Thyroid cancer: emerging role for targeted therapies

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Abstract: The histology and clinical behavior of thyroid cancer are highly diverse. Although most are indolent tumors with a very favorable outcome with the current standard of care therapy, a small subset of tumors may be among the most lethal malignancies known to man. While surgery and radioactive iodine are the standard of care for differentiated thyroid cancers (DTC) and are effective in curing a majority of such patients, those with iodine-resistant cancers pose a great challenge for clinicians, as these patients have limited treatment options and poor prognoses. Medullary thyroid carcinoma (MTC) has no effective systemic therapy despite the genetic and signaling defects that have been well characterized for the last two decades. Anaplastic thyroid cancer (ATC) is one of the most aggressive solid tumors that remains fatal despite conventional multimodality therapy. Increased understanding of the pathogenesis of papillary thyroid carcinoma, the most common type of DTC, as well as ATC, has led to the development of targeted therapies aimed at signaling pathways and angiogenesis that are critical to the development and/or progression of such tumors. Development of tyrosine kinase inhibitors targeting known pathogenetic defects in MTC has led to testing of such agents in the clinic. Numerous clinical trials have been conducted over the last 5 years to examine the effects of these targeted molecular therapies on the outcomes of patients with iodine-refractory DTC, MTC and ATC. Conduction of such trials in the last few years represents a major breakthrough in the field of thyroid cancer. Several trials testing targeted therapies offer promise for setting new standards for the future of patients with progressive thyroid cancer. The purpose of this paper is to outline the recent advances in understanding of the pathogenesis of thyroid cancer and to summarize the results of the clinical trials with these targeted therapies.

Keywords: targeted therapy, thyroid cancer, tyrosine kinase inhibitors

Introduction

The incidence of differentiated thyroid cancer (DTC) has risen 2.4-fold over the last 30 years [Davies and Welch, 2006]; among the largest increases of any type of cancer [Hayat *et al.* 2007]. This concerning trend has been documented across all stages of thyroid cancer [Enewold *et al.* 2009]. While the majority of these patients have highly favorable outcomes, with a 91% survival rate at 20 years [Chow *et al.* 2002] for stage I and II disease, a small contingency of patients have a poor prognosis. The traditional treatment algorithm for DTC consisting of surgery followed by radioactive iodine ablation and thyroid hormone suppressive therapy may be insufficient to prevent disease progression in patients with advanced tumors.

Likewise, patients with medullary thyroid cancer (MTC) and poorly differentiated tumors such as anaplastic thyroid cancer (ATC) have very limited treatment options. Conventional parenteral cytotoxic chemotherapy has been unsuccessful in controlling these tumors. Clinical trials with various combinations of doxorubicin, bleomycin, and a platinum-based agent have yielded unsatisfactory results, with response rates of 3–14% [Droz *et al.* 1990; Kolaric *et al.* 1977; Scherubl *et al.* 1990; Williams *et al.* 1986]. Similarly disappointing, the adverse event (AE) profile of these drugs is dose limiting, with patients experiencing significant neutropenia, nausea/vomiting, diarrhea, anorexia, even congestive heart failure [Argiris *et al.* 2008]. However, recent insights into the signaling

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pathways that promote tumorigenesis, allow metastatic spread, and inhibit programmed cell death have led to the development of multiple promising therapeutic agents that can target these specific mutations.

The majority of papillary thyroid carcinomas (PTC) are associated with a mutation in one of three genes involved in the signaling of the mitogen-activated protein (MAP) kinase pathway-RAS, BRAF, or RET/PTC rearrangement [Kondo *et al.* 2006]. These mutually exclusive genetic alterations result in downstream constitutive activation of the MAP kinase pathway, a key oncogenic event in the development of thyroid cancer [Jhiang *et al.* 1996; Kimura *et al.* 2003]. The majority of cases of inherited MTC, on the other hand, have been associated with germline mutations in the RET proto-oncogene. Several mutations have been identified; specific codons have been correlated with distinct phenotypic expressions of the multiple endocrine neoplasia (MEN) syndromes and familial MTC [Eng *et al.* 1996]. Approximately 50% of patients with sporadic MTC have also been found to have somatic mutations in RET at codon 918 [Gimm *et al.* 1999] in the tumor cells without expression in other tissues. The presence of this somatic mutation is associated with a high probability of lymph node metastases, higher likelihood of recurrent/persistent disease, and reduced survival [Elisei *et al.* 2007]. Targeting these oncogenic mutations is a logical choice for potential treatment of refractory thyroid cancers. Finally, mutations in the BRAF oncogene have been observed in 10–35% of ATC [Kondo *et al.* 2006]. Although the precise role of such mutations in ATC is unclear, further studies to evaluate BRAF as a potential target for therapy are ongoing.

Aberrations of normal physiologic processes also have been observed with increased frequency in thyroid cancer and appear to play an important role in the development of distant metastases, tumor growth, and inhibition of apoptosis. In particular, angiogenesis is a vital process for sustaining tumor growth through delivery of nutrients and removal of waste products. Thyroid cancers are associated with significantly higher vascularity than surrounding non-neoplastic thyroid tissue [Akslen and Livolsi, 2000]. Numerous growth factors are necessary for the development of new blood vessels; vascular endothelial growth factor (VEGF), basic

fibroblast growth factor, and platelet-derived growth factor (PDGF) have all been implicated in angiogenesis [Hoffmann *et al.* 2006b; Soh *et al.* 2000]. Levels of VEGF expression correlate with metastatic disease and other markers of tumor aggressiveness [Klein *et al.* 1999]. Levels of VEGF are highly expressed in thyroid tumor cell lines [Viglietto *et al.* 1995] and blocking VEGF results in inhibition of tumor growth [Soh *et al.* 2000]. These observations have led to the development of therapeutic agents targeting neovascularization through the various growth factors and their interactions with cell-surface receptors.

The recent progress in understanding of thyroid cancer pathogenesis has resulted in a renewed interest in the field and a surge in clinical trials to address these refractory tumors. This paradigm shift in management of thyroid cancer marks an exciting time for researchers and clinicians, with the hope that an effective new therapy awaits on the horizon. The purpose of this paper is to outline the recent advances in clarifying the pathogenesis of thyroid cancer and to summarize the results of the clinical trials with these targeted therapies.

Tyrosine kinase inhibitors

Tyrosine kinases are responsible for transferring phosphate from ATP to tyrosine residues of a protein. There are numerous tyrosine kinases in humans and they control vital cellular functions such as cell differentiation, survival, proliferation, function, and motility. Mutations in genes encoding these enzymes have been associated with various malignancies, including thyroid cancer. Small molecule tyrosine kinase inhibitors (TKI) are a new class of drugs which can combat the dysregulated tyrosine kinases at various steps in the activation pathway, including binding of the ligand to the receptor, prevention of dimerization of the receptor, or any one of the numerous steps in the intracellular cascade. These medications are partially selective, but many also have the ability to inhibit multiple kinases and thereby affect several signaling pathways. The majority are orally administered, making them more attractive to both clinicians and patients alike. These medications are generally well tolerated and can be administered on a chronic basis, but they do have a typical constellation of side effects which includes hypertension, diarrhea, fatigue, and myriad skin lesions.

Clinical trials in differentiated thyroid carcinoma

Several clinical trials conducted for DTC also included a small number of patients with MTC and/or ATC. However, in this section, we focus on the response data related to DTC (Table 1). The AE and correlative data are reported in this section due to the fact that a majority of patients treated on these trials had DTC and the published data are reported for the entire cohort of patients on the trials. The objective response data for the patients with MTC and ATC treated on these trials are not reported in this paper due to small sample size (less than 10 evaluable patients) in each of this cohort of patients.

