Thyroid cancer: pathogenesis and targeted therapy

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Abstract: Therapeutic options for advanced, unresectable radioiodine-resistant thyroid cancers have historically been limited. Recent progress in understanding the pathogenesis of the various subtypes of thyroid cancer has led to increased interest in the development of targeted therapies, with potential strategies including angiogenesis inhibition, inhibition of aberrant intracellular signaling in the MAPK and PI3K/AKT/mTOR pathways, radioimmunotherapy, and redifferentiation agents. On the basis of a recent positive phase III clinical trial, the RET, vascular endothelial growth factor receptor (VEGFR), and epidermal growth factor receptor (EGFR) inhibitor vandetanib has received FDA approval as of April 2011 for use in the treatment of advanced medullary thyroid cancer. Several other recent phase II clinical trials in advanced thyroid cancer have demonstrated significant activity, and multiple other promising therapeutic strategies are in earlier phases of clinical development. The recent progress in targeted therapy is already revolutionizing management paradigms for advanced thyroid cancer, and will likely continue to dramatically expand treatment options in the coming years.

Keywords: Thyroid cancer, molecular pathophysiology, novel therapies, kinase inhibitors, clinical trials

Introduction

Thyroid cancer is the most common endocrine neoplasm, and it is estimated that 44,670 new diagnoses of thyroid cancer (10,740 men and 33,930 women) were made in the USA in 2010 [Altekruse *et al.* 2010]. This represents nearly a 2.5-fold increase since the early 1970s [Davies and Welch, 2006]. The reasons for the increased incidence are unclear, with potential explanations including increased screening, more widespread diagnostic testing of asymptomatic thyroid nodules, changing demographics, and changing environmental risk factors. Regardless, the prognosis is generally considered favorable for individuals with localized disease, as most of these patients are cured with surgical resection. However, therapeutic options have historically been limited for patients with surgically unresectable, radioiodine-resistant disease. An estimated 1690 individuals died of thyroid cancer in 2010 [Altekruse *et al.* 2010]. Prognosis is closely associated with stage at diagnosis, 5-year survival being 99% for patients with localized disease compared with 58% for patients with distant metastatic cancer.

Thyroid cancers can be classified on the basis of histology into differentiated thyroid cancers (DTCs), including papillary (PTCs), follicular (FTCs), or Hürthle cell thyroid cancers (HTCs), medullary thyroid cancers (MTCs), and anaplastic thyroid cancers (ATCs). DTCs are derived from thyroid follicular cells and are by far the most common subtype, accounting for over 90% of all newly diagnosed cases [Davies and Welch, 2006]. In patients with localized disease, the standard of care for DTC is surgical resection, thyroid stimulating hormone (TSH) suppression with levothyroxine, and consideration for adjuvant radioiodine or external beam radiation therapy. Outcomes are generally favorable, but up to 30% of patients will relapse after initial treatment with curative intent and 15% of these will die of their disease [Mazzaferrri, 1999]. MTCs are derived from the parafollicular thyroid C cells and account for 2–8% of all thyroid cancers [Pacini *et al.* 2010; Ball *et al.* 2007]. They are intrinsically radioiodine-resistant, and patients with localized disease are typically treated with surgical resection with or without adjuvant

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external beam radiation therapy. Despite aggressive surgical therapy, nearly 50% of patients diagnosed with localized MTC and a palpable neck mass will have a local or distant recurrence [Moo-Young *et al.* 2009]. Median survival in patients with metastatic MTC is approximately 1 year [Pacini *et al.* 2010]. ATCs account for roughly 1.7% of all thyroid cancers in the USA [Hundahl *et al.* 1998] and are believed to arise from DTCs by the accumulation of genetic abnormalities that result in dedifferentiation and an aggressive phenotype. Despite intensive treatment with multimodal therapy, including surgery, cytotoxic chemotherapy, and external beam radiation therapy, outcomes remain poor, with a median survival of approximately 5 months and 1-year survival of around 20% [Smallridge and Copland, 2010; Kebebew *et al.* 2005]. Results with cytotoxic chemotherapy for all subtypes of thyroid cancer have been generally disappointing. Most regimens have used doxorubicin or cisplatin, singly or in combination, and though inferences are limited by study design, reported response rates have been in the range of 0–22% [Droz *et al.* 1990; Williams *et al.* 1986; Shimaoka *et al.* 1985]. This has led to a quest for improved therapeutic options for patients with unresectable radioiodine-refractory DTC, unresectable MTC, and all cases of ATC.

Molecular pathophysiology

Molecular studies have led to increased appreciation of the heterogeneity of thyroid neoplasms, with hereditary predisposition, somatic mutation, and epigenetic modulation all contributing to tumor behavior. However, despite the heterogeneity, there are several recurring mechanisms of tumorigenesis that have been identified in the various types of thyroid cancer.

