

Clinical Development of Kinase Inhibitors for the Treatment of Differentiated Thyroid Cancer

Alan L. Ho, MD, PhD, and Eric Sherman, MD

Dr. Ho and Dr. Sherman are Assistant Attendings in the Division of Solid Tumor Oncology at Memorial Sloan-Kettering Cancer Center in New York, New York.

Address correspondence to:
Alan L. Ho, MD, PhD
Memorial Sloan-Kettering Cancer Center
1275 York Avenue, Schwartz 1210B
New York, New York 10065
Phone: 212-639-3311
Fax: 212-717-3278
E-mail: hoa@mskcc.org

Abstract: The incidence of thyroid cancer is growing at a rapid rate, with the majority of cases being differentiated thyroid cancers. Although a significant number of patients with localized disease are cured, a paucity of effective therapies currently exists for patients with recurrent and/or metastatic disease. The translational bridging of critical biologic insights into the pathogenesis of thyroid cancer and the clinical development of specific kinase inhibitors that disrupt these oncogenic pathways has led to exciting progress in clinical thyroid cancer research. This review will present the scientific rationale and clinical trial data gathered to date with kinase inhibitors in differentiated thyroid cancers.

Introduction

Thyroid cancers of follicular origin consist of several histologic subtypes with diverse genetic and biologic features that directly influence clinical behavior and response to systemic therapies. Papillary thyroid cancer (PTC), representing 80% of all thyroid cancers, and follicular thyroid cancer (FTC) make up a group of malignancies known as *differentiated thyroid cancer* (DTC). DTCs can progress to more aggressive forms of disease categorized pathologically as poorly differentiated thyroid cancer (PDTC) and anaplastic thyroid cancer (ATC), a rapidly progressive and fatal disease with less than a 1-year survival in most cases. Hurthle cell cancer (HTC) is an oxyphilic variant of FTC that is generally considered to be a more aggressive subtype. Medullary thyroid cancers (MTCs) are not derived from follicular cells, but instead the parafollicular C cells. Hence, MTCs require separate consideration and will not be addressed in this review.

The incidence of new thyroid cancers is the fastest growing among all cancers for both men and women, with an estimated 44,670 new cases anticipated for 2010.¹ A total of 10–20% of thyroid patients develop distant metastasis.^{2,3} Surgical resection of recurrent and/or metastatic tumors, administration of radioactive iodine (RAI), and treatment with external beam irradiation are pal-

Keywords

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liative therapeutic options for a subset of patients. Once tumors lose the ability to accumulate RAI and are not amenable to locoregional treatments, therapeutic options are quite limited, as traditional chemotherapeutic agents are relatively ineffective.⁴ Adriamycin is the only drug approved by the US Food and Drug Administration (FDA) for the treatment of RAI-refractory thyroid cancer based on limited clinical data generated from the 1980s.

The discovery of exciting insights into the biology of thyroid cancer, including the existence of tumor-initiating genetic mutations, has greatly informed the conduct and interpretation of clinical investigations evaluating molecularly targeted therapies for thyroid cancer. The goal of this review will be to describe how the current understanding of thyroid cancer biology has been integrated into these clinical studies, and to provide an update of the clinical data generated with small molecule inhibitors. These drugs disrupt the activity of protein kinases, which are enzymes that modulate biologic activity by transferring a phosphate group from adenosine-5'-triphosphate (ATP) to amino acid residues on a protein substrate. Kinase inhibitors possess activity in a number of malignancies, and several have been FDA approved. Examples include imatinib (Gleevec, Novartis) for chronic myelogenous leukemia and gastrointestinal stromal tumors, erlotinib (Tarceva, OSI/Genentech) for lung and pancreatic cancers, sorafenib (Nexavar, Bayer) for kidney and liver cancers, and sunitinib (Sutent, Pfizer) for kidney cancer. While there is yet to be an FDA-approved indication for a kinase inhibitor in the treatment of thyroid cancer, many of these drugs have demonstrated promising activity against these tumors in either phase I or phase II studies (phase II data are presented in Table 1). This review will focus primarily on the kinase inhibitor data reported for DTCs, particularly PTCs.

