Review Article

Targeted Treatment of Differentiated and Medullary Thyroid Cancer

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Received 14 January 2011; Accepted 14 June 2011

1. Introduction

The incidence of thyroid cancer is increasing, with a concomitant increase in the number of patients with advanced and metastatic disease. Discoveries regarding the pathogenesis of thyroid cancer have led to the recent development of new therapeutic agents that are beginning to appear on the market. Many of these new agents are targeted kinase inhibitors primarily affecting oncogenic kinases (BRAF V600E, RET/PTC) or signaling kinases (VEGFR, PDGFR). Some of these agents report significant partial response rates, while others attain stabilization of disease as their best response. Their impact on survival is unclear. While these agents target similar pathways, a wide variety of differences exist regarding efficacy and side effect profile. Current expert opinion advises that these agents be used only in a specific subset of patients.

Patients with medullary thyroid cancer (MTC) are susceptible to early metastatic disease. Between 20 to 30% of patients with T1 tumors at the time of diagnosis already have metastasis to lymph nodes [6]. The mainstay of treatment for these patients is total thyroidectomy with aggressive lymph node dissection. For patients with a family history of MTC or multiple endocrine neoplasia 2A or 2B, prophylactic thyroidectomy is recommended as soon as possible, even in patients who are less than one-year-old [6].

Popular treatment options for advanced stages of DTC and MTC consist of radiotherapy and chemotherapy, which confer only a modest benefit on tumor burden and overall survival. Current treatment regimens for advanced thyroid cancer include bleomycin, doxorubicin, platinum-containing compounds, or a combination of these agents. For the most part, they result in minor responses, and their use is limited by their toxicities. Bleomycin is well known for its pulmonary toxicity, while doxorubicin can cause both cardiac arrhythmias and heart failure. Platinum-based therapies result in neuropathy, nausea, and renal toxicity [7].

However, recent research has shed light on the underlying molecular mechanisms of thyroid cancer and on the role of oncogenic kinases in metastatic thyroid cancer in particular...
2. Overview of the Molecular Pathways of Thyroid Cancer

Comprehensive studies of mutation pathways in DTC and MTC have been undertaken in the past two decades [9–21]. The knowledge gained from these analyses may render DTC and MTC amenable to designer therapeutics. The most important findings center on the discovery of oncogenic kinases, as well as the elucidation of various signaling pathway adaptations occurring in malignant cells. Of the oncogenic kinases, BRAF V600E mutation and RET/PTC mutations are being targeted as potential pathways for therapeutic intervention. Both of these mutations have the potential to activate the mitogen-activated protein kinase (MAPK) pathway downstream. Therapeutics targeting RET/PTC are being developed particularly for use in MTC. The vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) pathways, as well as the phosphatidylinositol-3-kinase-(PI3K-) phosphatase with tensin homology (PTEN) pathway are important signaling cascades being investigated for possible development of therapeutic kinase inhibitors (Figure 1).

2.1. Oncogenic Kinases. BRAF mutations are the most commonly encountered mutation in PTC [13, 22, 23]. BRAF mutations are present in 29–83% of cases of papillary thyroid cancer (PTC) [8, 24]. Anaplastic thyroid carcinoma (ATC) also has a high frequency of BRAF mutations, with up to 50% of ATC harboring a mutation in this entity [25]. The BRAF gene is located on chromosome 7q24. Oncogenic BRAF mutations in PTC commonly (approximately 80%) are comprised of a thymidine to adenine substitution in exon 15 (T1799A) resulting in an amino acid sequence change of valine to glutamate (V600E) [22, 26]. This change destabilizes the inactive conformation of BRAF, rendering it constitutively active [14, 26, 27]. Activated oncogenic mutant BRAF has a higher affinity for MEK1 and MEK2 and increases the phosphorylation of MEK. BRAF V600E also potently activates MAPK pathway directly. BRAF can be activated by another genetic rearrangement leading to formation of a fusion protein, AKAP9-BRAF, which can activate MAPK pathways. This rearrangement is present in approximately 11% of PTC [28]. The basis of these mutations is not known. The BRAF V600E mutant does not seem related to radiation exposure. In contrast, the AKAP9-BRAF is thought to be related to irradiation [28–30].