Multikinase inhibitors targeting angiogenesis

Axitinib. As discussed above, the agents that target VEGF signaling are attractive candidates for testing its efficacy in treatment of refractory thyroid cancer. Axitinib is a small molecule TKI of all VEGF receptors (VEGFR) and, to a lesser extent, it blocks PDGF receptor (PDGFR)- β and c-KIT as well. It inhibits VEGF-mediated autophosphorylation of the receptor and has shown dramatic reductions in microvascular formation in mouse thyroid tissue [Kamba *et al.* 2006]. In a phase I study [Rugo *et al.* 2005] one of five thyroid cancer patients had a decline in tumor burden that did not quite meet Response Evaluation Criteria in Solid Tumors (RECIST) for a partial response (PR). Subsequently, a phase II trial in 60 patients with all histologies of thyroid carcinoma was undertaken [Cohen *et al.* 2008]. Criteria for study entry included disease refractory to radioiodine therapy or inappropriate for radioiodine and at least one RECIST-defined lesion. Patients were started on 5 mg twice daily, with dose escalation to 10 mg twice daily as tolerated. Dose reductions to 2 mg twice daily were made as needed for treatment-related side effects. There were 47 patients with DTC; a PR was seen in 15 (31%), stable disease (SD) was seen in 20 (42%), and three (7%) patients had progressive disease (PD). As a result of patients not meeting response criteria and a lack of follow up scans, nine (20%) patients had indeterminate (IND) results. The median progression-free survival (PFS) for the entire cohort of 60 patients (including MTC and ATC) was 18 months; this statistic was not reported separately for the DTC patients.

Table 1. Summary of phase II clinical trials for differentiated thyroid cancer (DTC).

Name of the drug tested	Key targets	No. of DTC patients	No. of patients with PTC/FTC/-HTC/other	PR* n (%)	SD* n (%)	SD ≥ 6 months N (%)	Median duration of PR (months)	Median PFS (months)	Serum Tg response compared to baseline	Reference
Axitinib	VEGFRs 1–3, PDGFR, KIT	47	30/4/11/2	15 (31)	20 (42)	NR	NR	NR	Correlation with objective response not assessable	(Cohen <i>et al.</i> 2008)
Motesanib	VEGFRs 1–3, PDGFR, KIT	93	57/15/17/4	13 (14)	62 (67)	33 (35)	8	10	34/75 (45%) patients with $\geq 50\%$ decrease	(Sherman <i>et al.</i> 2008)
Sorafenib	VEGFRs 1–3, PDGFR, BRAF, RET	27	18/9/0/0	7 (26)	15 (56)	NR	4.5–21 (range)	21	17/19 (89%) patients with 70% mean decrease	(Gupta-Abramson <i>et al.</i> 2008)
Sorafenib	VEGFRs 1–3, PDGFR, BRAF, RET	52	41/2/9/0	6 (12)	34 (61)	29 (56)	9	15 (PTC patients)	No correlation with objective response	(Kloos <i>et al.</i> 2009)
Gefitinib	EGFR	18	11/6/1/0	0	NR	NR	NA	3.9	5/15 (33%) patients with $> 90\%$ decrease	(Pennell <i>et al.</i> 2008)
Vorinostat	HDAC	16	14/1/1/0	0	9 (56%)	6 (38%)	NA	NR	No correlation with objective response	(Woyach <i>et al.</i> 2009)
Rosiglitazone	PPAR γ	20	17/1/2/0	5 (25%)**	3 (15%)	NR	NR	NR	3/20 (15%) patients with decline	(Kebebew <i>et al.</i> 2009)
Rosiglitazone	PPAR γ	23	22/1/0/0	6 (26%)**	NR	NR	NR	NR	2/23 patients 51% and 97% decline	(Tepmongkol <i>et al.</i> 2008)
Cetecoxib [§]	COX-2	32	21/7/3/1	1	12	NR	9	NR	1/23 patients 30% decline	(Mrozek <i>et al.</i> 2006)

*Response criteria were RECIST unless otherwise specified; **Response was assessed based on iodine uptake or thyroglobulin levels; [§]Response criteria were based on RECIST or thyroglobulin levels.
 BRAF, V-raf murine sarcoma viral oncogene homolog B1; COX-2, cyclo-oxygenase-2 enzyme; EGFR, epidermal growth factor receptor; HDAC, histone deacetylases; PDGFR, platelet-derived growth factor receptor; PFS, progression-free survival; PPAR γ , peroxisome proliferator-activated receptor gamma; PR, partial response; RET, RET proto-oncogene; SD, stable disease; Tg, thyroglobulin; VEGFR, vascular endothelial growth factor receptor.
 NR, Not reported; NA, Not applicable.

Serum thyroglobulin levels were followed in nearly half of the patients with DTC. Most patients, including those with SD and PD, had initial declines in the thyroglobulin. However, the authors stated that given the small number of patients with PD whose thyroglobulin levels were measured, a statement could not be made as to the utility of this test as a marker of response to axitinib.

Median duration of therapy was 4.8 months; 32 patients discontinued axitinib. The majority — 10 patients (17%) — discontinued the drug because of no therapeutic benefit, eight patients (13%) withdrew as a result of AEs, four patients died as a consequence of AEs (but did not seem to be related to study treatment), and the remaining patients discontinued the axitinib for other reasons. Adverse events frequently seen with axitinib included fatigue, diarrhea, nausea, anorexia, hypertension, stomatitis, weight loss, and headache.

Pharmacodynamic markers of VEGFR-mediated signaling were measured at baseline and every 8 weeks during the study period. A 2.8-fold increase in VEGF concentration was seen after initiation of axitinib. Soluble (s) VEGFR-2 levels decreased by 32% and sVEGFR-3 levels declined by 35% ($p < 0.001$, $p < 0.0001$, respectively). Soluble KIT levels were also measured during the study and these declined by 13% ($p < 0.01$). Taken together, these findings of marked changes in VEGF, VEGFR-2 and -3, with a minimal change in serum KIT levels support the hypothesis that axitinib is a selective inhibitor of angiogenesis. However, the question of the true target at the level of the tumor tissue still remains open.

Motesanib. Motesanib is an oral multikinase inhibitor, with activity against VEGFRs 1–3, PDGFR and KIT. Thyroid cancer tissue expresses higher than normal levels of PDGFR; inhibition of this target *in vitro* resulted in reductions in cellular proliferation [Chen *et al.* 2006]. The multitargeted capabilities of motesanib make this an attractive candidate for treatment of refractory thyroid cancer. A phase I trial [Rosen *et al.* 2007] of this medication included seven patients with thyroid cancer of various histologies; three patients achieved a PR, three patients had SD. Based on these promising preliminary studies, an international multicenter phase II

study of 93 patients with DTC was undertaken [Sherman *et al.* 2008]. Requirements for study entry included metastatic or locally advanced DTC that was refractory to standard therapy with documented radiographic disease progression (by RECIST definition) in the 6 months prior to study entry. Patients were administered motesanib diphosphate 125 mg daily for 48 weeks or until PD or unacceptable toxicity was encountered. Thirty-two patients completed the entire course of treatment; the remainder discontinued the drug because of PD (35), AEs (12), death (5), or various other reasons (9). A PR was seen in 13 (14%), with SD in 62 patients (67%), and PD in seven (8%). The median duration of response was 8 months and estimated PFS was 10 months. Serum thyroglobulin levels decreased from baseline in 81% of patients whose values were measured; a correlation was observed between declines in thyroglobulin and reductions in tumor volume. The most common toxicities were diarrhea, hypertension, fatigue, and weight loss. There was an observed increase in thyrotropin concentrations, necessitating an increase in levothyroxine in a number of patients. It is interesting to note, as well, that five patients developed cholecystitis during motesanib therapy; the pathophysiologic mechanism is unclear.