Clinical Studies of Kinase Inhibitors in Thyroid Cancer

Inhibitors of the MAPK Pathway

The mitogen activated protein kinase (MAPK) signaling pathway is frequently activated in human malignancies. MAPK activation in cancer can result from alterations in upstream regulators such as receptor tyrosine kinases (RTKs), the RAS oncogene, and the RAF serine/threonine kinase. Signaling through these components leads to activation of the MAPK kinase MEK and subsequently the MAPK ERK (Figure 1). Approximately 70% of all carcinomas arising from thyroid follicular cells possess mutually exclusive genetic alterations in the upstream activators of MAPK, including the RTKs RET (rearranged during transfection) and NTRK (neurotrophic tyrosine kinase receptor), as well as the signaling molecules RAS and BRAF.⁵

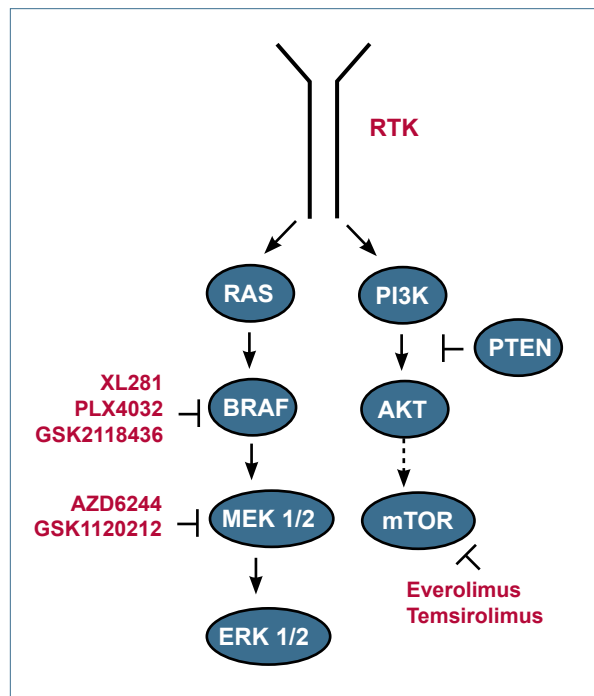


Figure 1. Schematic of mitogen-activated protein kinase and phosphatidylinositol 3-kinase pathway nodes inhibited by kinase inhibitors.

RTK=receptor tyrosine kinase.

Point mutation of the BRAF gene occurs in approximately 45% of PTCs, and hence is the most common genetic mutation for this malignancy.⁶ The mutation consists of a thymine to adenine nucleotide change at position 1799, resulting in a valine to glutamate substitution at codon 600. This is the same BRAF V600E mutation that is prevalent in cutaneous melanomas and that leads to constitutive activation of the BRAF enzyme.⁷ BRAF mutation is thought to be an early, initiating oncogenic event in thyroid cancer since it can be detected in early PTC microcarcinomas,⁸ and expression of the BRAF V600E mutant in mouse thyroid cells is sufficient to induce invasive PTCs and subsequently poorly differentiated carcinomas.⁹ Clinically, the BRAF V600E mutation has been associated with poor prognostic clinicopathologic features, including cancer recurrence¹⁰ and possibly mortality.¹¹

Hypothesized mechanisms by which mutant BRAF V600E induces oncogenesis include altering the dynamics of cell cycle progression and cell survival. Mutant BRAF V600E also suppresses the expression of thyroid differentiating genes, including those that regulate thyrocyte iodine uptake, such as the sodium-iodide symporter (NIS).^{12,13} Clinically, this translates into more RAI refractory disease in BRAF V600E mutant patients relative

to those with wild-type cancers,¹⁴ and exemplifies how tumor biology can directly affect the clinical utility of specific therapeutic approaches. Accordingly, MAPK activation has also been hypothesized to predict tumor susceptibility to targeted therapies directed at BRAF and MEK.¹⁵⁻¹⁷ As for MEK inhibition in thyroid cell lines,^{15,16} the published data have been consistent with the model initially formulated in BRAF V600E mutant cutaneous melanomas.¹⁸ In this model, BRAF V600E mutant cells possess enhanced susceptibility to the antiproliferative effects of a MEK inhibitor, whereas RAS mutant cell responses are more variable.¹⁵ Hence, the genetic lesions leading to MAPK activation are not necessarily equivalent with regard to susceptibility to MAPK pathway inhibition by targeted therapies.

Several agents have been clinically developed as BRAF or MEK inhibitors. While the MEK inhibitors are fairly selective for MEK1/2, the current BRAF inhibitors have variable selectivity for the various RAF isoforms and mutant BRAF. A particular concern that has come to light recently with the RAF inhibitors (sorafenib, XL281, and PLX4032) is an increased propensity for developing curable cutaneous keratoacanthomas and squamous cell carcinomas in patients receiving this class of drugs.¹⁹⁻²¹ Recent laboratory data have also demonstrated that BRAF inhibitors may paradoxically increase MAPK activation in specific genotypic contexts such as wild-type BRAF.²²⁻²⁴ These observations serve as a clear example of how targeted agents may disrupt baseline signaling pathways in normal tissues and reinforce the need to carefully delineate the therapeutic index of these strategies in patients.