Some authors suggest that PTCs with BRAF mutations are more aggressive and tend to present at a more advanced clinical stage and with extrathyroidal invasion [24, 31]. BRAF mutations are more frequently present in older patients with otherwise classical PTC, who are at a more advanced stage of the disease at the time of diagnosis [24, 31, 32]. This suggestion is also supported by the observation that the tall-cell variant of PTC has a high prevalence of BRAF mutations [33]. Additionally, BRAF mutation is common in aggressive microcarcinomas [34, 35]. These mutations occur rarely or not at all in follicular or medullary thyroid carcinomas, benign adenomas, or benign hyperplasias [23, 36, 37]. Many undifferentiated and anaplastic carcinomas arising from pre-existing PTC have BRAF mutations [32, 38]. Additionally, tumors with BRAF mutations tend to have decreased expression of NIS symporter, and leading the tumor to become refractory to radiodine treatment [39–41]. Interestingly, BRAF mutation is generally present without other common mutations found in PTC, suggesting that BRAF mutation alone may be sufficient for tumorigenesis [13, 36, 37].

The oncogenic RET/PTC mutation is also commonly found in PTCs, approximately 10–50% [21]. Familial forms of medullary thyroid carcinoma (MTC) also arise from inheritable activating mutations in RET (the most studied being the C634R change) [42, 43]. RET/PTC rearrangements are very common in thyroid tissue exposed to radiation, and are also commonly noted in pediatric PTC [44, 45]. Radiation has been shown to induce this recombination in thyroid cell lines and in normal human thyroid tissue transplanted onto SCID mice [46]. Twelve forms of RET/PTC mutations have been described, with forms 1 and 3 being the most common [16]. RET/PTC1 is typically associated with classical PTC, while RET/PTC3 rearrangement is associated with solid-variant PTCs [17]. These mutations result in the linking of the promoter and N-terminus to unrelated C-terminus fragments of RET, leading to a chimeric receptor that is constitutively active. RET/PTC mutations are uncommon in poorly differentiated cancers, suggesting that this mutation may imply a favorable prognosis [18]. Curiously, RET/PTC expression in thyroid cells has been found to be associated with impaired hormonogenesis and hypothyroidism, particularly Hashimoto’s thyroiditis (HT). Whether or not this predisposes an individual with HT to thyroid cancer is unclear [47–49].

2.2. Signaling Kinases. A few of the important signaling cascades being investigated for the possible development of therapeutic kinase inhibitors are the VEGF and PDGF pathways, as well as the PI3K/PTEN pathway. VEGF is a proangiogenic factor that binds to two receptor tyrosine kinases (VEGFR-1 and VEGFR-2), of which VEGFR-2 is widely recognized to be the primary mediator of angiogenesis. PDGF-B is required for the maturation of microvasculature, while tumor-derived PDGF-A recruits angiogenic stroma to the tissue. VEGFR and PDGFR mutually support the increased activity of each other [50]. Increased VEGF expression appears to be related to worse prognosis, increased risk of recurrence, and the presence of metastasis [51, 52].

The PI3K/PTEN pathway is responsible for regulating glucose metabolism, cell survival, adhesion, and motility [20, 53, 54]. It is found in some thyroid carcinomas (particularly follicular carcinomas) as well as other types of cancers [55–60]. Epigenetic methylation leads to silencing of the negative
3. New Agents for the Treatment of Thyroid Cancer

3.1. Agents Primarily Targeting Oncogenic Kinases. Given the increased frequency of BRAF mutations in PTC, a number of newer therapeutic agents have been developed that inhibit BRAF. The BRAF inhibitor studied most in thyroid cancer is sorafenib. Sorafenib (Nexavar, BAY 43-9006, Bayer) is an oral tyrosine kinase inhibitor which has been approved by the Food and Drug Administration for the treatment of advanced renal cell carcinoma and unresectable hepatocellular carcinoma. It inhibits VEGFR 2/3, RET including RET/PTC1 mutant, c-kit, PDGFR-beta, and BRAF (including the V600E mutation) [63, 64]. It is a biaryl compound that locks the mutant constitutively active kinase in an inactive state. It competitively inhibits ATP binding in the catalytic domains of both normal and mutant BRAF. This triggers G1 phase arrest.