Sorafenib. Sorafenib is a serine-threonine kinase inhibitor which targets VEGFRs 1–3, PDGFR, BRAF, and RET [Carlomagno *et al.* 2006]. It is Food and Drug Administration (FDA)-approved for use in renal cell carcinoma and hepatocellular carcinoma. Its ability to block the signaling of two key etiologic oncogenes in the MAP kinase pathway (RET and BRAF) as well as angiogenesis (VEGFR and PDGFR) has made sorafenib an attractive option in the treatment of refractory thyroid cancer. Our group conducted a National Cancer Institute (NCI) sponsored phase II trial of sorafenib [Kloos *et al.* 2009] for 56 patients with advanced thyroid cancer with the primary endpoint of examining objective response rates in patients with chemonaive PTC. Patients with chemonaive PTC were treated on Arm A of the trial and PTC patients with prior chemotherapy or patients with follicular thyroid carcinoma (FTC), hürthle cell thyroid carcinoma (HTC) and ATC were treated on Arm B as an exploratory arm. Patients with iodine-refractory, measurable disease were treated with 400 mg twice daily of sorafenib and RECIST was used to assess the objective response. Of the 33 chemonaive patients with PTC, 28 were

assessable for a response. Five patients (15%) showed a PR, 19 (57%) had SD, and four (12%) had PD. Response rates were similar in eight PTC patients with prior chemotherapy. Of the 10 evaluable patients with FTC/HTC, SD was the best response in nine and one had PD. Median PFS was 16 months in chemotherapy-naïve patients with PTC versus 10 months in PTC patients who had received prior chemotherapy. Although some patients with PRs and SDs had substantially reduced levels of thyroglobulin, there was no consistent correlation between thyroglobulin response and the objective radiological response in the entire study population. The most common AEs associated with sorafenib were hand/foot pain, hand–foot skin reaction (HFSR), fatigue, arthralgia, diarrhea and hypertension. Dose reduction was necessary in 52% of patients to control the toxicities; 14 patients discontinued the drug as a result of AEs.

To determine *in vivo* signaling inhibition, levels of ERK-, AKT-, and VEGFR-phosphorylation and VEGF expression were determined by immunohistochemistry on cell-block samples before and after initiation of treatment. Ten paired samples were analyzed. Four of 10 patients had major reductions in levels of pERK, pVEGFR, and pVEGF. Two of four patients had reductions in pAKT. All of the patients who demonstrated high basal levels of pERK and pVEGFR had significant inhibition with therapy. These results from the tumor biopsies demonstrate that sorafenib does target RAS-RAF kinase signaling in tumor tissues but do not prove inhibition of this signaling cascade as mechanism of action.

Another published phase II trial of sorafenib [Gupta-Abramson *et al.* 2008] examined its effects on all types of thyroid cancer; 27 patients with DTC were enrolled and treated for a minimum of 4 months. Entry criteria included metastatic or unresectable thyroid cancer for which curative measures were no longer possible. Patients were also required to have measurable disease by RECIST and have PD in the year prior to study entry. Sorafenib was administered as 400 mg orally twice daily and the dose was reduced by 200 mg increments as necessary for toxicities. At study completion, 22 DTC patients were evaluable for a response. Seven patients (26%) had a PR, 15 patients (56%) had SD. Median overall PFS was 21 months; median duration of treatment was 7 months. Of the 19

patients in whom serial thyroglobulin measurements were available, 17 (89%) of them had a mean reduction of 70% within 4 months of starting treatment. These reductions in thyroglobulin immediately preceded shrinkage of tumor on imaging, reflecting a biologic response. However, the authors have not been able to correlate the duration and degree of tumor reduction with declines in thyroglobulin. Six patients discontinued sorafenib as a result of AEs and 47% required dose reduction to control toxicities. The most common AEs included palmar-plantar erythema, rash, fatigue, weight loss, hypertension, musculoskeletal pain, diarrhea, and anorexia.

The disparity in objective response rates between these two studies with sorafenib may be attributable to differences in the populations of patients studied, dosing intensity, and/or intervals between response evaluations.

EGFR inhibitor. Gefitinib is an inhibitor of the epidermal growth factor receptor (EGFR) and has been extensively studied in nonsmall cell lung cancer. Thyroid cancer cells express high levels of EGFR on their surface and these levels have been associated with a more aggressive phenotype of DTC [Gorgoulis *et al.* 1992]. In addition, a phase I study of gefitinib [Fury *et al.* 2007] produced a PR in a patient with ATC. An open-label, phase II study of 27 patients was initiated [Pennell *et al.* 2008]. Patients with any histologic subtype were included, provided that there was evidence of locally advanced or metastatic disease which was not amenable to radioiodine and external beam radiotherapy. Patients were given gefitinib 250 mg daily which was continued until there was evidence of disease progression by RECIST or an unacceptable toxicity. There were 18 patients with DTC, five with ATC, and four with MTC. No patients had a complete response (CR) or PR. Median PFS for the DTC was 3.9 months; median overall survival (OS) was 27.4 months.

Of the 15 patients with detectable serum thyroglobulin (Tg), levels declined to <90% of baseline in 33% of patients. In four of the five patients whose Tg declined, these levels were clearly rising prior to initiation of therapy and seemed to correlate with radiographic reduction in tumor volume. Serum thyroglobulin also became appropriately elevated upon tumor progression,

indicating that this remains a useful marker of disease activity while taking gefitinib. Toxicities while on gefitinib were generally mild and included rash, diarrhea, nausea, and anorexia.

Median PFS for patients on this study was very short, indicating that patients who had SD had relatively short duration of response and that patients included in this trial had very aggressive clinical course. Genetic analysis of the EGFR in patients with nonsmall cell lung cancer who had a dramatic and rapid response to gefitinib reveals that these patients have specific mutations in the EGFR which are not present in patients who failed therapy [Lynch *et al.* 2004]. These findings suggest that certain mutations may confer susceptibility to the inhibition of EGFR signaling, and patients may need to be screened for these mutations to identify which tumors will benefit from gefitinib. It is not known whether the same is true for patients with thyroid cancer, but this question may warrant further investigation.

Histone deacetylase inhibitor

Histone deacetylases (HDAC) are enzymes which, working in concert with histone acetyltransferases, control the degree of acetylation of various histones and intracellular targets. The balance between these two opposing enzymes is essential to maintaining accessibility and function of transcription factors as well as structure of the nucleosome and chromatin [Mitsiades *et al.* 2005]. Dysregulation of HDAC has been associated with certain malignancies, as the cells have interference of the normal cell cycle and subsequently lose the ability to undergo normal differentiation. HDAC inhibitors are a class of molecules that repair this disequilibrium between acetylation and deacetylation and have favorable effects on cell cycle regulation, leading to apoptosis of the transformed neoplastic cells [Mitsiades *et al.* 2005]. In addition, these inhibitors have also been shown in cell culture to induce redifferentiation of cancer cells by restoring the function of the sodium-iodine symporter (NIS) [Zarnegar *et al.* 2002]. With this increased expression of NIS, it is postulated that the HDAC inhibitors may restore iodine uptake by the refractory thyroid tumors [Zarnegar *et al.* 2002].

Vorinostat, a small molecule inhibitor of HDAC, was found in a phase I study [Kelly *et al.* 2005] to yield a PR in a patient with refractory DTC. Consequently, our group undertook a NCI-sponsored phase II study [Woyach *et al.* 2009]

to examine specifically the cytotoxic effects in 16 patients with DTC. Patients were started on 200 mg orally twice daily and the dose was reduced for AEs as needed. The 3-week treatment cycle consisted of 2 weeks of therapy followed by 1 week off the medication. The medication was continued until there was evidence of disease progression by RECIST, or study withdrawal. The median duration of therapy was 4.2 months. No patients achieved a PR or CR, however. Nine patients (56%) had SD for a median duration of 6 months. Serum thyroglobulin levels did not correlate with objective response to therapy. Vorinostat's effects on radioiodine uptake were not evaluated in this study.

Redifferentiation agents

Peroxisomal proliferator-activated receptor gamma (PPAR γ) agonists have been shown to inhibit the growth of human thyroid carcinoma cells as well as increase the expression of thyroid-specific differentiation markers [Aiello *et al.* 2006]. A recent phase II trial [Kebebew *et al.* 2009] of 20 patients with DTC was undertaken to determine whether rosiglitazone increased uptake of radioiodine in previously resistant tumors. Patients with elevated thyroglobulin and negative post-treatment ^{131}I scans were given rosiglitazone 4 mg orally daily for 4 weeks followed by 8 mg daily for 7 weeks. After thyroid hormone withdrawal and a low-iodine diet, patients were given ^{131}I . Assessment of response was based on the presence of uptake on the post-treatment scan as well as thyroglobulin measurement up to a year after therapy. A PR was classified as either increased radioiodine uptake or a decreased thyroglobulin level. Five patients had uptake on the post-treatment scan. Unstimulated thyroglobulin levels after rosiglitazone treatment increased in five patients, remained stable in 12 patients, and decreased in three patients. Overall, 5 (25%) PR, 3 (15%) SD and 12 (60%) PD were noted. The authors suggested that despite some responses observed with this therapy, rosiglitazone did not result in clinically significant response on long-term follow up.

