimination is not affected by agents that inhibit CYP3A4 activity. A study examining concomitant administration of Ketoconazole, a CYP3A4 inhibitor, with Sorafenib in healthy male volunteers demonstrated no change in

the PK of Sorafenib [64].

Potent CYP3A4 inducers may increase Sorafenib metabolism, but no clinical studies have evaluated this potential interaction [65].

7.4 Protein p-glycoprotein (P-gp)-substrates

In vitro, Sorafenib has been shown to inhibit the transport P-gp. Increased plasma concentrations of P-gp substrates such as digoxin cannot be excluded with concomitant treatment with Sorafenib. *In vitro*, Sorafenib inhibited glucuronidation via UGT1A1 and UGT1A9 [63,66].

The clinical significance of this inhibition is not clear and drugs that are metabolized by these enzymes should be used with caution in patients treated with Sorafenib due to a potential risk of drug interactions.

Sorafenib was demonstrated to have a moderate affinity for several members of the ATP-binding cassette sub-family (ABC).

In LLC-PK1, Caco-2, K562, and MDCKII cells, it was shown that Sorafenib is a moderate substrate for the efflux transporter ABCB1 (P-glycoprotein) and ABCG2 (breast cancer resistance protein) [67].

In vivo, Sorafenib was also demonstrated to be a substrate of ABCG2 and perhaps, ABCB1. Mice completely lacking both functional ABCB1 and ABCB2 and administered with oral Sorafenib had a higher accumulation of this drug in the brain than mice lacking either of the transporters alone.

7.5 Combination with other anti-neoplastic agents

In clinical studies Nexavar has been administered with a variety of other anti-neoplastic agents at their commonly used dosing regimens including Gemcitabine, Cisplatin, Oxaliplatin, Paclitaxel, Carboplatin, Capecitabine, Doxorubicin, Irinotecan, Docetaxel and Cyclophosphamide. Sorafenib had no clinically relevant effect on the PK of Gemcitabine, Cisplatin, Oxaliplatin or Cyclophosphamide.

Sorafenib was given in phase I clinical trials in combination with Carboplatin, Dacarbazine, Gemcitabine, Oxaliplatin, and Paclitaxel, with no detectable drug interactions observed [65]. However, the Doxorubicin area under curve was 47% greater when coadministered with Sorafenib, although no significant difference in toxicity was observed despite the greater Doxorubicin exposure [68]. When coadministered with

Irinotecan, exposure was greater for both Irinotecan (26%–42%) and its active metabolite SN-38 (70%–120%), but diarrhea from Irinotecan was not appreciably worse with the combination [69].

7.6 *Sorafenib and other targeted agents*

A phase I dose-escalation trial of Sorafenib and Bevacizumab was performed on 39 patients with various cancers at below-recommended single-agent doses [70]. This combination showed promising clinical activity in particular in ovarian cancer, but the rapidity and frequency of dose reductions indicated an intolerable long-term dosage and the need for alternative dosing schedules.

Another phase I study reported that intermittent Sorafenib dosing with Bevacizumab had clinical activity, and fewer patients required a Sorafenib dose reduction and fewer side effects were observed [71].

The combination of Sorafenib with Erlotinib was investigated in a phase I trial: it was well-tolerated and showed promising activity [72]. In a phase II trial for the combination, a higher progression-free survival (PFS) and OS was seen in the epidermal growth factor receptor (EGFR) wild-type and the EGFR FISH-negative patients with advanced non-small cell lung cancer, compared to Erlotinib alone [73]. However, additional studies are needed to confirm the benefit of this combination. Recently, a pre-clinical study of the combination of Sorafenib with Erlotinib or Cetuximab showed synergistic antitumor activity in both colorectal cancer and non-small cell lung cancer [74].

A phase I study investigated Sorafenib plus interferon (IFN)-α-2a in advanced RCC and melanoma and it showed preliminary antitumor activity and the doses were well-tolerated [75]. Another study was conducted on the combination of Sorafenib and IFN-α-2b in advanced RCC patients and it showed substantial activity, but the toxicity exceeded that of either drug alone [76]. However, dose reductions and breaks between cycles allowed for long term therapy. In contrast, a more recent phase II study that investigated the combination of Sorafenib and pegylated IFN-α-2b in metastatic melanoma patients showed modest clinical activity and serious side effects, such as fatal bleeding complications [77]. This effect could have been linked to a different dosing schedule or the use of Peg-IFN-α-2b instead of conventional IFN-α.

7.7 Undesirable effects

The most common adverse reactions were diarrhoea, rash, alopecia and hand-foot syndrome, while the most important serious adverse reactions were myocardial infarction/ischaemia, gastrointestinal perforation, drug induced hepatitis, haemorrhage, and hypertension/hypertensive crisis.

Adverse reaction's frequencies are defined as: very common (≥1/10), common (≥1/100 to <1/10), uncommon (≥1/1,000 to <1/100), rare (≥1/10,000 to <1/1,000), not known (cannot be estimated from the data available).

Undesirable effects are presented in the **Table 3** [78].

8. Clinical studies in thyroid cancer

After previous studies showing that Sorafenib was able to inhibit oncogenic RET mutants, ^{V600E}BRAF, and angiogenesis and growth of orthotopic anaplastic thyroid cancer (ATC) xenografts in nude mice, some clinical trials proved the effectiveness of Sorafenib in advanced thyroid cancer [49,79].

There have been different phase II trials of Sorafenib [BAY 43-9006, 400 mg bis in die (b.i.d.)]. The first trial of Sorafenib (400 mg b.i.d.) was conducted on 30 DTC patients, and PR was reported in 7 patients and SD in 16 patients [28].

The second trial reported a PR in 6 patients and SD for 6 months or more in 23 of the 41 patients with PTC, but therapy was ineffective in the 11 patients with FTC or poorly DTC and in the 4 patients with ATC [29].

In the third trial, which was conducted on 32 DTC patients, a PR was reported in 8 patients and SD in 11 patients [30]. Greater efficacy of Sorafenib was seen in PTC in particular in the ones with a BRAF mutation, than in poorly DTC, and it had no effect on iodine uptake. A phase III trial comparing PFS is under way in which Sorafenib and placebo are being administered to patients with radioiodine resistant metastatic DTC.

A further phase II trial of Sorafenib in patients with advanced MTC (16 sporadic, or 25 hereditary) has been conducted. Among 16 patients with sporadic MTC, one achieved PR (6.3%), 14 had SD (87.5%), and one was not-evaluable. Median PFS (mPFS) was 17.9 months. The treatment in MTC hereditary patients was prematurely terminated because of slow accrual [37].