None of the four phase 1 trials of sorafenib included subjects with thyroid cancer, but there is in vitro data in thyroid cancer cell lines that demonstrated efficacy. The phase 1 trials established the optimum dosing regimen as 400 mg twice a day [65]. A number of phase II trials of sorafenib involved advanced or metastatic DTC. These patients' tumors demonstrated partial responses in 15–27% of participants, and stable disease in a little over 34–61% [66–68]. It should be noted that a recent retrospective review of thirteen patients with advanced DTC from MD Anderson demonstrated particular efficacy of this agent in lung metastasis, while it was less efficacious in bone metastasis [69]. Given its ability to interfere with RET and RET/PTC pathways, treatment with sorafenib was attempted in a phase II trial of MTC. Only a very small portion of patients achieved a partial response, although stable disease response rates were comparable to those seen in the DTC phase II trials [70].

While sorafenib is generally well tolerated with side effects including rash, diarrhea, hand-foot syndrome, and fatigue, treatment with sorafenib may be associated with an increased risk of cardiac toxicities, with up to 40% of patients experiencing EKG changes. Myocardial infarction has also been reported as a side effect of sorafenib in patients being treated for renal cell carcinoma [71, 72]. Increased risk for cutaneous squamous cell carcinomas has been ascribed to the entire class of BRAF inhibitors, and sorafenib is no exception [73].

PLX4032 (RG7204, a Plexxikon drug being codeveloped with Roche) is a 7-azaindole derivative that is currently in clinical trials. PLX4032 specifically inhibits BRAF V600E to a greater extent than wild-type BRAF [74, 75]. Unlike sorafenib which only binds to the inactive conformation of BRAF and keeps it inactive, PLX4032 binds to both the active form and inactive forms of BRAF. It has been shown to actively inhibit proliferation of BRAF-mutant-positive cell lines, particularly in melanoma; thus, most of the clinical trials have been focused on melanoma [76].

Notably, not all cell lines with BRAF V600E mutations respond equally to treatment with PLX4032. Although mutant BRAF V600E has been identified in ATC, PLX4032...
did not lead to apoptosis of the anaplastic thyroid carcinoma cell line ARO [77]. Different melanoma cell lines with BRAF V600E demonstrate differential response to PLX4032 as well; some are highly sensitive while some are essentially unresponsive to treatment with this agent. These differences might be explained by whether the cell line is homozygous or heterozygous for the BRAF V600E mutation. Variation in the upregulation of the PI3K/PTEN pathway in response to treatment with this agent potentially mediates the observed resistance in nonresponding cell lines [78].

An early clinical trial of PLX4032 demonstrated that one out of three participants with thyroid cancer achieved a partial response [79]. Rash is the most common side effect of this agent. Again noted is an increase in risk for development of cutaneous squamous cell carcinomas, likely owing to its anti-BRAF activity [79]. XL281 (Exelixis, Bristol-Meyers-Squibb BMS-908662) is another oral agent similar to PLX4032 in that it inhibits both wild-type and mutant BRAF kinases. Phase I clinical trials are ongoing and include subjects with thyroid carcinoma though early results are not encouraging [80].

Sunitinib (Sutent, SU11248, Pfizer) is a tyrosine kinase inhibitor affecting VEGFR 1/2/3, RET, RET/PTC1, and RET/PTC3 [81]. Of DTC and MTC patients enrolled in a phase II trial of sunitinib receiving 50 mg/day, partial response was observed in 13% of patients with DTC, while stable disease was the best response in 68% of patients with DTC. Eighty-three percent of patients with MTC achieved stable disease [82]. Additionally, there are case reports of patients with advanced MTC having a dramatic response to treatment with sunitinib with respect to both serum calcitonin levels and tumor burden [83]. Patients experience side effects primarily relating to fatigue, and diarrhea when treated with sunitinib. Another unique adverse effect of this agent is palmar-plantar erythrodesia.