Another study [Tepmongkol *et al.* 2008] investigated the effects of 8 mg of rosiglitazone daily for 6 weeks followed by radioiodine therapy in 23 patients with a previously negative post-treatment whole body scan (WBS). This study also evaluated the immunostaining of the tumors for PPAR γ . Seven patients had strongly positive staining; of this group, five had a positive

post-treatment WBS. Of the nine patients with weakly staining PPAR γ , one had a positive post-treatment WBS. Accordingly, none of the seven patients with absent staining had a positive post-treatment WBS. Six months after treatment with RAI, serum Tg levels declined in only two patients; both of them were in the strongly-positive staining group. In summary, patients with strongly-positive staining for PPAR γ were more likely to convert to a positive post-treatment WBS, but less than half of those patients correspondingly had a reduction in serum thyroglobulin 6 months later. It is unclear, however, if this redifferentiation of the tumor translates into a meaningful clinical benefit or improved outcome for patients with refractory thyroid cancer.

Cyclooxygenase 2 (COX-2) inhibitor

COX-2 levels are overexpressed in many tumors and may promote growth and development of neoplastic tissue [Specht *et al.* 2002]. Immunohistochemical analysis reveals that COX-2 levels are significantly higher in PTC than in benign thyroid tissue [Lo *et al.* 2005]. Patients with familial adenomatous polyposis (FAP) have a highly favorable response to high doses of the COX-2 inhibitor, celecoxib, with significant reductions in colonic polyp formation [Steinbach *et al.* 2000]. This promising finding prompted further study to determine whether the antineoplastic response seen with FAP extends to patients with refractory thyroid cancer. A phase II trial in 32 patients with iodine-refractory, progressive DTC was undertaken by our group to assess the PFS at 1 year after treatment with celecoxib [Mrozek *et al.* 2006]. Patients were administered 400 mg twice daily. Patients were eligible based on measurable disease or elevated serum thyroglobulin. Responses were defined based on either RECIST or thyroglobulin levels. Immunohistochemical analysis of the tissues before and after therapy was also performed to determine the effect COX-2 inhibition on levels of expression of COX-2. The study was negative in that 23 patients had PD by 1 year or had to withdraw from the study as a result of AEs. One PR was noted per RECIST and thyroglobulin levels; and one patient was progression-free based on RECIST since thyroglobulin antibody prevented this marker measurement. Due to the small number of responders to the therapy, differences in COX-2 expression by immunohistochemical analysis were difficult to interpret.

Clinical trials in medullary thyroid carcinoma

Imatinib

Imatinib (Glivec[®]), the TKI that garnered much attention for its highly favorable effect on chronic myeloid leukemia, has inhibitory action against PDGFR- α and - β , KIT, and RET. Imatinib has been shown to reduce RET-mediated cell growth of MTC cells [de Groot *et al.* 2006]. As such, it has become an attractive agent for study in patients with progressive MTC. A recent phase II study [de Groot *et al.* 2007] was performed in 15 patients with disseminated MTC for 12 months (Table 2). Imatinib 600 mg once daily was administered with the possibility of increasing to 800 mg for those with PD. Patients were evaluated for response every 2 months with RECIST. No objective responses were seen, although four patients had SD over 24 months. Three patients discontinued the medication as a result of AEs and four others required dose reductions as a result of toxicities. An ongoing phase I/II trial is investigating the effects of imatinib in combination with dacarbazine and capecitabine in patients with advanced metastatic MTC.

Motesanib

In a multicenter, international, open-label phase II trial of motesanib in MTC, 91 patients with locally advanced or metastatic, progressive or symptomatic MTC received motesanib diphosphate 125 mg daily for up to 12 months or until unacceptable toxicity or disease progression [Schlumberger *et al.* 2009]. A majority of patients (84%) had sporadic MTC while 14% of patients had MTC in setting of familial MTC, or MEN-2A or MEN-2B. RET mutation in tumor tissue was found in 28 of 39 (72%) patients with sporadic MTC. Of the 91 patients studied, two patients (2%) achieved PRs and 44 (48%) had SD of >6 months duration (Table 2). The Kaplan–Meier estimate of median PFS was 12 months (95% CI, 10.7–14). Serum calcitonin was decreased >50% of baseline levels in 37% of patients. The most common treatment-related AEs were diarrhea (41%), fatigue (41%), hypertension (27%), anorexia (27%), and nausea (26%). Interestingly, plasma trough concentrations of motesanib were reduced compared with previous monotherapy studies at the same dose level, which may impact efficacy. Due to high frequency of RET mutation positivity in tumor tissues obtained from patients with sporadic

Table 2. Summary of phase II clinical trials including patients with medullary thyroid carcinoma (MTC).

Name of the drug tested	Key targets	Number of patients (sporadic/hereditary/unknown)	PR* n (%)	SD n (%)	SD >6 months n (%)	Duration of PR (months)	Median PFS (months)	Serum calcitonin response to baseline	Reference
Imatinib	PDGFR; KIT, RET	15 (11/4/0)	0	4 (27)	4 (27)	NA	NR	No correlation with objective response	(Ide Groot et al. 2007)
Motesanib	VEGFRs 1–3, PDGFR, RET, KIT	91 (76/13/2)	2 (2)	74 (81)	44 (48)	8 months and 5 months	12	37% of patients with >50% decrease	(Schlumberger et al. 2009)
Vandetanib	VEGFRs, RET, EGFR	30 (0/30/0)	6 (20)	9 (30)	9 (30)	NR	NR	19/30 patients with 50% decrease	(Wells et al. 2007)
Sorafenib	VEGFRs 1–3, PDGFR, RET BRAF	16 (16/0/0)	1 (6)	14 (87)	10 (62)	18	NR	>25% reduction in 68% of patients, did not correlate with objective response	(Lam et al. 2009)
XL-184	VEGFRs 1–3, c-MET, RET	22 (0/0/22)	12 (55)	NR	NR	NR	NR	16 (72%) patients with >40% decrease	(Kurzrock et al. 2008)

BRAF, V-raf murine sarcoma viral oncogene homolog B1; EGFR, epidermal growth factor receptor; PDGFR, platelet-derived growth factor receptor; PFS, progression-free survival; PR, partial response; SD, stable disease; VEGFR, vascular endothelial growth factor receptor. c-MET, RET and KIT are proto-oncogenes. NR, Not reported; NA, Not applicable.

MTC, statistical analysis for correlation between genotype and response could not be performed.

Sorafenib

Due to the critical role of constitutive Ret signaling in MTC, the anti-RET activity of this multi-kinase inhibitor makes it an attractive agent for targeting MTC. Sorafenib inhibited the growth of TT cells (C634R RET mutation positive MTC cell line) *in vitro* and *in vivo*, and inhibited wild type and mutant Ret kinase activity [Carlomagno et al. 2006]. Our group performed a NCI-sponsored phase II trial of sorafenib in patients with hereditary (n=5) and sporadic MTC (n=16) [Lam et al. 2009]. Patients were given sorafenib 400 mg orally twice a day and tumor markers and imaging studies were followed every 2 months. While accrual in the hereditary group is still ongoing, results for the sporadic group have been fully analyzed. Nine of the 12 (75%) sporadic MTC patients tested were positive for RET M918T mutation. Sorafenib showed a PR in 1 (6%) and SD of >6 months duration in 10 of 16 (62%) patients with sporadic MTC (Table 2). Serum calcitonin and carcinoembryonic antigen (CEA) levels declined by >25% in 62% and 44% of patients, respectively; these reductions did not correlate with objective response. The drug was generally well tolerated; there were two grade 4 AEs including a pulmonary embolus and hypokalemia. Common AEs which consisted of hypertension, HFSR, diarrhea, joint pain, infections, hyponatremia, and thrombocytopenia.