Ahmed et al. [32] evaluated Sorafenib in a UK population with progressive locally advanced/metastatic MTC or DTC with non-radioiodine-avid disease. Thirty-four patients were recruited (15 medullary and 19 differentiated) and were treated with Sorafenib 400 mg twice daily. After 6 months, the radiological response rate (RR) was 15% and a further 74% of patients achieved SD in the first 6 months. After 12 months of treatment, the radiological RR was 21%. In the MTC patients, the radiological RR at 12 months was 25% and OS was 100%. In DTC patients corresponding rates were 18 and 79% respectively. Median overall and PFS points were not reached at 19 months. The most common adverse events included hand-foot syndrome, other skin toxicities, diarrhoea and alopecia. Dose reduction was required in 79% patients. Median time on treatment was 16.5 months. The majority of the patients showed radiological and biochemical stabilization of disease despite dose reductions [32].

Capdevila et al. [31] tested Sorafenib in patients with advanced thyroid cancer among 30 evaluable patients and they observed PRs in 9 and SD in 13 patients. With a median follow-up of 11 months, the mPFS was 11.6 months. Interestingly, six out of 12 (50%) patients with MTC obtained a PR, indicating that the drug might be effective in MTC, although the small number of patients requires further prospective studies.

Due to these results, Koh et al. [50] tried to identify potential combinatorial strategies to improve the efficacy of Sorafenib in patients with progressive MTC. Two human MTC cell lines, TT and MZ-CRC-1, which harbor endogenous C634W and M918T RET mutations respectively, were tested with Sorafenib, Everolimus and AZD6244 (Mek inhibitor) alone and in combination. The results showed that Sorafenib was the most active compound and had a synergistic effect with AZD6244 ($p<0.001$ for each cell line) [50].

Recently, Capdevila et al. [33] designed a retrospective analysis of patients with metastatic thyroid cancer treated with Sorafenib in seven Spanish referral centers. Thirthy-four patients were included between June 2006 and January 2010. They were all not suitable for any traditional therapies and received 400 mg twice a day. The primary end point was objective RR, secondary was toxicity, mPFS, mOS and correlation between tumor marker levels (Tg, calcitonin and carcinoembryogenic antigen). Sixteen patients presented DTC (7 papillary, 9 follicular), 15 MTC, and 3 ATC. Eleven patients (32%) achieved PR and 14 (41%) had SD beyond 6 months. RRs were 47% (7 of 15) for MTC, 19% (3 of 16) for DTC and 33% (1of 3) for ATC. With a median follow up of 11.5 months, mPFS were 13.5, 10.5 and 4.4 months for DTC, MTC and ATC

respectively. A significant decrease in tumor markers was also reported. In this trial, Sorafenib showed antitumor efficacy in all histological subtypes of thyroid cancer [33].

Thanks to the phase II trial results, it has been initiated a multicenter, double-blind randomized phase III study evaluating the efficacy and safety of Sorafenib compared to placebo in locally advanced/metastatic radioactive iodine (RAI)-refractory DTC that is still ongoing [80].

9. The DECISION trial

The DECISION trial was designed to assess the ability of Sorafenib to improve PFS in patients with locally advanced or metastatic, RAI-refractory DTC. The duration of the trial is expected to be 30 months. The primary endpoint is PFS; secondary includes OS, time to disease progression, disease control rate, RR, duration of response, safety and PK analysis. The efficacy and safety are evaluated respectively every 56 days (two cycles) and 28 days (1 cycle) for the first 8 months and every 56 days after.

About 380 patients, enrolled in 19 countries (USA, Italy, Germany, France, Poland, UK, Denmark, Spain, Sweden, Netherlands, Austria, Belgium, China, South Korea, Japan, Russia, Slovakia, Bulgaria and Saudi Arabia) and stratified according to age (<60 vs >60 years) and geographic region (North America vs Europe vs Asia) will be randomized 1:1 to receive placebo or Sorafenib. The inclusion criteria are: age >18, lie expectancy of at least 12 weeks, locally advanced or metastatic DTC (papillary, follicular, Hurtle cell or poorly differentiated cancer) with at least one measurable lesion as measured by computer tomography or magnetic resonance imaging and disease progression within 14 months. All patients must have RAI-refractory disease, defined as a target lesion with no iodine uptake on a post RAI scan performed under conditions of a low iodine diet and adequate TSH elevation or recombinant human TSH stimulation. Some patients who have had some iodine uptake may be also eligible.

Addictional inclusion criteria are a performance status < 2 according to Eastern Cooperative Oncology Group, adequate TSH suppression (<0.5 mU/L) absence of renal ad liver failure and adequate bone marrow function. Patients who have yet received any treatment with TKI, monoclonal antibodies against VEGFRs or other targeted agents, cytotoxic chemotherapy or Thalidomide were excluded.

Patients are being randomized to receive Sorafenib 400 mg or matching placebo, twice daily in a double-blind fashion. The treatment will be continued until radiographically documented disease progression,

unacceptable toxicity or the study endpoint. In the case of confirmed disease progression, patients who receive placebo were crossed-over to Sorafenib.

Dose modifications or interruptions are feasible according to specific criteria: grade 2-3 hand-foot skin reaction and other adverse events [81].

A recent press release has communicated that DECISION trial has met its primary endpoint increasing mPFS.

10. Latest results

Schneider et al. [34] recently published a long term analysis on the efficacy of Sorafenib (400 mg twice a daily) in 31 patients with progressive metastatic or locally advanced RAI-refractory DTC. Encouraging results have been reported: mPFS was 18 months (95% confidence interval: 7–29 months) and mOS was 34.5 months (95% confidence interval: 19–50 months). Eight patients (31%) achieved a PR and 11 patients (42%) showed SD after a median follow-up of 25 months (range 3.5–39 months). Toxicity mostly included hand foot syndrome, weight loss, diarrhoea and rash [34].

Furthermore, the therapeutic effect of Sorafenib has been recently shown in a patient with brain metastasis from FTC [82].

In another study, 17 patients with progressive DTC refractory to RAI were treated with Sorafenib used off-label independently from their performance status. Median follow-up was 15.5 months. Clinical benefit was obtained in 71% of subjects (30% PR and 41% SD). Sorafenib was mostly well tolerated but a high incidence of fatal events was reported (3 patients died from severe bleeding events and 2 from cardiac arrest). The best responses were observed in lymph nodes and lung. Baseline Tg levels and the Tg response to treatment were correlated to both radiological response and PFS. Baseline fluorodeoxyglucose positron emission tomography assessment and early fluorodeoxyglucose positron emission tomography response were correlated to radiological response [35].

Savvides et al. [36] treated twenty patients with ATC with Sorafenib 400 mg twice daily. Two of 20 patients had a PR (10%) and an additional 5 of 20 (25%) had SD. The duration of response in the two responders was 10 and 27 months respectively. For the patients with SD the median duration was 4 months (range 3–11 months). The overall mPFS was 1.9 months with a median and 1-year survival of 3.9 months

and 20% respectively. Toxicity was manageable as previously described for Sorafenib including hypertension and skin rash [36].