XL281 XL281 (Exelixis) is a pan RAF inhibitor. It inhibits BRAF, BRAF V600E mutant, and CRAF. It is ATP competitive and reversible. Results of the first 48 patients enrolled in a phase I study were reported in 2009; 7 of the 48 patients had thyroid cancer (6 papillary subtype, 1 Hurthle cell subtype).²⁵ Two of the PTC patients had a BRAF V600E mutation, and the other 4 did not have tissue available for genotyping. Although no responses have been noted, both patients with BRAF mutant PTC have remained on study for at least 84 weeks with stable disease. Interestingly, another subject with a Hurthle cell thyroid cancer (BRAF wild-type) was on study for longer than 60 weeks. An expansion cohort in patients with PTC has been completed and results are pending.

PLX4032 PLX4032 (Plexxikon) is a small molecule inhibitor that is selective for the BRAF V600E mutation. Results of a phase I study were presented in 2009.²⁶ Although the majority of patients in the study had melanoma, 3 subjects with thyroid cancer—all with a

BRAF V600E mutation—were accrued. Among these 3 patients, 1 had a confirmed partial response and the other 2 subjects had stable disease. Twenty-one subjects with melanoma were treated at a dose felt sufficient to induce a meaningful biologic effect. Sixteen of these patients had a BRAF V600E mutation, with 9 of 16 having a partial response. All patients with melanoma that did not have BRAF V600E mutations had progressive disease within the first 3 months of treatment. A randomized phase III study is currently ongoing in subjects with BRAF V600E mutant melanoma. A study in thyroid cancer is currently being planned.

GSK2118436 GSK2118436 (GlaxoSmithKline) is an ATP-competitive, reversible inhibitor of RAF that appears to be selective against BRAF-mutant cell lines. A phase I/II clinical study evaluated 76 subjects with BRAF V600-mutant melanoma and 2 subjects with BRAF V600E-mutant thyroid cancer.²⁷ Toxicity included cutaneous squamous cell cancer (9%). Overall, 72% of subjects experienced skin toxicity, although a minimal number of subjects had any toxicity of grade 3 or greater.

In the evaluable BRAF V600 melanoma cohort that received what was considered a biologically active dose (150 mg twice a day or greater), the major response rate was 63% (10/16). In the evaluable BRAF V600 melanoma cohort that received a lower dose, the major response rate was 39% (16/41), with 1 complete response. BRAF wild-type and K601E mutant melanoma tumors did not appear to respond to drug treatment, as an 80% rate of disease progression was observed in this group at the first restaging point. In the 2 subjects with BRAF-mutant PTC, 1 had a partial response, with 31% reduction by response, evaluation, criteria in solid tumors (RECIST). The second subject had a mixed response, with a 66% reduction in the size of the target lesion, but was nonetheless classified as developing progressive disease given the detection of a new lesion concomitant with tumor response.

AZD6244 AZD6244 (AstraZeneca/Array Biopharma) is a small molecule inhibitor of MEK1 and 2. A multi-institutional phase II study evaluated the efficacy of AZD6244 in the treatment of PTC.²⁸ In the study, there were 32 evaluable patients. Results included 1 subject with a partial response, 21 with stable disease, and 10 with progressive disease. Rash was the most common side effect, with 18% of subjects developing grade 3/4 rash.

Interestingly, median progression-free survival (PFS) was 32 weeks (95% confidence interval [CI] 30–34 weeks) in the patient group with tumors that possessed a BRAF V600E mutation, whereas the BRAF wild-type group had a PFS of only 11 weeks (95% CI, 6–16 weeks).