Vandetanib

Vandetanib (ZD6474) is an orally active TKI with multiple targets including RET, EGFR and VEGFR. MTC cells treated with vandetanib lose their proliferative autonomy conferred by RET/PTC3 mutations [Carlomagno et al. 2002]. Likewise, vandetanib inhibits EGFR-mediated cellular proliferation and survival [Carlomagno et al. 2002] and blocks VEGFR-mediated angiogenesis [Hoffmann et al. 2006a]. This multi-targeted approach has made vandetanib an attractive agent for evaluation in multiple types of malignancy, including nonsmall cell lung cancer and MTC. In a multi-institutional phase II trial [Wells et al. 2007] of vandetanib in patients with hereditary MTC, 30 patients were treated with 300 mg once daily. Patients with hereditary MTC had measurable disease which was unresectable, locally advanced, or metastatic. Responses were assessed using RECIST every

3 months. Six patients (20%) achieved a PR; nine (30%) had SD after a median of 5.7 months of treatment (Table 2). Plasma calcitonin levels declined by 50% for at least 6 weeks in 19 patients (63%). Adverse events included rash, diarrhea, fatigue, and nausea. Because of the promising initial results, an international, randomized, placebo-controlled phase III trial has recently completed its accrual of patients with hereditary and sporadic MTC.

XL-184

XL-184 is a TKI with activity against VEGFR-2, MET, and RET. It has potent effects on blocking cellular proliferation and migration. In a phase I study that included 13 patients with MTC, three (23%) had a PR; seven (54%) patients had SD. All 13 patients had significant reductions in serum calcitonin and CEA levels. Based on the encouraging initial results, the trial was expanded to include additional patients with MTC in a phase II, dose-fixed schedule. At the time of the preliminary report, 22 of 36 patients with MTC had response assessment performed at the 3-month timepoint [Kurzrock *et al.* 2008]. Twelve of 22 (55%) patients achieved PR and 16 (72%) of patients had >40% decline in plasma calcitonin levels (Table 2). In a small subset of patients with prior TKI therapy such as vandetanib or sorafenib, XL-184 induced PRs. This vigorous response has resulted in the conduction of an international multi-institutional, randomized, double-blinded, placebo-controlled Phase III study of XL-184.

Clinical trials in anaplastic thyroid carcinoma

Anaplastic thyroid carcinoma is a nearly-uniformly lethal malignancy with a median survival of less than 6 months [Neff *et al.* 2008]. Traditional cytotoxic chemotherapies have been highly toxic and largely ineffective at prolonging survival in this challenging group of patients. The advent of targeted molecular therapies has brought new hope for finding a more effective treatment for a condition that has had little, if any, reason for optimism to this point. All patients with ATC should be considered for entry into a clinical trial [Cooper *et al.* 2006]. There are few studies with significant numbers of patients enrolled, due to the rarity of this tumor. The results of recent phase II trials of targeted molecular therapy that focuses specifically on patients with ATC are outlined below.

Combretastatin A4 phosphate

Combretastatin A4 phosphate (CA4P) is a tubulin-binding compound isolated from the bark of the African bush willow tree. It is a vascular disrupting agent, resulting in reduced central blood flow to the tumor. Though the exact mechanism is unknown, there is evidence suggesting it disrupts cell–cell adhesion in vascular endothelium by destabilizing microtubules. The consequence of this disordered endothelial cell architecture is vascular collapse. In phase I studies, the effects of this agent were greatest in tumors with the highest vascularity; as such, thyroid cancer is a prime target. In a phase I study, a single patient with ATC had a durable CR of more than 9 years with CA4P monotherapy [Dowlati *et al.* 2002].

A phase II trial in 26 patients with ATC was recently reported [Mooney *et al.* 2009]. The primary endpoint was doubling of overall survival. Patients were administered 45 mg/m² CA4P as a 10-minute infusion on days 1, 8, and 15 of a 28-day cycle. No objective responses were seen and doubling of survival time was not observed. Median survival was 4.7 months; in seven patients median duration of SD was 12.3 months (Table 3). Soluble intracellular adhesion molecule-1 (sICAM), an endothelial cell-specific marker, levels were measured at baseline and at several time points on the study. It was predicted that vascular endothelial cell damage and apoptosis would lead to release of sICAM. Low baseline sICAM levels were predictive of survival. Although the study did not achieve its primary endpoint, the authors recommend further study of CA4P in combination with other agents as a more effective method of controlling disease progression in patients with ATC. Currently, this agent in combination with cytotoxic chemotherapy is being tested in clinical trial in patients with ATC.

Imatinib

A preclinical study of imatinib showed efficacy in inhibiting growth of ATC cell lines [Podtcheko *et al.* 2003]. Although the molecular target of this agent is not clearly defined [Mitsiades *et al.* 2003; Podtcheko *et al.* 2003], proposed mechanisms include inhibition of PDGF, KIT, and c-ABL. A single-institution study of imatinib 400 mg twice daily orally in 11 patients with ATC was recently reported [Ha *et al.* 2009]. Of the eight evaluable patients, two had a PR, four had SD, and two had PD. Six month PFS was 27%; 6 month survival

Table 3. Summary of clinical trials including patients with anaplastic thyroid carcinoma (ATC).

Name of the drug tested	Key targets	Number of patients	PR <i>n</i> (%)	SD <i>n</i> (%)	SD >6 months <i>n</i> (%)	Median duration of SD and PR (months)	Median survival (months)	Reference
Combretastatin A4 phosphate	Tubulin binding;	26	0	7 (27)	NR	12.3	4.7	Mooney <i>et al.</i> 2009
Imatinib	vascular disruption PDGF- α , - β ; KIT, RET	11	2 (18)	4 (36)	3 (27)	NR	6-month survival 46%	Ha <i>et al.</i> 2009
Sorafenib	VEGFR 1–3, BRAF, PDGF, RET	16	2 (13)	4 (27)	NR	5	3.5	Nagaiah <i>et al.</i> 2009

BRAF, V-raf murine sarcoma viral oncogene homolog B1; PDGF, platelet-derived growth factor; PR, partial response; SD, stable disease; VEGFR, vascular endothelial growth factor receptor. NR, Not reported. RET and KIT are proto-oncogenes.

was 46% (Table 3). Frequent toxicities included lymphopenia, edema, anemia, and hyponatremia. Because of poor accrual, however, the trial was prematurely terminated.

Sorafenib

BRAF mutations have been observed in 10–35% of patients with ATC [Kondo *et al.* 2006]. Multikinase inhibitor sorafenib targets BRAF kinase in addition to its angiogenic targets. Sorafenib has shown efficacy in inhibiting cell proliferation in ATC cell lines while reducing tumor growth and angiogenesis in orthotopic ATC xenografts [Kim *et al.* 2007]. Further, sorafenib improved survival of the test animals [Kim *et al.* 2007]. A phase II trial of sorafenib for patients with ATC is actively enrolling patients [Nagaiah *et al.* 2009]. The dosing schedule is 400 mg twice daily orally on a 28-day cycle. Thus far, 16 patients have been studied. Median time on the study is 2 months. Of the 15 evaluable patients, two (13%) had a PR, four (27%) had SD; median duration of PR/SD is 5 months. Median time to progression is 1.5 months and median survival is 3.5 months (Table 3). Since sorafenib demonstrates objective tumor response in patients with ATC, accrual for this study continues.