Cohen et al. evaluated the combination therapy (Withaferin+Sorafenib) against anaplatic (SW1736) and papillary (BCPAP) carcinoma cell lines. Combination therapy with Sorafenib+Withaferin showed synergistic efficacy in papillary and anaplastic cancers in vitro with significant induction of apoptosis. This combination achieved potent anticancer activity with lower overall doses of Sorafenib, indicating a potential strategy to decrease Sorafenib toxicity in future translational studies [83].

Iyer et al. described the case of an 8-year old boy with respiratory failure secondary to diffuse micronodular PTC requiring mechanical ventilation and subsequent delay in definitive therapy. The boy was treated with Sorafenib and subsequently with radioiodine-131, with a good response to the therapy. The Authors suggested that Sorafenib could be considered for gap therapy while the patient recovers pulmonary function [84].

In Sorafenib-treated patients with metastic cancer, disease stabilization and tumor shrinkage wer short-lived and drug resistance occured. A study explored the Sorafenib treatment escape mechanisms to overcome their drawbacks, showing that mammalian target of Rapamycin (mTOR) complex 2 activation is an escape mechanism from Sorafenib treatment. When Sorafenib is combinend with Everolimus, its antitumor activity is increased by complete inhibition of mTOR pathway [85].

It has been recently shown, the AKT/mTOR pathway is particularly overactivated in human cPTC harboring the V600EBRAF mutation. These results suggest that the mTOR pathway could be a good target to enhance therapy effects in certain types of thyroid carcinoma, namely in those harboring the V600EBRAF mutation [86].

We include the current clinical researches with Sorafenib in different types of thyroid cancer (**Table 4**; [87]).

11. Targeted Therapies Limits

Tumor cells often devise strategies to bypass the effects of anti-neoplastic agents and selection of therapy-resistant clones is frequently the reason for treatment failure. However, the possibility of testing the sensitivity of primary DePTC cells from each subject to different TKIs could increase the effectiveness of

the treatment. In fact, *in vitro* chemosensitivity tests are able to predict *in vivo* effectiveness in 60% of cases [88]; while, it is well known that a negative chemosensitivity test *in vitro* is associated with a 90% of ineffectiveness of the treatment *in vivo*, allowing the administration of inactive chemotherapeutics to these patients to be avoided [88].

It has been recently demonstrated that it is possible to test the anti-neoplastic activity of different compounds in primary ATC cells obtained from each patient [51, 89-91]. Interestingly, more recently, two new multi-targeted kinase inhibitors (CLM3 and CLM29 inhibit several targets, including RET, EGFR, and VEGFR and have an antiangiogenic effect) have been shown to have an anti-tumoral effect not on the normally used cell lines, that are quite different from the tumor of the patients themselves, but directly on primary DePTC of patients refractory to the radioiodine therapy [48], or in ATC opening the way to the possibility of personalizing the kinase inhibitors therapy in each patient.

12. Conclusions

Sorafenib is an oral multikinase inhibitor with antiproliferative, antiangiogenic and proapoptotic effects that seems to be a promising therapeutic option in patients with advanced thyroid cancer not responsive to tradionally therapeutic strategies.

Nowadays, several studies are ongoing to evaluate long term efficacy and tolerability of Sorafenib, especially on thyroid tumors.

Further research is needed to determine the ideal targeted therapy based on the molecular characterization of the tumor and of the host factors to obtain the best response in terms of survival and quality of life [92].

References

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. CA Cancer J Clin. 2007;57:43-66.

2. American Thyroid Association (ATA) Guidelines Taskforce on Thyroid Nodules and Differentiated Thyroid Cancer, Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, Mazzaferri EL, McIver B, Pacini F, Schlumberger M, Sherman SI, Steward DL, Tuttle RM. Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid. 2009; 19:1167-214.

3. Antonelli A, Miccoli P, Ferdeghini M, Di Coscio G, Alberti B, Iacconi P, Baldi V, Fallahi P, Baschieri L. Role of neck ultrasonography in the follow-up of patients operated on for thyroid cancer. Thyroid. 1995; 5:25-8.

4. Antonelli A, Miccoli P, Fallahi P, Grosso M, Nesti C, Spinelli C, Ferrannini E. Role of neck ultrasonography in the follow-up of children operated on for thyroid papillary cancer. Thyroid. 2003;13: 479-84.

5. Antonelli A, Miccoli P, Derzhitski VE, Panasiuk G, Solovieva N, Baschieri L. Epidemiologic and clinical evaluation of thyroid cancer in children from the Gomel region (Belarus). World J Surg. 1996;20:867-71.

6. Spinelli C, Bertocchini A, Antonelli A, Miccoli P. Surgical therapy of the thyroid papillary carcinoma in children: experience with 56 patients < or =16 years old. J Pediatr Surg. 2004;39:1500-5.

7. Haugen BR. Management of the patient with progressive radioiodine non-responsive disease. Semin Surg Oncol. 1999;16:34-41.

8. Krause DS, Van Etten RA. Tyrosine kinases as targets for cancer therapy. N Engl J Med. 2005;353: 172-87.

9. Antonelli A, Fallahi P, Ferrari SM, Carpi A, Berti P, Materazzi G, Minuto M, Guastalli M, Miccoli P. Dedifferentiated thyroid cancer: a therapeutic challenge. Biomed Pharmacother. 2008;62:559-63.

10. Lemoine NR, Mayall ES, Wyllie FS, Williams ED, Goyns M, Stringer B, Wynford-Thomas D. High frequency of ras oncogene activation in all stages of human thyroid tumorigenesis. Oncogene. 1989;4:159-64.

11. Garcia-Rostan G, Zhao H, Camp RL, Pollan M, Herrero A, Pardo J, Wu R, Carcangiu ML, Costa J, Tallini G. ras mutations are associated with aggressive tumor phenotypes and poor prognosis in thyroid cancer. J Clin Oncol. 2003;21:3226-35.

12. Dwight T, Thoppe SR, Foukakis T, Lui WO, Wallin G, Höög A, Frisk T, Larsson C, Zedenius J. Involvement of the PAX8/peroxisome proliferator-activated receptor gamma rearrangement in follicular thyroid tumors. J Clin Endocrinol Metab. 2003;88:4440-5.

13. Nikiforov YE, Nikiforova MN. Molecular genetics and diagnosis of thyroid cancer. Nat Rev Endocrinol 2011;7:569-80.

14. Anders J, Kjar S, Ibanez CF. Molecular modeling of the extracellular domain of the RET receptor tyrosine kinase reveals multiple cadherin-like domains and calcium-binding site. J Biol Chem. 2001;276: 35808-17.