Table 1. Phase II Clinical Data for Kinase Inhibitors in Thyroid Cancer

Drug	Targets	Reference	Non-DTC Histologies Included	Number of Evaluable Patients*	ORR	% SD	Comments
Angiogenesis Inhibitors							
Sorafenib	VEGFR1–3, PDGFR β , KIT, RET, BRAF, CRAF, FLT3	Gupta-Abramson et al ⁴⁹	MTC, ATC	25 (from 1st stage of 2-stage design)	23%	53%	All evaluable DTC pts had some decrease in tumor size; 2 pts with POD had ATC and poorly differentiated disease; 63% required drug holiday; 1 pt death (hepatic failure)
		Kloos et al ⁵⁰	ATC	56	11%	63%	15% ORR in PTC pts; 52% required dose reduction
		Capdevila et al ⁵¹	ATC	18	17%	—	Compassionate use program in Spain
Sunitinib	VEGFR1–3, PDGFR, KIT, RET, FLT3, CSF-1R	Cohen et al ⁵⁶	None (MTC data separate)	35	17%	74%	2 deaths; 7 grade 4 events
		Carr et al ⁵⁷	MTC	29	32% (7% CR)	—	No grade 4 events; 1 death from GI bleed on anticoagulation
		Ravaud et al ⁵⁸	MTC	20 (all pts)	13% (DTC pts only)	—	ORR among MTC patients not clear
Axitinib	VEGFR1–3, PDGFR β , KIT	Cohen et al ⁵⁹	MTC, ATC	45 (DTC pts only)	31% (DTC pts only)	42% (DTC pts only)	20% not evaluable for response; 13% (total 60 patients) discontinued treatment due to AEs
Motesanib	VEGFR1–3, PDGFR, KIT, RET	Sherman et al ⁶⁰	None	93	14%	67%	13% discontinued due to AEs; 5 pts with grade 4 events; 2 pts died of hemorrhage
Pazopanib	VEGFR1–3, PDGFR, KIT	Bible et al ⁶¹	None	37	49%	—	ATC being evaluated in separate study
MAPK Pathway Inhibitors							
AZD6244	MEK 1/2	Lucas et al ²⁸	None	32	3%	66%	Median PFS: mutant BRAF pts, 32 wks; wild-type BRAF pts, 11 wks; 18% with grade 3/4 rash
EGFR Inhibitors							
Gefitinib	EGFR	Pennell et al ⁷⁶	MTC, ATC	27	0%	48%	2 patients with SD for 12 mos or longer (1 with ATC)

AEs=adverse events; ATC=anaplastic thyroid carcinoma; CSF-1R=colony stimulating factor-1 receptor; DTC=non-differentiated thyroid carcinoma; EGFR=epidermal growth factor receptor; FLT3=fms-like tyrosine kinase 3; GI=gastrointestinal; KIT=stem cell factor receptor; MEK1/2=mitogen-activated protein kinases 1/2; MTC=medullary thyroid carcinoma; ORR=overall response rate (complete response + partial response); PDGFR=platelet-derived growth factor receptor; PFS=progression-free survival; PTC=papillary thyroid cancer; RET=rearranged during transfection; SD=stable disease; VEGFR=vascular endothelial growth factor receptor.

*Includes all evaluable patients of all histologic subtypes pooled together unless otherwise specified.

GSK1120212 GSK1120212 (GlaxoSmithKline) is a reversible, allosteric inhibitor of MEK1 and 2. A phase I study was done with an expansion study of melanoma, pancreatic cancer, colorectal cancer, and non-small cell lung cancer (NSCLC).²⁹ It was noted that there were 3 cases of central serous retinopathy in 162 patients. All

cases were reversible upon withholding the drug. Otherwise, rash and diarrhea were the most common adverse events, though these were rarely reported to be grade 3.

The preliminary report in 20 patients with BRAF V600E-mutant melanoma showed 2 complete responses and 6 partial responses (major response rate of 40%).

Seven additional subjects had stable disease, 2 of whom had previously been treated with PLX4032. This is in contrast to the 22 melanoma patients with BRAF wild-type tumors, amongst whom only 2 patients had partial responses, and 9 subjects experienced progression of disease. Unfortunately, no patients with thyroid cancer were included in the study.

Inhibitors of Angiogenesis

Thyroid malignancies are highly vascular tumors. Studies examining the relationship between microvessel density and clinical outcomes have demonstrated that hypervascular tumors correspond with worse disease-free survival relative to less vascularized tumors.^{30,31} Vascular endothelial growth factor (VEGF) has been identified as a critical activator of angiogenesis in the tumor microenvironment via stimulation of tumor-associated endothelial cell growth and survival. Several isoforms of VEGF have been discovered (VEGF-A, -B, -C, -D) as well as several different VEGF-specific transmembrane receptor tyrosine kinases (FLT-1, KDR/FLK-1, FLT-4). Multiple studies have reported higher VEGF expression in primary thyroid cancer specimens³²⁻³⁶ relative to normal thyroid tissue. Both normal and malignant thyroid cells can secrete VEGF into cell culture medium,^{35,37} and serum VEGF levels are elevated in patients with recurrent or metastatic well differentiated thyroid cancers relative to normal patient controls.^{38,39} Higher VEGF expression has been correlated with larger tumor size,³² higher tumorigenic potential,³⁶ metastatic disease,⁴⁰ and shorter recurrence-free survival.⁴¹ Furthermore, overexpression of VEGF in poorly tumorigenic cell lines can enhance tumor formation in nude mice via increased tumor vascularity, whereas suppression of VEGF expression via an antisense strategy decreased tumorigenic potential in an oncogenically aggressive tumor cell line.⁴² Taken together, these data suggest that the high levels of VEGF observed in tumors may be a biologically relevant contributor to oncogenic progression in thyroid cancer.