Discussion

Targeted therapies offer the promise of setting new standards for the future for patients with iodine-resistant DTC and MTC. For example, off-label use of sorafenib in patients with advanced iodine-refractory DTC and MTC has been recently endorsed by expert panel of National Comprehensive Cancer Network (NCCN). While significant progress has been

made in understanding some of the mechanisms underlying tumorigenesis and in translating that knowledge into various treatment modalities, numerous challenges remain in testing targeted therapies against refractory thyroid cancer. First, selecting a primary endpoint for phase II and III trials is difficult. Because of the cytostatic nature of targeted therapies, stabilization of cancer is anticipated as a beneficial effect of such therapies. However, due to the slow-growing pattern of a subset of thyroid cancers, even in advanced stages, attributing disease stabilization to the targeted therapy is difficult. Thus, objective responses using RECIST or PFS as an endpoint in phase II trials or overall survival as an endpoint in a phase III trial may not be optimal. Likewise, many of the studies are measuring serum levels of thyroglobulin, calcitonin, or CEA to determine if these biomarkers may be used as an additional tool to evaluate response to therapy. As seen in the studies above, however, these markers are only variably useful and may not be a reliable indicator of disease responsiveness. Many of the tumors in these studies are dedifferentiated, and as such, do not produce significant quantities of tumor markers. Further study is needed, as well, to understand the relationship between targeted molecular therapies and their direct effects on the synthesis or secretion of tumor marker proteins.

The second challenge is selecting appropriate patients for phase II and III clinical trials. An argument can be made to restrict eligibility of patients into clinical trials to those with PD in the 6- or 12- months prior study entry so that attribution of SD as an objective response to targeted therapy may be interpretable.

Furthermore, patients with an overall indolent cancer may be spared the toxicities of targeted therapies. A significant limitation of this approach, however, is that patients diagnosed at an advanced stage with severe or symptomatic tumor burden who desperately need therapy may not be eligible for the trials due to inability to prove PD at the study entry.

The third complexity which needs to be addressed when designing future clinical trials is whether to select tumor type based on histology or based on genotype. For example, when testing sorafenib, which targets the common genetic defects of RET proto-oncogene in PTC and MTC, should the trial be conducted only in PTC and MTC patients that have RET mutations? Or should separate trials be conducted for PTC and MTC regardless of the type of genetic aberration, with the assumption that the true target of sorafenib is unknown and that targeting VEGFRs in either PTC or MTC regardless of the RET gene aberration may be sufficient?

The fourth obstacle facing clinicians when utilizing TKIs in thyroid cancer patients is selecting appropriate response assessment criteria. RECIST is not applicable to many thyroid cancer patients due to bony metastases, subcentimeter bilateral multiple lung metastases, and calcifications in the tumor. Therefore, the current mechanism for assessing a response to therapy is imperfect for thyroid cancer patients.

An additional challenge facing clinicians is monitoring compliance and safety of oral targeted therapies that are used on a chronic basis. Patients may not report toxicities due to concerns of being taken off the trial therapy that may be their only treatment option. Careful patient education is vital to maintaining compliance and to ensuring that the medications are not causing untoward effects. It should be explained to the patients that early reporting of the AEs will ensure proper treatment and may actually reduce the likelihood of discontinuation of the medication. Likewise, clinicians require education regarding the safety of these medications. The oral targeted molecular therapies have been viewed as safe and simple in comparison to the traditional parenteral chemotherapies. However, these agents are not without serious toxicities. In fact, a significant proportion of patients in the trials discussed above prematurely terminated their participation as a result of AEs

[Cohen *et al.* 2008; Gupta-Abramson *et al.* 2008; Kloos *et al.* 2009]. Those patients who remained in the studies, however, also reported noteworthy side effects. Across all of these studies fatigue, diarrhea, and nausea were a common theme, although generally mild, grade 1 or 2. It should be noted, as well, that these medications are typically continued as long as an effect is seen, in some cases as long as several years. In these cases, the cumulative effect of many months with 'mild' AEs should not be overlooked. Furthermore, uncommon but serious AEs such as bowel perforation, bleeding and thrombosis have been attributed to the class of targeted therapies that target VEGFRs. This is particularly important with thyroid cancer as the majority of patients are asymptomatic when off treatment, even with progressive metastatic disease. The clinician must discuss with the patient the distinct possibility of side effects before initiating a targeted molecular therapy; the benefit of halting disease progression should outweigh the risk of toxicities.

Another issue that requires attention is prioritizing which type of targeted agent merits further testing. Due to the relatively small pool of patients with advanced thyroid cancer, as compared to patients with lung cancers or breast cancers, we must prioritize testing of targeted agents based on their mechanism of action. It may not be efficient or wise to test several compounds that target a similar spectrum of tyrosine kinases, because we may miss an opportunity to examine a distinct class of drugs due to a paucity of patients.

It should be noted that the above-mentioned considerations do not necessarily apply to patients with ATC. Because of the rapidly progressive nature and dismal prognosis of the disease, it is currently recommended that all patients be considered for entry into a clinical trial upon diagnosis [Cooper *et al.* 2006]. These targeted agents offer a glimmer of hope for patients who otherwise have little to none. However, targeted therapies alone may not be sufficient to induce durable responses for this very aggressive cancer and the combination of cytotoxic chemotherapy along with targeted therapies (based on preclinical data) will need to be tested. With a cause-specific mortality of 69% at 6 months and 80% at 1 year [Kebebew *et al.* 2005], overall survival is a desirable endpoint. Because it is such a rare tumor, enrolling

sufficient numbers of patients to power a study focusing on ATC alone is difficult and would require a multi-institutional trial.

In spite of these challenges and limitations, this remains an exciting time in the management of patients with refractory thyroid cancer. Tremendous progress has been made in understanding the mechanisms of tumor formation and progression. New agents are being investigated for their efficacy in thyroid cancers with successful collaborations between academia, industry and government agencies at national and international levels. The dramatic increase in the number of available phase II clinical trials and the development of phase III clinical trials are critical milestones in development of targeted therapies for advanced thyroid cancer. No longer is refractory thyroid cancer a diagnosis associated with supportive care. The explosion of knowledge gives researchers, clinicians and patients hope that a targeted therapy will improve quality of life and survival.

Conflict of interest statement

None declared.