15. de Groot JW, Links TP, Plukker JT, Lips CJ, Hofstra RM. RET as a diagnostic and therapeutic target in sporadic and hereditary endocrine tumors. Endocr Rev. 2006;27:535-60.

16. Adeniran AJ, Zhu Z, Gandhi M, Steward DL, Fidler JP, Giordano TJ, Biddinger PW, Nikiforov YE. Correlation between genetic alterations and microscopic features, clinical manifestations, and prognostic characteristics of thyroid papillary carcinomas. Am J Surg Pathol. 2006;30:216-22.

17. Nikiforov YE, Rowland JM, Bove KE, Monforte-Munoz H, Fagin JA. Distinct pattern of ret oncogene rearrangements in morphological variants of radiation-induced and sporadic thyroid papillary carcinomas in children. Cancer Res. 1997;57:1690-4.

18. Xing M. BRAF mutation in thyroid cancer. Endocr Relat Cancer. 2005;12:245-62.

19. Xing M, Westra WH, Tufano RP, Cohen Y, Rosenbaum E, Rhoden KJ, Carson KA, Vasko V, Larin A, Tallini G, Tolaney S, Holt EH, Hui P, Umbricht CB, Basaria S, Ewertz M, Tufaro AP, Califano JA, Ringel MD, Zeiger MA, Sidransky D, Ladenson PW. BRAF mutation predicts a poorer clinical prognosis for papillary thyroid cancer. J Clin Endocrinol Metab. 2005;90:6373-79.

20. Hara H, Fulton N, Yashiro T, Ito K, DeGroot LJ, Kaplan EL. N-ras mutation: an independent

prognostic factor for aggressiveness of papillary thyroid carcinoma. Surgery. 1994;116:1010-6.

21. Antonelli A, Ferrari SM, Fallahi P, Minuto M, Corrado A, Bruno R, Miccoli P. Medullary thyroid cancer: new targeted molecular therapies. Recent Pat Endocr Metab Immune Drug Discov. 2010;4:10-4(5).

22. Kebebew E, Clark OH. Medullary thyroid cancer. Curr Treat Options Oncol. 2000;1:359-67.

23. Weber T, Shilling T, Buchler MW. Thyroid carcinoma. Curr Opin Oncol. 2006;18:30-5.

24. Santoro M, Melillo RM, Carlomagno F, Fusco A, Vecchio G. Molecular mechanisms of RET activation in human cancer. Ann N Y Acad Sci. 2002;963:116-21.

25. Drosten M, Putzer BM. Mechanisms of Disease: cancer targeting and the impact of oncogenic RET for medullary thyroid carcinoma therapy. Nat Clin Pract Oncol. 2006;3:564-74.

26. Lorusso PM, Eder JP. Therapeutic potential of novel selective-spectrum kinase inhibitors in oncology. Expert Opin Investig Drugs. 2008; 17:1013-28.

27. Antonelli A, Ferri C, Ferrari SM, Sebastiani M, Colaci M, Ruffilli I, Fallahi P. New targeted molecular therapies for dedifferentiated thyroid cancer. J Oncol. 2010;2010:921682.

28. Gupta-Abramson V, Troxel AB, Nellore A, Puttaswamy K, Redlinger M, Ransone K, Mandel SJ, Flaherty KT, Loevner LA, O'Dwyer PJ, Brose MS. Phase II trial of sorafenib in advanced thyroid cancer. J Clin Oncol. 2008;26:4714-9.

29. Kloos RT, Ringel MD, Knopp MV, Hall NC, King M, Stevens R, Liang J, Wakely PE Jr, Vasko VV, Saji M, Rittenberry J, Wei L, Arbogast D, Collamore M, Wright JJ, Grever M, Shah MH. Phase II trial of sorafenib in metastatic thyroid cancer. J Clin Oncol. 2009;27:1675-84.

30. Hoftijzer H, Heemstra KA, Morreau H, Stokkel MP, Corssmit EP, Gelderblom H, Weijers K, Pereira AM, Huijberts M, Kapiteijn E, Romijn JA, Smit JW. Beneficial effects of sorafenib on tumor progression, but not on radioiodine uptake, in patients with differentiated thyroid carcinoma. Eur J Endocrinol. 2009; 161:923-31.

31. Capdevila J, Iglesias L, Halperin I, Segura A, Vaz M, Corral J, Grau JJ, Obiols G, Hitt R, Tabernero J. Sorafenib in patients (pts) with advanced thyroid carcinoma (TC): a compassionate use program [abstract no. 5590]. J Clin Oncol 2010; 28 Suppl.15. Presented at the 46th Annual Meetingof the American Society of Clinical Oncology; 2010 June 4-8; Chicago Illinois.

32. Ahmed M, Barbachano Y, Riddell A, Hickey J, Newbold KL, Viros A, Harrington KJ, Marais R, Nutting CM. Analysis of the efficacy and toxicity of sorafenib in thyroid cancer: a phase II study in a UK based population. Eur J Endocrinol. 2011;165:315-22.

33. Capdevila J, Iglesias L, Halperin I, Segura A, Martínez-Trufero J, Vaz MÁ, Corral J, Obiols G, Grande E, Grau JJ, Tabernero J. Sorafenib in metastatic thyroid cancer. Endocr Related Cancer. 2012;19:209-16.

34. Schneider TC, Abdulrahman RM, Corssmit EP, Morreau H, Smit JW, Kapiteijn E. Long term analysis of the efficacy and tolerability of sorafenib in advanced radio-iodine refractory differentiated thyroid carcinoma: final results of a phase II trial. Eur J Endocrinol. 2012;167:643-50.

35. Marotta V, Ramundo V, Camera L, Del Prete M, Fonti R, Esposito R, Palmieri G, Salvatore M, Vitale M, Colao A, Faggiano A. Sorafenib in advanced iodine-refractory differentiated thyroid cancer: efficacy, safety and exploratory analysis of role of serum thyroglobulin and FDG-PET. Clin Endocrinol (Oxf). 2013;78:760-7.

36. Savvides P, Nagaiah G, Lavertu PN, Fu P, Wright JJ, Chapman R, Wasman J, Dowlati A, Remick SC. Phase II Trial of Sorafenib in Patients with Advanced Anaplastic Carcinoma of the Thyroid. Thyroid. 2013; 23:600-4.

37. Lam ET, Ringel MD, Kloos RT, Prior TW, Knopp MV, Liang J, Sammet S, Hall NC, Wakely PE Jr, Vasko VV, Saji M, Snyder PJ, Wei L, Arbogast D, Collamore M, Wright JJ, Moley JF, Villalona-Calero MA, Shah MH. Phase II clinical trial of sorafenib in metastatic medullary thyroid cancer. J Clin Oncol. 2010;28:2323-30.