Consistent with data suggesting a reliance upon VEGF activation for the tumorigenic phenotype, antibodies^{38,43,44} and small molecules^{45,46} targeting VEGF signaling reduce thyroid tumor cell line growth in xenograft models. In addition to blocking VEGF-induced endothelial cell mitogenesis, the antitumor effects of these strategies may also be related to a direct effect on thyroid cancer cells, which also express VEGF receptors (VEGFR) FLT-1 and KDR/FLK-1.^{47,48} These data support the clinical hypothesis that VEGF-targeted agents may be effective in this disease. Indeed, many of the targeted inhibitors with activity in thyroid cancer disrupt the VEGF pathway, and these data are summarized below. Common side effects seen with these agents include hypertension, proteinuria, and thrombosis.

Sorafenib Sorafenib is a multitargeted small molecule inhibitor that inhibits several molecules involved in angiogenesis, including VEGFRs 1–3 and PDGFR β . Sorafenib also inhibits RAF kinase, but the relevance of RAF inhibition to sorafenib-related activity in thyroid cancer and other malignancies is much debated. Other targets include KIT, RET, and FLT3. Currently, the FDA has approved the use of sorafenib in the treatment of kidney and liver cancers.

There have been several studies that have evaluated the use of sorafenib in the treatment of thyroid cancer. Investigators at the University of Pennsylvania have published the first stage (total of 30 patients, although 5 patients were not evaluable) of a 2-stage phase II study design evaluating sorafenib at a dose of 400 mg twice a day in patients with all subtypes of thyroid cancer (DTC, ATC, and MTC).⁴⁹ It was noted that “All patients who were enrolled had evidence of disease progression in the year before initiation of treatment,” although it is not clear if this was a formal entry criteria. Of the 30 subjects, 18 (60%) had PTC, 2 had PDTC/ATC, and 1 had MTC. The investigators reported a response rate of 23%, with a stable disease rate of 53%. Interestingly, the 2 evaluable subjects that developed progressive disease had either ATC or PDTC. All evaluable DTC patients, in contrast, experienced a decrease in tumor size.

Investigators at Ohio State University have also published a phase II study evaluating sorafenib at a dose of 400 mg twice a day in patients with RAI-refractory thyroid cancer (DTC and ATC).⁵⁰ In this study, 58 patients were enrolled, but 2 subjects who never started treatment were not included in any analyses. A total of 41 patients had PTC and 4 had ATC. Among the 56 evaluable patients, a partial response by RECIST criteria was seen in 6 patients (11%). All 6 responses were detected in patients with PTC (total response rate, 15%). Furthermore, stable disease was noted in 35 patients (63%), of which 25 had PTC (61% in this group).

Several other studies with sorafenib in thyroid cancer have also been reported. In a compassionate use program in Spain, partial responses were reported in 3 of 18 (17%) patients with DTC or ATC.⁵¹ A phase II study in the Netherlands evaluated whether sorafenib would cause reuptake of RAI in tumors after 26 weeks of treatment.⁵² In 31 patients, no uptake was seen; however, the authors reported partial responses in 25% and stable disease in 34% of patients.

Though both the Ohio State University and University of Pennsylvania studies reported discouraging results for sorafenib in the treatment of ATC, there is an ongoing study evaluating the drug in ATC.⁵³ In 2009, Nagaiah and colleagues reported 2 major responses (13%) and 4 patients with stable disease (27%) among 15 evaluable patients with ATC treated with sorafenib 400 mg

twice a day. Though the median time to progression (1.9 months) and median overall survival (3.5 months) were not encouraging, PFS was reportedly 6.3% at 24 months. All the reported patients died, but 25% were alive 1 year after starting treatment.

Toxicities have been reported with sorafenib. One patient on the University of Pennsylvania study died of liver failure due to the drug.⁴⁹ Sixty percent of patients experienced weight loss. In total, 63% of the patients required a drug holiday during the study. In the Ohio State University study, 52% of the patients required some type of dose reduction due to difficulties tolerating the drug.⁵⁰ The 1 death observed in this study was not thought to be drug related, though no other explanation was provided.