References

- Aiello, A., Pandini, G., Frasca, F., Conte, E., Murabito, A., Sacco, A. *et al.* (2006) Peroxisomal proliferator-activated receptor-gamma agonists induce partial reversion of epithelial-mesenchymal transition in anaplastic thyroid cancer cells. *Endocrinology* 147: 4463–4475.
- Akslen, L.A. and Livolsi, V.A. (2000) Increased angiogenesis in papillary thyroid carcinoma but lack of prognostic importance. *Hum Pathol* 31: 439–442.
- Argiris, A., Agarwala, S.S., Karamouzis, M.V., Burmeister, L.A. and Carty, S.E. (2008) A phase II trial of doxorubicin and interferon alpha 2b in advanced, non-medullary thyroid cancer. *Invest New Drugs* 26: 183–188.
- Carlomagno, F., Vitagliano, D., Guida, T., Napolitano, M., Vecchio, G., Fusco, A. *et al.* (2002) The kinase inhibitor PP1 blocks tumorigenesis induced by RET oncogenes. *Cancer Res* 62: 1077–1082.
- Carlomagno, F., Anaganti, S., Guida, T., Salvatore, G., Troncone, G., Wilhelm, S.M. *et al.* (2006) BAY 43-9006 inhibition of oncogenic RET mutants. *J Natl Cancer Inst* 98: 326–334.
- Chen, K.T., Lin, J.D., Liou, M.J., Weng, H.F., Chang, C.A. and Chan, E.C. (2006) An aberrant autocrine activation of the platelet-derived growth factor alpha-receptor in follicular and papillary thyroid carcinoma cell lines. *Cancer Lett* 231: 192–205.
- Chow, S.M., Law, S.C., Mendenhall, W.M., Au, S.K., Chan, P.T., Leung, T.W. *et al.* (2002) Papillary thyroid carcinoma: prognostic factors and the role of radioiodine and external radiotherapy. *Int J Radiat Oncol Biol Phys* 52: 784–795.
- Cohen, E.E., Rosen, L.S., Vokes, E.E., Kies, M.S., Forastiere, A.A., Worden, F.P. *et al.* (2008) Axitinib is an active treatment for all histologic subtypes of advanced thyroid cancer: results from a phase II study. *J Clin Oncol* 26: 4708–4713.
- Cooper, D.S., Doherty, G.M., Haugen, B.R., Kloos, R.T., Lee, S.L., Mandel, S.J. *et al.* (2006) Management guidelines for patients with thyroid nodules and differentiated thyroid cancer. *Thyroid* 16: 109–141.
- Davies, L. and Welch, H.G. (2006) Increasing incidence of thyroid cancer in the United States, 1973–2002. *JAMA* 295: 2164–2167.
- de Groot, J.W., Plaza, M., Schepers, I., Drenth-Diephuis, H., Osinga, L.J., Plukker, J.J.T. *et al.* (2006) Cellular effects of imatinib on medullary thyroid cancer cells harboring multiple endocrine neoplasia type 2A and 2B associated RET mutations. *Surgery* 139: 806–814.
- de Groot, J.W., Zonnenberg, B.A., Quarles, U.-M., de Vries, M.M., Links, T.P., Lips, C.J. *et al.* (2007) A phase-II trial of imatinib therapy for metastatic medullary thyroid carcinoma. *J Clin Endocrinol Metab* 92: 3466–3469.
- Dowlati, A., Robertson, K., Cooney, M., Petros, W.P., Stratford, M., Jesberger, J. *et al.* (2002) A phase I pharmacokinetic and translational study of the novel vascular targeting agent combretastatin a-4 phosphate on a single-dose intravenous schedule in patients with advanced cancer. *Cancer Res* 62: 3408–3416.
- Droz, J.P., Schlumberger, M., Rougier, P., Ghosn, M., Gardet, P. and Parmentier, C. (1990) Chemotherapy in metastatic nonanaplastic thyroid cancer: experience at the Institut Gustave-Roussy. *Tumori* 76: 480–483.
- Elisei, R., Cosci, B., Romei, C., Bottici, V., Renzini, G., Molinaro, E. *et al.* (2007) Prognostic significance of somatic RET oncogene mutations in sporadic medullary thyroid cancer: a 10 years follow up study. *J Clin Endocrinol Metab* 93: 682–687.
- Enewold, L., Zhu, K., Ron, E., Marrogi, A.J., Stojadinovic, A., Peoples, G.E. *et al.* (2009) Rising thyroid cancer incidence in the United States by demographic and tumor characteristics, 1980–2005. *Cancer Epidemiol Biomarkers Prev* 18: 784–791.
- Eng, C., Clayton, D., Schufenecker, I., Lenoir, G., Cote, G., Gagel, R.F. *et al.* (1996) The relationship between specific RET proto-oncogene mutations and disease phenotype in multiple endocrine neoplasia type 2: international RET mutation consortium analysis. *JAMA* 276: 1575–1579.

- Fury, M.G., Solit, D.B., Su, Y.B., Rosen, N., Sirotinak, F.M., Smith, R.P. *et al.* (2007) A phase I trial of intermittent high-dose gefitinib and fixed-dose docetaxel in patients with advanced solid tumors. *Cancer Chemother Pharmacol* 59: 467–475.
- Gimm, O., Neuberg, D.S., Marsh, D.J., Dahia, P.L.M., Cuong, H.V., Raue, F. *et al.* (1999) Over-representation of a germline *RET* sequence variant in patients with sporadic medullary thyroid carcinoma and somatic *RET* codon 918 mutation. *Oncogene* 18: 1369–1373.
- Gorgoulis, V., Aninos, D., Priftis, C., Evagelopoulou, C., Karameris, A., Kanavaros, P. *et al.* (1992) Expression of epidermal growth factor, transforming growth factor- α and epidermal growth factor receptor in thyroid tumors. *In Vivo* 6: 291–296.
- Gupta-Abramson, V., Troxel, A.B., Nellore, A., Puttaswamy, K., Redlinger, M., Ransone, K. *et al.* (2008) Phase II trial of sorafenib in advanced thyroid cancer. *J Clin Oncol* 26: 4714–4719.
- Ha, H.T., Lee, J.S., Urba, S., Koenig, R.J., Sisson, J., Giordano, T. *et al.* (2009) Phase II trial evaluating imatinib (I) in patients (pts) with anaplastic thyroid carcinoma (ATC). *J Clin Oncol* 27: Abstract 6057.
- Hayat, M.J., Howlader, N., Reichman, M.E. and Edwards, B.K. (2007) Cancer statistics, trends, and multiple primary cancer analyses from the Surveillance, Epidemiology, and End Results (SEER) Program. *Oncologist* 12: 20–37.
- Hoffmann, S., Glaser, S., Wunderlich, A., Lingelbach, S., Dietrich, C., Burchert, A. *et al.* (2006a) Targeting the EGF/VEGF-R system by tyrosine-kinase inhibitors—a novel antiproliferative/antiangiogenic strategy in thyroid cancer. *Langenbecks Arch Surg* 391: 589–596.
- Hoffmann, S., Wunderlich, A., Celik, I., Maschuw, K., Hassan, I., Hofbauer, L.C. *et al.* (2006b) Paneling human thyroid cancer cell lines for candidate proteins for targeted anti-angiogenic therapy. *J Cell Biochem* 98: 954–965.
- Jhiang, S.M., Sagartz, J.E., Tong, Q., Parker-Thornburg, J., Capen, C.C., Cho, J.Y. *et al.* (1996) Targeted expression of the *ret/PTC1* oncogene induces papillary thyroid carcinomas. *Endocrinology* 137: 375–378.
- Kamba, T., Tam, B.Y., Hashizume, H., Haskell, A., Sennino, B., Mancuso, M.R. *et al.* (2006) VEGF-dependent plasticity of fenestrated capillaries in the normal adult microvasculature. *Am J Physiol Heart Circ Physiol* 290: H560–H576.
- Kebebew, E., Greenspan, F.S., Clark, O.H., Woeber, K.A. and McMillan, A. (2005) Anaplastic thyroid carcinoma. Treatment outcome and prognostic factors. *Cancer* 103: 1330–1335.
- Kebebew, E., Lindsay, S., Clark, O., Woeber, K.A., Hawkins, R. *et al.* (2009) Results of rosiglitazone therapy in patients with thyroglobulin-positive and radioiodine-negative advanced differentiated thyroid cancer. *Thyroid* 19: 953–956.
- Kelly, W.K., O'Connor, O.A., Krug, L.M., Chiao, J.H., Heaney, M., Curley, T. *et al.* (2005) Phase I study of an oral histone deacetylase inhibitor, suberoylanilide hydroxamic acid, in patients with advanced cancer. *J Clin Oncol* 23: 3923–3931.
- Kim, S., Yazici, Y.D., Calzada, G., Wang, Z.Y., Younes, M.N., Jasser, S.A. *et al.* (2007) Sorafenib inhibits the angiogenesis and growth of orthotopic anaplastic thyroid carcinoma xenografts in nude mice. *Mol Cancer Ther* 6: 1785–1792.
- Kimura, E.T., Nikiforova, M.N., Zhu, Z., Knauf, J.A., Nikiforov, Y.E. and Fagin, J.A. (2003) High prevalence of BRAF mutations in thyroid cancer: genetic evidence for constitutive activation of the RET/PTC-RAS-BRAF signaling pathway in papillary thyroid carcinoma. *Cancer Res* 63: 1454–1457.
- Klein, M., Picard, E., Vignaud, J.M., Marie, B., Bresler, L., Toussaint, B. *et al.* (1999) Vascular endothelial growth factor gene and protein: strong expression in thyroiditis and thyroid carcinoma. *J Endocrinol* 161: 41–49.
- Kloos, R.T., Ringel, M.D., Knopp, M.V., Hall, N.C., King, M., Stevens, R. *et al.* (2009) Phase II trial of sorafenib in metastatic thyroid cancer. *J Clin Oncol* 27: 1675–1684.
- Kolaric, K., Maricic, Z., Nola, P. and Krusic, J. (1977) Modified administration schedule of adriamycin in solid tumors. *Z Krebsforsch Klin Onkol Cancer Res Clin Oncol* 88: 255–260.
- Kondo, T., Ezzat, S. and Asa, S.L. (2006) Pathogenetic mechanisms in thyroid follicular-cell neoplasia. *Nat Rev Cancer* 6: 292–306.
- Kurzrock, R., Sherman, S., Hong, D., Ng, C., Frye, J., Janisch, L. *et al.* (2008) A phase 1 study of XL184, a MET, VEGFR2, and RET kinase inhibitor, administered orally to patients (pts) with advanced malignancies, including a subgroup of pts with medullary thyroid cancer (MTC). The AACR-NCI-EORTC International Conference, Molecular Targets and Cancer Therapeutics, Discovery, Biology, and Clinical Applications October 21–24, 2008, Geneva, Switzerland.
- Lam, E.T., Ringel, M.D., Kloos, R.T., Collamore, M., Arbogast, D., Liang, J. *et al.* (2009) A phase II study of sorafenib in patients with metastatic medullary thyroid carcinoma. 100th AACR Annual meeting, Abstract 4513, April 18–22, San Diego, CA.
- Lo, C.Y., Lam, K.Y., Leung, P.P. and Luk, J.M. (2005) High prevalence of cyclooxygenase 2 expression in papillary thyroid carcinoma. *Eur J Endocrinol* 152: 545–550.
- Lynch, T.J., Bell, D.W., Sordella, R., Gurubhagavatula, S., Okimoto, R.A., Brannigan, B.W. *et al.* (2004) Activating mutations in the epidermal growth factor receptor underlying responsiveness of