38. de Groot JW, Zonnenberg BA, van Ufford-Mannesse PQ, de Vries MM, Links TP, Lips CJ, Voest EE. A phase II trial of imatinib therapy for metastatic medullary thyroid carcinoma. J Clin Endocrinol Metab. 2007;92:3466-9.

39. Wells SA Jr, Gosnell JE, Gagel RF, Moley J, Pfister D, Sosa JA, Skinner M, Krebs A, Vasselli J, Schlumberger M. Vandetanib for the treatment of patients with locally advanced or metastatic hereditary medullary thyroid cancer. J Clin Oncol. 2010;28:767-72.

40. Robinson BG, Paz-Ares L, Krebs A, Vasselli J, Haddad R. Vandetanib (100 mg) in patients with locally advanced or metastatic hereditary medullary thyroid cancer. J Clin Endocrinol Metab. 2010;95:2664-71.

41.Carlomagno F, Vitagliano D, Guida T, Ciardiello F, Tortora G, Vecchio G, Ryan AJ, Fontanini G, Fusco A, Santoro M. ZD6474, an orally available inhibitor of KDR tyrosine kinase activity, efficiently blocks oncogenic RET kinases. Cancer Res. 2002;62:7284-90.

42. Cuccuru G, Lanzi C, Cassinelli G, Pratesi G, Tortoreto M, Petrangolini G, Seregni E, Martinetti A, Laccabue D, Zanchi C, Zunino F. Cellular effects and antitumor activity of RET inhibitor RPI-1 on MEN2A-associated medullary thyroid carcinoma. J Natl Cancer Inst. 2004;96:1006-14.

43. Strock CJ, Park JI, Rosen M, Dionne C, Ruggeri B, Jones-Bolin S, Denmeade SR, Ball DW, Nelkin BD. CEP-701 and CEP-751 inhibit constitutively activated RET tyrosine kinase activity and block medullary thyroid carcinoma cell growth. Cancer Res. 2003;63:5559-63.

44. Carlomagno F, Vitagliano D, Guida T, Basolo F, Castellone MD, Melillo RM, Fusco A, Santoro M. Efficient inhibition of RET/papillary thyroid carcinoma oncogenic kinases by 4-amino-5-(4-chloro-phenyl)-7-(t-butyl)pyrazolo[3,4-d]pyrimidine (PP2). J Clin Endocrinol Metab. 2003;88:1897-902.

45. Verbeek HH, Alves MM, de Groot JW, Osinga J, Plukker JT, Links TP, Hofstra RM. The Effects of Four Different Tyrosine Kinase Inhibitors on Medullary and Papillary Thyroid Cancers Cells. J Clin Endocrinol Metab. 2011;96: E991-5.

46. Coxon A, Bready J, Estrada J, Estrada J, Osgood T, Canon J, Wang L, Radinsky R, Kendall R, Hughes P, Polverino A. Antintumor Activity of Motesanib in a Medullary Thyroid Cancer Model. J Endocrinol Invest. 2012;35:181-90.

47. Samady AK, Mukerji R, Shah A, Timmermann BN, Cohen MS. A novel RET inhibitor with potent efficacy against medullary thyroid cancer in vivo. Surgery. 2010;148:1228-36.

48. Antonelli A, Bocci G, La Motta C, Ferrari SM, Fallahi P, F ioravanti A, Sartini S, Minuto M, Piaggi S, Corti A, Alì G, Berti P, Fontanini G, Danesi R, Da Settimo F, Miccoli P. Novel pyrazolopyrimidine derivatives as tyrosine kinase inhibitors with antitumoral activity in vitro and in vivo in papillary dedifferentiated thyroid cancer. J Clin Endocrinol Metab. 2011; 96: E288-96.

49. Kim S, Yazici YD, Calzada G, Wang ZY, Younes MN, Jasser SA, El-Naggar AK, Myers JN. Sorafenib inhibits the angiogenesis and growth of orthotopic anaplastic thyroid carcinoma xenografts in nude mice. Mol Cancer Ther. 2007;6:1785-92.

50. Koh YW, Shah MH, Agarwal K, McCarty SK, Koo BS, Brendel VJ, Wang C, Porter K, Jarjoura D, Saji M, Ringel MD. Sorafenib and Mek inhibition is synergistic in medullary thyroid carcinoma in vitro. Endocr Relat Cancer 2012;19: 29-38.

51. Antonelli A, Bocci G, La Motta C, Ferrari SM, Fallahi P, Ruffilli I, Di Domenicantonio A, Fioravanti A, Sartini S, Minuto M, Piaggi S, Corti A, Alì G, Di Desidero T, Berti P, Fontanini G, Danesi R, Da Settimo F, Miccoli P. CLM94, a Novel Cyclic Amide with Anti-VEGFR-2 and Antiangiogenic Properties, Is Active against Primary AnaplasticThyroid Cancer in Vitro and in Vivo. J Clin Endocrinol Metab. 2012; 97:E528-36.

52. Vieira JM, Santos SC, Espadinha C, Correia I, Vag T, Casalou C, Cavaco BM, Catarino AL, Dias S, Leite V. Expression of vascular endothelial growth factor (VEGF) and its receptors in thyroid carcinomas of follicular origin: a potential autocrine loop. Eur J Endocrinol. 2005;153:701-9.

53. Schlumberger M. Kinase inhibitors for refractory thyroid cancers. Lancet Oncol. 2010;11:912-3.

54. Verbeek HH, Alves MM, de Groot JW, Osinga J, Plukker JT, Links TP, Hofstra RM. The effects of four different tyrosine kinase inhibitors on medullary and papillary thyroid cancer cells. J Clin Endocrinol Metab. 2011;96:E991-5.

55. Kurzrock R, Sherman SI, Ball DW, Forastiere AA, Cohen RB, Mehra R, Pfister DG, Cohen EE, Janisch L, Nauling F, Hong DS, Ng CS, Ye L, Gagel RF, Frye J, Müller T, Ratain MJ, Salgia R. Activity of XL184 (Cabozantinib), an oral tyrosine kinase inhibitor, in patients with medullary thyroid cancer. J Clin Oncol. 2011;29:2660-6.

56. Smalley KS, Xiao M, Villanueva J, Nguyen TK, Flaherty KT, Letrero R, Van Belle P, Elder DE, Wang Y, Nathanson KL, Herlyn M. CRAF inhibition induces apoptosis in melanoma cells with non-V600E BRAF mutations. Oncogene. 2009;28:85-94.

57. Wilhelm SM, Adnane L, Newell P, Villanueva A, Llovet JM, Lynch M. Preclinical overview of sorafenib, a multikinase inhibitor that targets both Raf and VEGF and PDGF receptor tyrosine kinase signaling. Mol Cancer Ther. 2008;7:3129-40.

58. Ibrahim N, Yu Y, Walsh WR, Yang JL. Molecular targeted therapies for cancer: sorafenib mono-therapy and its combination with other therapies (review). Oncol Rep. 2012; 27:1303-11.