Sunitinib can also cause hypothyroidism like many of the tyrosine kinase inhibitors. The mechanism is thought to be related to a destructive thyroiditis when administered for the treatment of renal cell carcinoma [84, 85]. However, this is unlikely to be the cause of hypothyroidism in thyroid cancer patients, as they have all presumably undergone total thyroidectomy. There is other evidence suggesting that increases in TSH in athyreotic patients are associated with increased type 3 deiodination and augmented peripheral thyroid hormone metabolism [86]. Interestingly, some studies suggest that development of hypothyroidism during treatment for other cancers other than thyroid cancer may actually be an encouraging prognostic factor [87, 88].

Heart failure may also be a serious adverse effect sunitinib, occurring in 2.7% of patients from a retrospective study of 600 patients at MD Anderson [69]. A different retrospective analysis including 75 patients involved in phase I and II trials with sunitinib at several centers around the United States reported an 11% cardiac event rate, and a decrease in left ventricular ejection fraction of greater than 10% in 47% of included subjects. Half of the included patients developed hypertension [89]. While the mechanism of heart failure associated with tyrosine kinase inhibitors may be related to mitochondrial damage, recent studies postulate that myocyte damage occurs secondary to a lack of target selectivity of binding to both tyrosine kinases and serine-threonine kinases [89–91]. Of the clinically available tyrosine kinase inhibitors used in one comparison study, sunitinib, sorafenib, and pazopanib induced the highest degree of myocyte damage as measured by lactate dehydrogenase leakage [90].

Vandetanib (Zactima, ZD6474, iPR Pharmaceuticals, AstraZeneca Pharmaceuticals) is an oral tyrosine kinase inhibitor that targets VEGFR 2/3, RET, and EGFR [92, 93]. It is a heteroaromatic-substituted anilinoquinalzine. It specifically inhibits RET/PTC1 and RET/PTC3 in PTC, and M918R RET mutations in MEN2B [94, 95]. Recent investigations into the mechanism of action of vandetanib in cell culture revealed that the agents ability to block both RET and EGFR simultaneously can prevent escape from RET blockade [96]. A completed phase II trial demonstrated efficacy in metastatic familial MTC [97]; 21% of patients treated with 300 mg/day showed a partial response, while 53% patients had stable disease at 24 weeks. There was a decrease in levels of calcitonin in most patients. Adverse effects were significant enough to require dose reductions in several subjects and consisted of diarrhea, severe rash, fatigue, and QTc prolongation [97].

The vandetanib safety database, which accrues data from treatment of multiple cancer types, noted a potential increase in other serious entities such as cerebrovascular accidents and interstitial lung disease [98]. Recent US Food and Drug Administration review cites concern regarding the side effect profile of this agent and propose limiting the indications to progressive symptomatic disease [98]. Another recently published study of subjects with locally advanced or metastatic hereditary MTC administered only 100 mg/day of the drug with nearly similar response rates compared to the above study, and was somewhat better tolerated regarding side effects [99]. Other phase II trials for familial MTC and DTC are underway, as are phase III trials for metastatic MTC. Based on the above mentioned trials as well as other recent data, in April 2011, the US Food and Drug Administration approved vandetanib for use in late-stage MTC. This is the first medication approved by the FDA for the treatment of MTC [100].

Imatinib Mesylate (STI571, Gleevec, Novartis) is an oral tyrosine kinase inhibitor (TKI) that suppresses c-ABL mutation, c-KIT, and inhibits RET autophosphorylation [101]. It was first utilized in the 1990s for treatment of BCRABL-positive leukemias. In anaplastic thyroid cancer cell lines (FRO and ARO), it caused growth inhibition, but did not inhibit growth in papillary thyroid cancer cell lines [102, 103]. Two small phase II trials of patients with MTC showed only a small percentage of subjects achieving a stable disease as their best tumor response [104, 105]. These patients were treated with 600 mg daily of imatinib. Over half of the patients were noted to have profound hypothyroidism and required significant increases in their need for thyroid hormone.

New agents are also on the horizon, particularly in RET-mutant MTC. Withaferin A (WA) is a novel compound which appears effective against MTC cell proliferation in
culture. WA inhibits both activation and phosphorylation of RET as well as total RET expression. The investigators recently published evidence of its efficacy in a murine model of MTC. Treatment with WA resulted in 80% regression of tumor volume in the treated animals with a corresponding significant decrease in calcitonin levels. Additionally, all the treated animals were alive at 6 weeks, while essentially all the control animals died by this point in time [106].