Even though sorafenib appears to have some activity in thyroid cancer, it is unclear if the benefit of the drug is related to inhibition of the BRAF kinase or disruption of tumor-related angiogenesis. In melanoma, the activity observed with sorafenib is independent of BRAF mutation status.⁵⁴ In the Ohio State University study, 22 patients with PTC had tissue with sufficient DNA for analysis, and 17 of these patients had a mutation on exon 15 of BRAF. Unfortunately, because of the high rate of BRAF mutations in this cohort, the authors felt a comparison of outcomes based on BRAF mutation could not be done.⁵⁰ In the University of Pennsylvania study, 22 patients were genotyped for BRAF mutation. Despite the fact that PFS was not significantly different between the wild-type and mutant BRAF groups, there was a “trend” in favor of superior outcome in the group with a BRAF mutation.⁵⁵

Sunitinib Sunitinib can effectively inhibit VEGF and PDGF receptors in addition to other tyrosine kinase receptors such as KIT, RET, CSF-1R, and FLT3. At this time, a completed sunitinib study in thyroid cancer has yet to be published. The University of Chicago Consortium Phase II trial did present early data from a phase II study evaluating sunitinib in both differentiated and medullary thyroid cancer.⁵⁶ At the time of the presentation, data were available on 35 subjects with DTC, with a response rate of 17% and a stable disease rate of 74%. However, a dosing schedule of 50 mg daily (4 weeks on/2 weeks off) was associated with significant toxicity, with 2 deaths (due to hepatic failure) and 7 grade 4 events (congestive heart failure, fatigue, hypertension, hematologic). Carr and colleagues presented preliminary data on their phase II study of sunitinib in differentiated and medullary thyroid cancer.⁵⁷ They reported a response rate of 32% (7% with complete response) for 29 evaluable subjects with both DTC and MTC. They did not report any grade 4 toxicities, but did have 1 patient die of gastrointestinal bleed while on enoxaparin. Ravaud and associates reported early results of their phase II study, with a 13% response rate

in DTC patients, although their study only included 20 patients with both DTC and MTC.⁵⁸ Both the Ravaud and Carr studies used the same dosing as the University of Chicago Consortium Phase II study.

Axitinib Axitinib (Pfizer) is an oral agent that selectively inhibits VEGFR1–3 and less potently inhibits PDGFR β and KIT. A multi-institutional phase II study was reported in subjects with DTC, ATC, and MTC.⁵⁹ The response rate for the 45 subjects with DTC was 31% in the intent-to-treat group, with a 42% rate of stable disease. It is notable that 20% of patients were not evaluable for response, and only 4% were evaluated as having progression of disease. However, 8 (13%) of the total 60 patients in the study discontinued treatment due to adverse events, though only 3 patients experienced a grade 4 toxicity (stroke, reversible posterior leukoencephalopathy syndrome related to hypertension, and proteinuria).

There was an attempt to follow-up this phase II study with a registration phase II study in subjects with doxorubicin-refractory thyroid cancer but, unfortunately, accrual to the study was difficult.

Motesanib Motesanib (Amgen/Takeda) is an oral agent that inhibits VEGFR1–3, PDGFR, and KIT. A phase II clinical study was performed by the Motesanib Thyroid Cancer Study Group in 93 subjects with DTC.⁶⁰ The response rate was 14%, with another 67% of patients having stable disease. Twelve (13%) of the 93 patients had to discontinue treatment due to toxicities. Fifty-one (55%) of the subjects had at least 1 grade 3 event, and 5 patients had at least 1 grade 4 event. Additionally, 2 subjects died of hemorrhage.

Pazopanib Pazopanib (Votrient, GlaxoSmithKline) is an oral agent that inhibits VEGFR1–3, PDGFR, and KIT. It has recently been approved for the treatment of kidney cancer. A study of pazopanib at the Mayo Clinic consisted of 37 patients with evaluable differentiated thyroid cancer.⁶¹ Confirmed partial responses were noted in 18 (49%) subjects. The highest response rate was seen in follicular thyroid cancer (73%), followed by Hurthle cell (45%) and papillary (33%) subtypes. Seventeen (46%) subjects were on treatment for over 12 months. A correlation between plasma concentration of pazopanib and tumor response was noted. Two subjects died during the study.

Epidermal Growth Factor Receptor Inhibitors

The epidermal growth factor receptor (EGFR) has been implicated in the pathogenesis of many tumor types. Mutational activation of this RTK in NSCLC has been correlated to clinical response with EGFR tyrosine kinase inhibitors (TKIs). EGFR is the first member of what is

known as the ErbB family, which consists of 4 RTKs. EGFR can be activated via gene mutation, EGFR ligand(s) overexpression, EGFR overexpression, and transactivation by other RTKs. Reports of whether or not EGFR is overexpressed in PTCs vary, with some groups reporting higher levels than those observed in normal thyroid tissue,^{62,63} and others demonstrating minor to no differences.^{64,65} Nonetheless, simple immunohistochemical detection of EGFR has also been linked to poorer prognostic features for PTC.^{66,67} One Japanese group recently reported that 7 of 23 analyzed PTC patient tumor samples possessed EGFR drug-sensitive mutations commonly found in NSCLC.⁶⁸ These findings are in contradiction to an earlier report identifying only 2 EGFR-mutant tumors of 62 thyroid cancer specimens examined,⁶⁵ and more recent data in anaplastic carcinomas demonstrating no EGFR mutations.⁶⁹⁻⁷¹ Further studies are warranted to determine the rate of EGFR mutations in different patient populations and their potential role in PTC biology.