VEGF-associated neovascularization

Thyroid cancers have long been recognized as highly vascular tumors, and in DTC tumor vascularity has been correlated with overall disease-free survival [Dhar *et al.* 1998]. Vascular endothelial growth factor (VEGF) and the associated VEGF-specific receptor tyrosine kinases have been implicated as key molecular mediators of tumor neovascularization. Preclinical studies have demonstrated that thyroid neoplasms have increased expression of VEGF compared with normal thyroid tissue [Capp *et al.* 2010; Soh *et al.* 1997, 1996; Viglietto *et al.* 1995]. Serum VEGF levels have been shown to be elevated in patients with metastatic DTC compared with

normal controls [Pasiaka *et al.* 2003; Tuttle *et al.* 2002], and VEGF expression has been correlated with tumor cell proliferation in PTC and FTC [Klein *et al.* 1999] and shorter progression-free survival in PTC [Lennard *et al.* 2001]. Preclinical studies with mouse xenograft models have demonstrated the efficacy of VEGF-targeted therapies in PTC and ATC [Schoenberger *et al.* 2004; Bauer *et al.* 2003, 2002], further validating the role of VEGF and neovascularization in thyroid cancer pathobiology.

Aberrant cell signaling

Among the major signaling pathways implicated in thyroid cancer are the RAS/RAF/MEK/ERK pathway (MAPK signaling pathway) and the PI3K/Akt/mTOR signaling pathway. These pathways are closely involved in promoting cell survival, cell cycle progression, migration, proliferation, metabolism, tumorigenesis, and angiogenesis [Brzezianska and Pastuszak-Lewandoska, 2011]. Selected aberrations in the MAPK and PI3K/Akt/mTOR pathways seen in thyroid cancer are described below.

RET. Mutations involving *RET* have been implicated as the dominant genetic abnormality in almost all forms of hereditary MTC. Activation of the *RET* proto-oncogene results in constitutive activation of the MAPK pathway, promoting tumorigenesis. In patients with hereditary MTC syndromes, such as multiple endocrine neoplasia type 2A or 2B (MEN 2A/2B) or familial MTC, approximately 96% of families will have identifiable germline mutations in the *RET* gene [Eng *et al.* 1996]. The frequency of *RET* mutations in sporadic MTC reported in the literature has been variable, ranging from 25% to 50%, a recent study reporting somatic *RET* mutations in up to 65% of individuals tested [Moura *et al.* 2009]. In patients with hereditary forms of MTC, the specific mutation and associated degree of *RET* activation is closely associated with tumor aggressiveness, a fact that is currently reflected in the American Thyroid Association risk stratification guidelines for hereditary MTC [Kloos *et al.* 2009a]. *RET* has also been implicated in the pathogenesis of DTC. Though *RET* is normally expressed only in cells of neural crest origin, including thyroid C cells, translocations of *RET* and *PTC* (*RET/PTC* rearrangements) have been documented in 3–60% of PTCs [Kondo *et al.* 2006; Santoro *et al.* 2006; Nikiforov, 2002; Tallini and Asa, 2001]. This results in expression

of activated RET and downstream activation of MEK/ERK. There are at least 15 known translocations of *RET/PTC* documented in PTC. Preclinical studies have confirmed that these translocations are oncogenic in thyroid follicular cell cultures and in mouse xenograft models [Buckwalter *et al.* 2002; Santoro *et al.* 1996].

RAF. The Raf family of proteins plays an essential gateway function in transmitting signals from cell membrane receptors to nuclear transcription factors. Mutations in the *BRAF* gene are the most commonly described mutations in DTC, being observed in 36–69% of cancers [Xing, 2005; Trovisco *et al.* 2004; Cohen *et al.* 2003; Kimura *et al.* 2003; Nikiforova *et al.* 2003]. The most common mutation results in the substitution of a single glutamic acid for valine in the B-Raf protein (V600E); other activating mutations have been described but are rare. The *BRAF* V600E mutation is characteristic of PTC, but is also seen in patients with poorly differentiated thyroid cancer and ATC [Xing, 2005; Begum *et al.* 2004; Soares *et al.* 2004; Nikiforova *et al.* 2003]. Mouse models with *BRAF* V600E have confirmed that this is an oncogenic mutation [Knauf *et al.* 2005]. Several studies have looked at the correlation of *BRAF* V600E with clinicopathologic features of PTC [Riesco-Eizaguirre *et al.* 2006; Fugazzola *et al.* 2006, 2004; Kim *et al.* 2005; Namba *et al.* 2003; Nikiforova *et al.* 2003], and a recent meta-analysis has documented significant associations between the *BRAF* V600E mutation and certain adverse clinical features, including extrathyroidal extension, lymph node metastasis, and advanced stage of disease [Xing, 2007a].

RAS. Mutations in Ras proteins have been well documented in patients with thyroid cancer, the characteristic aberration typically being an activating point mutation. Between 20% and 50% of all patients with follicular thyroid adenomas or carcinomas have point mutations in codon 61 of *RAS*, approximately 0–15% of patients with PTC sharing this mutation [Vasko *et al.* 2003; Motoi *et al.* 2000]. *RAS* activation *in vitro* in a thyrocyte cell line has been associated with increased proliferation and decreased expression of follicular cell specific genes [Portella *et al.* 1999; Monaco *et al.* 1995]. *RAS* activation has also been correlated clinically with progression and a more aggressive phenotype of DTC [Garcia-Rostan *et al.* 2003]. *RAS* also appears

clinically significant in subsets of patients with MTC. In patients with *RET* mutation-negative sporadic MTC, a recent study identified *RAS* mutations in 68% of patients; only 2.5% of patients with sporadic MTC and an activating *RET* mutation demonstrated a coexisting *RAS* mutation [Moura *et al.* 2011].