3.2. Agents Primarily Targeting Signaling Kinases. Pazopanib (Votrient, GlaxoSmithKline, GW786034) is a second-generation oral small molecule kinase inhibitor that targets VEGFR-1, 2, and 3, as well as alpha and beta PDGFR [107]. There is new data from studies of breast cancer indicating that it also targets multiple forms of Raf, though it likely does not affect the common BRAF V600E mutant [108]. It is approved for use in renal cell carcinoma and is likely effective in other forms of cancer including ovarian cancer, and nonsmall cell lung carcinoma [109–111].

A phase II study completed in early 2009 of thyroid cancer patients led by the Mayo Clinic demonstrated a confirmed partial response rate by RECIST criteria in 49% of enrolled subjects (18 patients). There were no complete responses [112]. Starting dose was 800 mg per day. Patients able to tolerate maximum doses of the medication significantly decreased their tumor size as compared to those patients unable to tolerate maximum doses of the agent. Although not statistically significant, the subset of patients with FTC attained a partial response more frequently than subjects with PTC. Forty-three percent (43%) required dose reductions, owing most frequently to fatigue, skin and hair hypopigmentation, diarrhea, and nausea. Nearly 66% of patients doubled their TSH concentrations. Also of note, three patients (8%) developed grade 3 lower gastrointestinal hemorrhage, which according to the authors is similar to the rate noted in trials with Sorafenib [112].

Motesanib (AMG706, Amgen) is an oral tyrosine-kinase inhibitor that inhibits autophosphorylation of RET and also targets VEGFR 1, 2, and 3, PDGFR, and c-KIT. It demonstrates both direct antitumor and antiangiogenic properties [113]. Phase 1 trials were encouraging with 3 DTC patients registering a partial response [114]. A subsequent phase II trial administering 125 mg/day to patients with DTC demonstrated a partial response in 14% of patients, while 35% of patients had stable disease after 48 weeks [115]. A separate arm of this study examined a cohort of patients with advanced, progressive, symptomatic, or metastatic MTC. In this MTC cohort, 2% of patients showed an objective response, 81% maintained stable disease, and an overall 76% of patients showed decrease in the size of their target lesions [116]. Motesanib was generally well tolerated in both cohorts with fatigue, nausea, diarrhea, and hypertension comprising the majority of adverse side effects. As a result of treatment with motesanib, greater than 60% of patients experienced a TSH elevation out of the desired therapeutic range at some time during the study [116]. A recent study of both DTC and MTC revealed that a decrease in soluble VEGFR-2 and a concurrent increase in placental growth factor (PIGF) during the course of treatment with motesanib predicted which patients would respond to treatment with this agent [117].

Axitinib (AG-013736) inhibits VEGFR more specifically than the agents discussed above. A phase I study included patients with thyroid cancer though none demonstrated partial responses [118]. A phase II study using a dose of 5 mg orally two times per day noted partial responses in 31% of the patients with DTC and in 18% of the patients with MTC. Side effects included fatigue, stomatitis, and hypertension [119]. Further trials are ongoing.

XL 184 (BMS-907351) inhibits VEGF 1 and 2, C-MET, RET, c-kit, fms-related tyrosine kinase 3 (FLT3), and TIE-2. A unique aspect of this agent is its activity against hepatocyte growth factor (HGF) and C-MET, both of which are overexpressed in PTC [120]. A phase 1 trial was promising; 55% of 36 patients MTC demonstrated a partial response, and 84% overall had stable disease [121]. Interestingly, patients both with and without RET mutations responded. A phase III trial exploring XL 184 in MTC is currently underway.

Other recently evaluated novel agents include pyrazolopyrimidine derivatives like CLM3 and CLM29, which also appear to be widely effective against cytoplasmic and receptor ATP competitive tyrosine kinases including RET, EGFR, VEGFR, and angiogenesis pathways. These agents are unique because they induce apoptosis and decrease tumor volume in murine models of differentiated PTC, irrespective of BRAFV600E mutation [122].