The discrepancy amongst these reports regarding the expression profile and mutational status of EGFR in PTC has also carried over into preclinical studies addressing the potential importance of EGFR targeting in thyroid cancer. Several studies have reported that anti-EGFR antibodies⁷² and TKIs (AG 1478,⁷³ gefitinib [Iressa, AstraZeneca],^{63,74} and AEE788 [Novartis]⁷⁵) can slow or block thyroid cancer cell line growth. Mitsiades and coworkers, however, argued that the degree of the antiproliferative effect with EGFR targeting is modest when drug concentrations are kept to submicromolar concentrations, which effectively inhibits the growth of an EGFR mutant NSCLC cell line.⁶⁵

At this time, there are several FDA-approved drugs that inhibit EGFR. Two are monoclonal antibodies (cetuximab [Erbix, ImClone/Bristol-Myers Squib] and panitumumab [Vectibix, Amgen]). In addition, erlotinib is a TKI specific for EGFR, and is approved for the treatment of lung and pancreatic cancer. Lapatinib (Tykerb, Glaxo SmithKline) is a dual EGFR and HER2/neu inhibitor that recently was approved for the treatment of breast cancer. Most of these agents are well tolerated, with a common side effect of folliculitis.

Gefitinib Gefitinib is a selective oral TKI of EGFR that binds to the ATP binding site of the enzyme. As a selective TKI, its tolerability appears to be better than the inhibitors discussed above. Acne is common, but grade 4 adverse events are not.

Massachusetts General Hospital completed a phase II study of gefitinib 150 mg daily in 27 subjects with DTC (n=18), ATC (n=5), or MTC (n=4).⁷⁶ The main inclusion criterion was metastatic or locally advanced thyroid cancer (any histologic subtype) that was not amenable to surgery and/or RAI therapy. There were no responses seen, though

stable disease was reported in 48% of patients (decreased to 24% at 6 months). Only 2 subjects continued to have stable disease for 12 months or longer, but one of the subjects had ATC (the other had DTC). By the waterfall plot, 8 subjects had at least a slight reduction in tumor size by RECIST criteria. It was noted that all patients with MTC had progression of disease at the earliest time point for evaluation. The treatment was very well tolerated, with no grade 4 toxicities and only 3 patients experiencing a grade 3 toxicity.

What was not evaluated in the study was RAS mutational status. More recent studies have shown that EGFR inhibitors, including gefitinib, are ineffective in tumors with RAS mutations.^{77,78} Furthermore, studies have started to show a lack of response to EGFR inhibitors amongst tumors with BRAF mutations.⁷⁹ Since the frequency of either a BRAF or a RAS mutation in thyroid cancer is high, this might be one explanation for the ineffectiveness of gefitinib as a single agent.

Mammalian Target of Rapamycin Inhibitors

The mammalian target of rapamycin (mTOR) is a serine/threonine kinase that serves as a critical downstream mediator of growth factor, nutrient, and energy signaling in the cell. Many of the signaling pathways altered in oncogenesis lead to the activation of mTOR. mTOR exists in 2 different multimeric complexes. mTOR complex 1 (TORC1) phosphorylates ribosomal S6 kinase 1/2 (S6K1/2) and eukaryotic initiation factor 4E (eIF-4E)-binding protein (4E-BP1) to promote mRNA translation. TORC1 is targeted by the drug rapamycin and its structural analogues. A second mTOR complex known as TORC2 has more recently been demonstrated to phosphorylate and activate Akt at serine 473.^{80,81} RTK signaling primarily activates mTOR via activation of phosphatidylinositol 3-phosphate kinase (PI3K) and Akt (protein kinase B/PKB; Figure 1). Briefly, RTK activation results in recruitment of PI3K to the membrane where it phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-triphosphate (PIP3). This event results in recruitment of Akt to the membrane where it is activated by phosphorylation. Phosphatase and tensin homolog deleted on chromosome ten (PTEN) can counter these signals by dephosphorylating PIP3 back to PIP2.