59. Matsuse M, Mitsutake N, Tanimura S, Ogi T, Nishihara E, Hirokawa M, Fuziwara CS, Saenko VA, Suzuki K, Miyauchi A, Yamashita S. Functional characterization of the novel BRAF complex mutation, BRAF(V600delinsYM), identified in papillary thyroid carcinoma. Int J Cancer. 2013;132:738-43.

60. FDA Approval letter for use of sorafenib in advenced renal cancer .

61. European Commission – Enterprise and industry. Nexavar Retrieved April 24, 2007.

62. " Nexavar (Sorafenib) Approved for Hepatocellular Carcinoma in Europe" 2007.

63. Package insert sorafenib (Nexavar). In: Bayer HelthCare and Onyx Pharmaceuticals.

64. Lathia C, Lettieri J, Cihon F, Gallentine M, Radtke M, Sundaresan P. Lack of effect of ketoconazole-mediated CYP3A inhibition on sorafenib clinical pharmacokinetics. Cancer Chemother Pharmacol. 2006;57:685-92.

65. Grandinetti CA, Goldspiel BR. Sorafenib and sunitinib: Novel targeted therapies for renal cell cancer. Pharmacotherapy. 2007;27:1125-44.

66. Keating GM, Santoro A. Sorafenib: A Review of its Use in Advanced Hepatocellular Carcinoma. Drugs. 2009;69:223-40.

67. Lagas JS, van Waterschoot RA, Sparidans RW, Wagenaar E, Beijnen JH, Schinkel AH. Breast cancer resistance protein and P-glycoprotein limit sorafenib brain accumulation. Mol Cancer Ther. 2010;9:319-26.

68. Richly H, Henning BF, Kupsch P, Passarge K, Grubert M, Hilger RA, Christensen O, Brendel E, Schwartz B, Ludwig M, Flashar C, Voigtmann R, Scheulen ME, Seeber S, Strumberg D. Results of a phase I trial of sorafenib (BAY 43–9006) in combination with doxorubicin in patients with refractory solid tumors. Ann Oncol. 2006;17:866-73.

69. Mross K, Steinbild S, Baas F, Gmehling D, Radtke M, Voliotis D, Brendel E, Christensen O, Unger C. Results from an in vitro and a clinical/pharmacological phase I study with the combination irinotecan and sorafenib. Eur J Cancer. 2007;43:55-63.

70. Azad NS, Posadas EM, Kwitkowski VE, Steinberg SM, Jain L, Annunziata CM, Minasian L, Sarosy G, Kotz HL, Premkumar A, Cao L, McNally D, Chow C, Chen HX, Wright JJ, Figg WD, Kohn EC. Combination targeted therapy with sorafenib and bevacizumab results in enhanced toxicity and antitumor activity. J Clin Oncol. 2008;26:3709-14.

71. Lee JM, Sarosy GA, Annunziata CM, Azad N, Minasian L, Kotz H, Squires J, Houston N, Kohn EC.

Combination therapy: intermittent sorafenib with bevacizumab yields activity and decreased toxicity. Br J Cancer. 2010;102:495-9.

72. Duran I, Hotté SJ, Hirte H, Chen EX, MacLean M, Turner S, Duan L, Pond GR, Lathia C, Walsh S, Wright JJ, Dancey J, Siu LL. Phase I targeted combination trial of sorafenib and erlotinib in patients with advanced solid tumors. Clin Cancer Res. 2007;13:4849-57.

73. Spigel DR, Burris HA III, Greco FA, Shipley DL, Friedman EK, Waterhouse DM, Whorf RC, Mitchell RB, Daniel DB, Zangmeister J, Bass JD, Hainsworth JD. Randomized, double-blind, placebo-controlled, phase II trial of sorafenib and erlotinib or erlotinib alone in previously treated advanced non-small cell lung cancer. J Clin Oncol. 2011;29:2582-9.

74. Martinelli E, Troiani T, Morgillo F, Rodolico G, Vitagliano D, Morelli MP, Tuccillo C, Vecchione L, Capasso A, Orditura M, De Vita F, Eckhardt SG, Santoro M, Berrino L, Ciardiello F. Synergistic antitumor activity of sorafenib in combination with epidermal growth factor receptor inhibitors in colorectal and lung cancer cells. Clin Cancer Res. 2010;16:4990-5001.

75. Escudier B, Lassau N, Angevin E, Soria JC, Chami L, Lamuraglia M, Zafarana E, Landreau V, Schwartz B, Brendel E, Armand JP, Robert C. Phase I trial of sorafenib in combination with IFN α-2a in patients with unresectable and/or metastatic renal cell carcinoma or malignant melanoma. Clin Cancer Res. 2007;13:1801-9.

76. Gollob JA, Rathmell WK, Richmond TM, Marino CB, Miller EK, Grigson G, Watkins C, Gu L, Peterson BL, Wright JJ. Phase II trial of sorafenib plus interferon α2b as first-or second-line therapy in patients with metastatic renal cell cancer. J Clin Oncol. 2007;25:3288-95.

77. Egberts F, Gutzmer R, Ugurel S, Becker JC, Trefzer U, Degen A, Schenck F, Frey L, Wilhelm T, Hassel JC, Schadendorf D, Livingstone E, Mauch C, Garbe C, Berking C, Rass K, Mohr P, Kaehler KC, Weichenthal M, Hauschild A. Sorafenib and pegylated interferon-α2b in advanced metastatic melanoma: a multicenter phase II DeCOG trial. Ann Oncol. 2011;22:1667-74.

78. www.ema.europa.eu/ema/index

79. Carlomagno F, Anaganti S, Guida T, Salvatore G, Troncone G, Wilhelm SM, Santoro M. BAY 43-9006 inhibition of oncogenic RET mutants. J Natl Cancer Inst. 2006;98:326-34.

80. Nexavar® Versus Placebo in Locally Advanced/Metastatic RAI-Refractory Differentiated Thyroid Cancer (NCT00984282). Available at URL: http://clinicaltrials.gov/ct2/show/NCT00984282?term=NCT00984282&rank=1

81. Brose MS, Nutting CM, Sherman SI, Shong YK, Smit JW, Reike G, Chung J, Kalmus J, Kappeler C, Schlumberger M. Rational and design of decision: a double-blind, randomized, placebo-controlled phase III trial evaluating the efficacy and safety of sorafenib in patients with locally advanced or metastatic radioactive iodine (RAI)-refractory, differentiated thyroid cancer. BMC Cancer. 2011;11: 349.

82. Shen Y, Ruan M, Luo Q, Yu Y, Lu H, Zhu R, Chen L. Brain metastasis from follicular thyroid carcinoma: treatment with sorafenib. Thyroid. 2012;22:856-60.