4. Conclusion

Recent increased incidence of thyroid cancer is associated with a rise in the number of patients with metastatic disease and tumors that are resistant to the effect of radioiodine. Presently, there are no consensus guidelines about safe and effective methods to treat advanced-stage thyroid cancers. However, the recent elucidation of the pathogenesis of thyroid cancer has facilitated the development of new targeted agents intended to have activity against specific biochemical and oncologic pathways. Many of these newer agents being developed and tested are kinase inhibitors that show a promise for improved treatment of advanced DTC, as well as MTC.

In general, options for the chemotherapeutic treatment of advanced-stage thyroid cancers remain limited. The most promising agents display activity against VEGFR, including pazopanib, motesanib, sorafenib, sunitinib, and vandetanib. There is structural similarity between VEGFR and RET kinases, and cross-activity likely occurs perhaps increasing the efficacy of these agents. Interestingly, axitinib (a tyrosine kinase inhibitor that more specifically targets VEGFR) garnered similar promising tumor responses to the above noted multitargeted kinase inhibitors [119]. In addition, the effective targeted kinase inhibitors not only demonstrate specific activity against VEGFR, but also exhibit activity against a wide array of cellular pathways.

Perhaps owing to their wide ranging cellular targets, there are also numerous concerning side effects of these multitargeted kinase inhibitors. Several trials of the above listed agents reported a significant percentage of patients...
requiring a dose reduction during the study period for general tolerability. The most concerning adverse effects are increases in the incidence of cardiomyopathy and associated hypertension and stroke. Additionally, minor-to-severe bleeding (often in the form of gastrointestinal bleeding) should not be overlooked. Trials of motesanib and sunitinib noted increasing TSH values during the course of treatment, placing patients at risk for being on subtherapeutic doses of suppressive thyroid hormone for a period of time.

Other targeted kinase agents have been shown less effective than previously hoped. Imatinib does not appear to be a candidate for further study in MTC, nor does gefitinib which was not discussed in detail because a phase II trial did not demonstrate any partial responses [123]. Agents specifically targeting the BRAF pathway and BRAF V600E are in earlier stages of clinical trials; however, stable disease appears to be the best response achieved in this class of agents, including PLX4032 as well as XL281. The more specific BRAF inhibitors also have concerning side effects, including an increased incidence of squamous cell neoplasms.

Overall, options for targeted therapy of patients with advanced thyroid cancer remain limited. While these agents may improve radiographic tumor response, change in survival is unclear. Most trials have demonstrated that only small percentages of patients achieved partial responses. There has been a lack of complete responses [124]. Current expert opinion advises that these agents be used only in a specific subset of patients. They should be administered only to patients with rapidly progressive radioiodine refractory metastatic disease. Locally recurrent, unresectable cancer which is unresponsive to radiation may also be considered appropriate for treatment [125].

Other lines of research must be pursued including immunotherapy with vaccines and interferon administration, as well as efforts to induce redifferentiation of tumor cells to take up radioiodine with histone deacetylase inhibitors Romidespin and Vorinostat, for example [126–131]. Another area that warrants further investigation is the exploration of biomarkers that may be able to predict response to a given agent, which may help tailor treatment to an individual. Additionally, both in vivo and in vitro chemosensitivity testing is becoming more common, and is currently available in several clinical trials. These tests appear to be most useful in terms of negative predictability, meaning a treatment is very likely to be unsuccessful in vivo if it is unsuccessful in vitro. Unfortunately the positive predictability of such tests is not as robust. There are many diverse challenges to be addressed before chemosensitivity becomes routine [122, 132]. Promising new studies are being performed investigating combinations of tyrosine kinase inhibitors with other conventional modalities of treatment, like radiation [133]. Much new data is required before such agents are offered routinely for the treatment of advanced or dedifferentiated thyroid cancer.

Conflict of Interests

The authors declare that there is no conflict of interests.

References


[121] R. Kurzrock, S. Sherman, D. Hong et al., “A Phase I study of XL184, a MET, VEGFR2, and RET kinase inhibitor, administered orally to patients with advanced malignancies, including a subgroup of patients with medullary thyroid carcinoma,” in the EORTC-NCI-AACR International Conference on Molecular Targets and Cancer Therapeutics, Geneva, Switzerland, October 2008, poster number 379.