Clinical genetics provide the most convincing evidence that activation of the PI3K/Akt pathway can play a critical role in thyroid tumorigenesis. Germline loss of PTEN leads to Cowden syndrome, a multiple hamartoma syndrome that is characterized by various neoplasias, including both benign and malignant thyroid tumors. Mice harboring PTEN loss indeed spontaneously develop thyroid cancer.⁸² Mouse models have demonstrated that conditional loss of PTEN in thyroid follicular cells leads

to hyperplastic thyroid growth that is dependent on mTOR activation.⁸³ While somatic PTEN mutations are relatively rare,⁸⁴ alternative mechanisms of downregulating PTEN expression, such as promoter hypermethylation, may play a role in thyroid cancer.⁸⁵ PIK3CA mutations and amplifications have also been described both in primary differentiated and anaplastic thyroid cancers.⁸⁶⁻⁸⁸ The higher prevalence of PIK3CA mutations in ATC relative to well-differentiated tumors has led to speculation that these alterations are more critical to later thyroid cancer progression. Indeed, recent mutational profiling of PDCs and ATCs revealed not only PIK3CA mutations, but also, for the first time, Akt1 mutations exclusively in RAI-refractory, [18F]fluorodeoxyglucose (FDG) positron emission tomography–positive recurrent/metastatic tumors.⁸⁹ Invariably, PIK3CA and Akt1 mutations were found concomitantly with BRAF mutations, suggesting cooperativity between these 2 pathways in advanced thyroid cancer.

Preclinical data in other tumor types suggest that activation of the PI3K/Akt pathway is sufficient to confer susceptibility to mTOR inhibition by rapamycin analogues.⁹⁰⁻⁹² Studies in thyroid tumor cell lines have also confirmed that those possessing mutations in this pathway possess increased susceptibility to the antiproliferative effects of a rapamycin analogue.⁹³

Beyond activation via growth factor receptor–mediated signaling and the PI3K/Akt pathway, mTOR activation may play a particularly critical role in thyrocyte mitogenesis (and hence tumorigenesis), given that mTOR is activated via TSH-initiated signaling in a manner independent of Akt.⁹⁴ Hence, thyroid cancer may be uniquely dependent upon mTOR activation beyond the common mechanisms of oncogenic dependence ascribed to other malignancies, further strengthening the rationale for the trials described below.

Everolimus and Temozolomide These 2 mTOR inhibitors have been approved by the FDA for the treatment of kidney cancer. Importantly, each of these rapamycin structural analogues is considered pharmacodynamically equivalent with regards to TORC1 targeting.⁹⁵

Everolimus (Afinitor, Novartis) is currently undergoing a phase II study in thyroid cancer. Furthermore, the combinations of sorafenib/temozolomide (Torisel, Wyeth) and sorafenib/everolimus are both being studied in separate phase II studies.

Conclusion

Although a number of kinase inhibitors have been evaluated in small, single-arm phase II studies and have shown promising response rates and PFS intervals, there are no

completed randomized phase III studies to date. Because overall survival may be excellent in patients with differentiated thyroid cancer without any treatment, response rates and PFS are inadequate surrogates to prove clinical benefit. Currently, the guidelines set by both the National Comprehensive Cancer Network (NCCN) and the American Thyroid Association (ATA) recommend the use of TKIs such as sorafenib and sunitinib. Neither sorafenib nor sunitinib is FDA-approved for the treatment of thyroid cancer, although they are approved by the FDA for the treatment of other cancers. Because both the NCCN and ATA thyroid cancer guidelines recommend sorafenib and sunitinib, insurance companies are more likely to pay for these agents outside of a clinical study, allowing medical oncologists to use these in the treatment of thyroid cancer when clinical trials are not available. Though the motivation of the guideline committees is well-meaning, we do have concerns about the deficit of data (as noted above) to support the benefit (in terms of overall survival or quality of life) of these drugs in the treatment of thyroid cancer and about the danger of significant morbidity, including death. Furthermore, the potential availability of these agents off clinical trials will lead to difficulty in clinical trial accrual and may make patients ineligible for certain studies.

A final issue is the lack of data determining who should receive systemic therapy. At this time, the basic entry criterion for these studies is RAI-refractory thyroid cancer, yet this population has an average survival rate in excess of 5 years. The presence of FDG-avid tumors can help determine who needs treatment, but even this is not accurate enough. Further research in this area is desperately needed.

Fortunately, the last several years have revealed exciting developments in the understanding of thyroid cancer biology and evidence of clinical activity in thyroid cancer patients with small molecule inhibitors that target active oncogenic pathways. The ongoing challenges will be to continue to translate scientific developments into clinical studies, as well as to better interrogate the clinical data to provide physiologically meaningful insights into these diseases. With an integration of these perspectives, future, rationally designed studies are sure to yield more expansive therapeutic options for these patients.

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