83. Cohen SM, Mukerji R, Timmermann BN, Samadi AK, Cohen MS. A novel combination of withaferin A and sorafenib shows synergistic efficacy against both papillary and anaplastic thyroid cancers. Am J Surg. 2012;204:895-900.

84. Iyer P, Mayer JL, Ewig JM. Response to Sorafenib in a Pediatric Patient with Papillary Thyroid Carcinoma with Diffuse Nodular Pulmonary Disease Requiring Mechanical Ventilation. Thyroid. 2013 Apr 2

85. Pignochino Y, Dell'aglio C, Basiricò M, Capozzi F, Soster M, Marchiò S, Bruno S, Gammaitoni L, Sangiolo D, Torchiaro E, D'Ambrosio L, Fagioli F, Ferrari S, Alberghini M, Picci P, Aglietta M, Grignani G. The Combination of Sorafenib and Everolimus Abrogates mTORC1 and mTORC2 Upregulation in Osteosarcoma Preclinical Models. Clin Cancer Res. 2013;19:2117-31.

86. Faustino A, Couto JP, Pópulo H, Rocha AS, Pardal F, Cameselle-Teijeiro JM, Lopes JM,Sobrinho-Simões M, Soares P. mTOR pathway overactivation in BRAF mutated papillary thyroid carcinoma. J Clin Endocrinol Metab. 2012;97:E1139-49.

87. www.clinicaltrials.gov

88. Blumenthal RD, Goldenberg DM. Methods and goals for the use of in vitro and in vivo chemosensitivity testing. Mol Biotechnol. 2007;35:185-97.

89. Antonelli A, Ferrari SM, Fallahi P, Berti P, Materazzi G, Minuto M, Giannini R, Marchetti I, Barani L, Basolo F, Ferrannini E, Miccoli P. Thiazolidinediones and antiblastics in primary human anaplastic thyroid cancer cells. Clin Endocrinol (Oxf). 2009;70:946-53.

90. Antonelli A, Ferrari SM, Fallahi P, Berti P, Materazzi G, Marchetti I, Ugolini C, Basolo F, Miccoli P, Ferrannini E. Evaluation of the sensitivity to chemotherapeutics or thiazolidinediones of primary anaplastic thyroid cancer cells obtained by fine-needle aspiration. Eur J Endocrinol. 2008;159:283-91.

91. Antonelli A, Ferrari SM, Fallahi P, Berti P, Materazzi G, Barani L, Marchetti I, Ferrannini E, Miccoli P. Primary cell cultures from anaplastic thyroid cancer obtained by fine-needle aspiration used for chemosensitivity tests. Clin Endocrinol (Oxf). 2008;69:148-52.

92. Cabanillas ME, Hu MI, Durand JB, Busaidy NL. Challenges Associated with Tyrosine Kinase Inhibitor Therapy for Metastatic Thyroid Cancer. J Thyroid Res. 2011;2011:985780.

Acknowledgments and Disclosures section.

None.

Table 1. Clinical trials with targeted therapies against RET in patients with thyroid cancer.

Drug	Pathway inhibited	Thyroid cancer	Responses	Author, reference n°
		Follicular derived Thyroid Cancer		
Sorafenib	RAF, RET, VEGFR	30 DTC	7(23%)PR; 16(53%)SD (DTC)	Gupta-Abramson V et al. [28]
Sorafenib	RAF, RET, VEGFR	41 PTC 11 FTC 4 ATC	6(15%)PR; 23(56%)SD (PTC) No response (FTC) No response (ATC)	Kloos RT et al. [29]
Sorafenib	RAF, RET, VEGFR	32 DTC	8(25%)PR; 11(34%)SD (DTC)	Hoftijzer H et al. [30]
Sorafenib	RAF, RET, VEGFR	30 advanced thyroid cancer	9(30%)PR; 13(36%)SD	Capdevila J et al. [31]
Sorafenib	RAF, RET, VEGFR	19 DTC	Twelve months radiological RR: DTC 18%	Ahmed M et al. [32]
Sorafenib	RAF, RET, VEGFR	7 PTC, 9 FTC, 3 ATC)	7 PTC, 9 FTC : RR 19%, 3 ATC : RR 33%	Capdevila J et al. [33]

Sorafenib	RAF, RET, VEGFR	31 advanced thyroid cancer	PR 8 (31%) SD 11 (42%)	Schneider TC et al. [34]
Sorafenib	RAF, RET, VEGFR	17 progressive DTC	PR 30%, SD 41%	Marotta V et al. [35]
Sorafenib	RAF, RET, VEGFR	20 ATC	2 PR (10%) duration of response respectively 10 and 27 months, 5 SD (25%) median duration 4 months (range 3-11 months)	Savvides P et al. [36]
		Medullary Thyroid Cancer		
Sorafenib	RAF, RET, VEGFR	16 sporadic MTC	1(6%)PR; 14(88%)SD	Lam ET et al. [37]
Imatinib	VEGFR-2, RET	15 MTC	4(27%)SD (MTC)	de Groot JW et al. [38]
Vandetanib	VEGFR-2, EGFR, RET	30 MTC hereditary	6(20%)PR, 16(53%)SD (MTC)	Wells SA Jr et al. [39]
Vandetanib	VEGFR-2, EGFR, RET	19 MTC hereditary	3(16%)PR, 10(53%)SD (MTC)	Robinson BG et al. [40]
Sorafenib	RAF, RET, VEGFR	15 MTC	Twelve months radiological RR:	Ahmed M et al. [32]

Sorafenib	RAF, RET, VEGFR	15 MTC	MTC 25%; 15 MTC : PR 11(32%), SD 14 (41%), RR 47%	Capdevila J et al. [33]

VEGFR = vascular endothelial growth factor receptor; **EGFR** = epidermal growth factor receptor; **DTC** = differentiated thyroid cancer; **ATC** = anaplastic thyroid cancer; **PTC** = papillary thyroid cancer; **FTC** = follicular thyroid cancer; **MTC** = medullary thyroid cancer; **PR** = partial response; **SD** = stable disease; **CR** = complete response; **RR**= rate response.

Table 2. *In vitro* studies with targeted therapies against RET in papillary, medullary and anaplastic thyroid cancer.