- non-small-cell lung cancer to gefitinib. *N Engl J Med* 350: 2129–2139.
- Mitsiades, C.S., Sykoutri, D., McMullan, C., Poulaki, V. and Mitsiades, N. (2003) Effect of imatinib mesylate (Gleevec) on anaplastic thyroid carcinoma cell lines. *J Clin Endocrinol Metab* 88: 5043–5044.
- Mitsiades, C.S., Poulaki, V., McMullan, C., Negri, J., Fanourakis, G., Goudopoulou, A. *et al.* (2005) Novel histone deacetylase inhibitors in the treatment of thyroid cancer. *Clin Cancer Res* 11: 3958–3965.
- Mooney, C.J., Nagaiah, G., Fu, P., Wasman, J.K., Cooney, M.M., Savvides, P.S. *et al.* (2009) A phase II trial of fosbretabulin in advanced anaplastic thyroid carcinoma and correlation of baseline serum-soluble intracellular adhesion molecule-1 with outcome. *Thyroid* 19: 233–240.
- Mrozek, E., Kloos, R.T., Ringel, M.D., Kresty, L., Snider, P., Arbogast, D. *et al.* (2006) Phase II study of celecoxib in metastatic differentiated thyroid carcinoma. *J Clin Endocrinol Metab* 91: 2201–2204.
- Nagaiah, G., Fu, P., Wasman, J.K., Cooney, M.M., Mooney, C., Afshin, D. *et al.* (2009) Phase II trial of sorafenib (bay 43-9006) in patients with advanced anaplastic carcinoma of the thyroid (ATC). *J Clin Oncol* 27: Abstract 6058.
- Neff, R.L., Farrar, W.B., Kloos, R.T. and Burman, K.D. (2008) Anaplastic thyroid cancer. *Endocrinol Metab Clin North Am* 37: 525–538, xi.
- Pennell, N.A., Daniels, G.H., Haddad, R.I., Ross, D.S., Evans, T., Wirth, L.J. *et al.* (2008) A phase II study of gefitinib in patients with advanced thyroid cancer. *Thyroid* 18: 317–323.
- Podtcheko, A., Ohtsuru, A., Tsuda, S., Namba, H., Saenko, V., Nakashima, M. *et al.* (2003) The selective tyrosine kinase inhibitor, STI571, inhibits growth of anaplastic thyroid cancer cells. *J Clin Endocrinol Metab* 88: 1889–1896.
- Rosen, L.S., Kurzrock, R., Mulay, M., Van Vugt, A., Purdom, M., Ng, C. *et al.* (2007) Safety, pharmacokinetics, and efficacy of AMG 706, an oral multikinase inhibitor, in patients with advanced solid tumors. *J Clin Oncol* 25: 2369–2376.
- Rugo, H.S., Herbst, R.S., Liu, G., Park, J.W., Kies, M.S., Steinfeldt, H.M. *et al.* (2005) Phase I trial of the oral antiangiogenesis agent AG-013736 in patients with advanced solid tumors: pharmacokinetic and clinical results. *J Clin Oncol* 23: 5474–5483.
- Scherubl, H., Raue, F. and Ziegler, R. (1990) Combination chemotherapy of advanced medullary and differentiated thyroid cancer. Phase II study. *J Cancer Res Clin Oncol* 116: 21–23.
- Schlumberger, M.J., Elisei, R., Bastholt, L., Wirth, L.J., Martins, R.G., Locati, L.D. *et al.* (2009) Phase II study of safety and efficacy of motesanib in patients with progressive or symptomatic, advanced or metastatic medullary thyroid cancer. *J Clin Oncol* 27: 3794–3801.
- Sherman, I.S., Wirth, L.J., Droz, J.P., Hofmann, M., Bastholt, L., Martins, R.G. *et al.* (2008) Motesanib diphosphate in progressive differentiated thyroid cancer. *N Engl J Med* 359: 31–42.
- Soh, E.Y., Eigelberger, M.S., Kim, K.J., Wong, M.G., Young, D.M., Clark, O.H. *et al.* (2000) Neutralizing vascular endothelial growth factor activity inhibits thyroid cancer growth *in vivo*. *Surgery* 128: 1059–1065.
- Specht, M.C., Tucker, O.N., Hovever, M., Gonzalez, D., Teng, L. and Fahey III, T.J. (2002) Cyclooxygenase-2 expression in thyroid nodules. *J Clin Endocrinol Metab* 87: 358–363.
- Steinbach, G., Lynch, P.M., Phillips, R.K., Wallace, M.H., Hawk, E., Gordon, G.B. *et al.* (2000) The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. *N Engl J Med* 342: 1946–1952.
- Tepmongkol, S., Keelawat, S., Honsawek, S. and Ruangvejvorachai, P. (2008) Rosiglitazone effect on radioiodine uptake in thyroid carcinoma patients with high thyroglobulin but negative total body scan: a correlation with the expression of peroxisome proliferator-activated receptor-gamma. *Thyroid* 18: 697–704.
- Viglietto, G., Maglione, D., Rambaldi, M., Cerutti, J., Romano, A., Trapasso, F. *et al.* (1995) Upregulation of vascular endothelial growth factor (VEGF) and downregulation of placenta growth factor (PlGF) associated with malignancy in human thyroid tumors and cell lines. *Oncogene* 11: 1569–1579.
- Wells Jr, S.A., Gosnell, J.E., Gagel, R.F., Moley, J.F., Pfister, D.G., Sosa, J.A. *et al.* (2007) Vandetanib in metastatic hereditary medullary thyroid cancer: follow-up results of an open-label phase II trial. *J Clin Oncol* 25: Abstract 6018.
- Williams, S.D., Birch, R. and Einhorn, L.H. (1986) Phase II evaluation of doxorubicin plus cisplatin in advanced thyroid cancer: a Southeastern Cancer Study Group Trial. *Cancer Treat Rep* 70: 405–407.
- Woyach, J.A., Kloos, R.T., Ringel, M.D., Arbogast, D., Collamore, M., Zwiebel, J.A. *et al.* (2009) Lack of therapeutic effect of the histone deacetylase inhibitor vorinostat in patients with metastatic radioiodine-refractory thyroid carcinoma. *J Clin Endocrinol Metab* 94: 164–170.
- Zarnegar, R., Brunaud, L., Kanauchi, H., Wong, M., Fung, M., Ginzinger, D. *et al.* (2002) Increasing the effectiveness of radioactive iodine therapy in the treatment of thyroid cancer using Trichostatin A, a histone deacetylase inhibitor. *Surgery* 132: 984–990.