Drugs	Cell type	Results	Xenografts (yes/no)	Anti-neoplastic effect in xenografts (yes/no)	Author, reference n°
ZD6474	PTC cell line,	Prol+	Yes	Yes	Carlomagno F et al. [41]
RPI-1	MTC cell line	Prol+	Yes	Yes	Cuccuru G et al. [42]
CEP-701, CEP-751, CEP-2563	MTC cell line	Prol+	Yes	Yes	Strock CJ et al. [43]
PP2	PTC cell line	Prol+	No	-	Carlomagno F et al. [44]
Vandetanib, XL184	MTC cell line	Prol+	No	-	Verbeek HH et al. [45]
XL184	PTC cell line		No	-	Verbeek HH et al. [45]
Motesanib	MTC cell line		Yes	Yes	Coxon A et al. [46]
Withaferin A	MTC cell line	Prol+, apo+	Yes	Yes	Samady AK et al. [47]
CLM3, CLM29	DePTC cell line	Prol+, apo+	Yes	Yes	Antonelli A et al. [48]
Sorafenib	ATC cell	Prol +, apo +	Yes	Yes	Kim S et al. [49]

	lines				
Sorafenib Everolimus, AZD 6244	MTC cell lines (TT, MZ-CRC-1)				Koh YW et al. [50]
CLM94	ATC cells, ATC cell line (AF) in CD nu/nu mice; HMVEC-d, 8305 C cells	Prol +, apo +	Yes	Yes	Antonelli A et al. [51]

PTC = papillary thyroid cancer; **DePTC** = dedifferentiated papillary thyroid cancer; **MTC** = medullary thyroid cancer; **ATC** = anaplastic thyroid cancer.

Inhibition of proliferation (prol+), induction of apoptosis (apo+).

Table 3. Adverse reactions from several clinical study of patients treated with Sorafenib, and from post marketing report (from EMA).

Classification (systems or organ)	Very common ≥ 1/10	Common ≥ 1/100, <1/10	Uncommon ≥1/1000, <1/100	Rare
Infections			Infection Folliculitis	
Hemolymphopoietic Diseases	Lymphopenia	Leukopenia Neutropenia Thrombocytopenia		
Immune system disorders			Hypersensivity (skin reaction and nettle rash)	Angioedema, anaphylaxis shock
Endocrine disorders			Hypo/hyperthyroidism	
Metabolic and nutritional disorders	Hypophosphatemia	Anorexia Hypocalcemia	Hyponatriemia Dehydration	
Psychiatric disorder		Depression		
Neurological Diseases		Periferical sensorial neurophaty	Reversible white matter encephalopathy	
Ear disease		Tinnitus		
Cardiac diseases		Congestive hearth failure, myocardial ischaemia		QT prolungation

Vascular diseases	Hypertension, bleeding (from the gastrointestinal tract, respiratory tract; cerebral haemorrhage)		Hypertensive crisis	
Respiratory tract end mediastinic diseases		Raucousness	Rhinorrhea, interstitial pneumonial like diseases	
Gastrointestinal tract diseases	Diarrhea, nausea, vomit	Constipation, stomatitis, dyspepsia, dysphagia	Gastro-oesophageal reflux, gastritis, pancreatitis, bowel perforation	
Hepatobiliary diseases			Jaundice, cholecystitis	Drug related hepatitis
Skin	Rash, alopecia, hand-foot syndrome, itch	Skin dryness, exfoliative dermatitis, acne	Erythema multiforme, eczema	Stevens Johnsons syndrome, vasculitis
Muscle skeletal system and connettive tissue disease		Arthralgia, myalgia		Rhabdomyolysis
Renal diseases		Renal failure		
Diseases of reproductive system and breast		Erectile dysfunction	Gynecomastia	

Diseases related to the way of administration	Fatigue, pain (mouth, abdomen, bone, oncologic), headache	Asthenia, fever, influenza like syndrome		
Laboratory exams	Increasing levels of amylase and lipase	Weight loss, increasing levels of hepatic enzyme	Alteration of INR value	

Table 4. Current clinical research with Sorafenib in different types of thyroid cancer (from www.clinicaltrials.gov).

Status/Phase	Study	Condition	Intervention
Active, not recruiting/Phase II	Combination of Temsirolimus and Sorafenib in the Treatment of Radioactive Iodine Refractory Thyroid Cancer	Thyroid Cancer	Drug: Temsirolimus and Sorafenib
Unknown/Phase II	Phase II Trial of Sorafenib (Nexavar) in Patients With Advanced Thyroid Cancer	Metastatic Differentiated Thyroid Cancer; Metastatic Poorly Differentiated Thyroid Cancer; Metastatic Anaplastic Thyroid Cancer; Metastatic Medullary Thyroid Cancer	Drug: Sorafenib
Unknown/Phase II	Study of Everolimus and Sorafenib in Patients With Advanced Thyroid Cancer Who Progressed on Sorafenib Alone	Differentiated Thyroid Cancer	Drug: Everolimus; Drug: Sorafenib
Active, not recruiting/Phase II	Sorafenib Tosylate in Treating Patients With Metastatic, Locally Advanced, or Recurrent Medullary Thyroid Cancer	Multiple Endocrine Neoplasia; Recurrent Thyroid Cancer; Thyroid Gland Medullary Carcinoma	Drug: Sorafenib tosylate; Genetic: molecular genetic technique and mutation analysis; immunohistochemistry staining method; diagnostic laboratory biomarker analysis; pharmacological study
Active, not recruiting/Phase II	Evaluating the Combination of Everolimus and Sorafenib in the Treatment of Thyroid Cancer	Thyroid Cancer	Drug: Sorafenib with Everolimus

Terminated Has Results/Phase II	Sorafenib in Treating Patients With Advanced Anaplastic Thyroid Cancer	Anaplastic Thyroid Cancer; Recurrent Thyroid Cancer	Drug: Sorafenib tosylate
Completed /Phase II	Sorafenib Tosylate in Treating Patients With Locally Advanced, Metastatic, or Locally Recurrent Thyroid Cancer	Anaplastic Thyroid Cancer; Insular Thyroid Cancer; Recurrent Thyroid Cancer; Stage III Follicular Thyroid Cancer; Stage III Papillary Thyroid Cancer; Stage IV Follicular Thyroid Cancer; Stage IV Papillary Thyroid Cancer	Drug: Sorafenib tosylate; Other: laboratory biomarker analysis; pharmacological study; Radiation: fludeoxyglucose F 18; Procedure: positron emission tomography; Procedure: dynamic contrast-enhanced magnetic resonance imaging
Completed/Phase II	Sorafenib as Adjuvant to Radioiodine Therapy in Non-Medullary Thyroid Carcinoma	Thyroid Cancer	Drug: Sorafenib (Nexavar)
Withdrawn/Phase II	Efficacy and Safety Study of Sorafenib to Treat Advanced Medullary Thyroid Carcinoma	Medullary Thyroid Carcinoma	Drug: Sorafenib
Active, not recruiting/Phase III	Nexavar® Versus Placebo in Locally Advanced/Metastatic RAI-Refractory Differentiated Thyroid Cancer	Thyroid Neoplasms	Drug: Sorafenib (Nexavar, BAY43-9006); Drug: Placebo
Recruiting/Phase II	RAD001 for Patients With Radioiodine Refractory Thyroid Cancer	Thyroid Cancer	Drug: RAD001