PI3K/PTEN. Mutations and amplifications in PI3K subunit genes (*PIK3CA*, *PIK3CB*, *PIK3R1*) have been well described in many cancers, and inhibition of PI3K in thyroid cancer mouse models does inhibit thyroid tumor growth, reduce cell proliferation, promote apoptosis, and impair metastatic spread [Furuya *et al.* 2007b]. A recent study of patients with benign adenomas as well as more advanced thyroid cancers demonstrated that 31% of follicular adenomas, 55% of FTCs, 24% of PTCs, and 58% of ATCs harbored at least one abnormality in *PI3K* or the associated tumor suppressor *PTEN* [Hou *et al.* 2007]. PI3K/Akt activation appears to be correlated to tumor invasiveness in PTC [Shinohara *et al.* 2007; Ringel *et al.* 2001], and may be a later event in these tumors that is associated with dedifferentiation and a progression toward an anaplastic phenotype [Hou *et al.* 2007].

Epigenetic modulation

DNA methylation, histone deacetylation, and chromatin remodeling all have impacts on gene expression and have been implicated in tumorigenesis. In DTC, promoter hypermethylation and silencing of the TSH receptor gene (*TSHR*) and the sodium–iodine symporter gene (*NIS*), which are integral in normal thyrocyte function and iodine uptake, have been documented in both DTC cell lines and in tissue samples [Xing, 2007b; Hoque *et al.* 2005; Xing *et al.* 2003; Venkataraman *et al.* 1999]. Histone deacetylation has also been implicated in the downregulation of *NIS*, thyroperoxidase, and thyroglobulin mRNA [Puppini *et al.* 2005; Furuya *et al.* 2004]. These changes are at least partially responsible for the loss of radioiodine sensitivity in certain DTCs. Inactivation of tumor suppressor genes in thyroid cancer has also been identified as an important mechanism of tumorigenesis. *PTEN* promoter hypermethylation has been documented in roughly 46% of papillary carcinomas and 85% of follicular adenomas and carcinomas [Alvarez-Nunez *et al.* 2006]. Other important tumor suppressors

that are epigenetically suppressed by hypermethylation include p16 in nearly 30% of DTCs [Elisei *et al.* 1998], RAS association family 1A tumor suppressor gene in roughly 38% of all thyroid carcinomas [Nakamura *et al.* 2005], and Rap1 GTPase-activating protein (Rap1GAP), an inhibitor of the RAS superfamily protein Rap1, in up to 71% of PTC [Zuo *et al.* 2010].

Novel approaches to the treatment of thyroid cancer

In patients with advanced, unresectable, radioiodine-refractory thyroid cancer, effective options for systemic therapy have historically been limited. With progress in the understanding of the pathogenesis of thyroid cancer over the last decade has come a corresponding interest in translating this knowledge into rational clinical therapies. Recent published clinical trials in thyroid cancer are summarized in Table 1.

FDA-approved novel therapies

Vandetanib. Vandetanib (AZD6474/ZD6474, Zactima) is an orally bioavailable inhibitor of RET, VEGFR-2 and epidermal growth factor receptor (EGFR) [Carlomagno *et al.* 2002; Wedge *et al.* 2002]. Preclinical studies have demonstrated activity *in vitro* against the *RET* mutant MTC cell lines TT and MZ-CRC-1, with corollary studies suggesting activity against oncogenic RET as well [Vitagliano *et al.* 2010; Vidal *et al.* 2005]. Orthotopic mouse xenograft models of ATC have also suggested activity [Gule *et al.* 2011]. Two recent phase II trials investigated vandetanib at different doses in patients with hereditary MTC. Wells and colleagues reported the results of a phase II study of vandetanib initiated at 300 mg daily in patients with locally advanced or metastatic MTC [Wells *et al.* 2010]. Evidence of disease progression at enrollment was not required. A total of 30 patients were enrolled in the study, of whom 29 were evaluable for response. Objective responses were confirmed in 6 patients (20%), all of whom had radiographic partial response (PR); an additional 16 patients (53%) had stable disease (SD) for at least 24 weeks. The median progression-free survival (PFS) period was 27.9 months. The most common adverse events were diarrhea (70%), rash (67%), fatigue (63%), and nausea (63%); the most common grade 3 or higher adverse events were diarrhea (10%), nausea (10%), and hypertension (10%). Dose reductions were

required in 80% of patients and five patients (17%) discontinued treatment for drug associated adverse events.

Robinson and colleagues studied patients with locally advanced or metastatic hereditary MTC initiated on vandetanib at the lower dose of 100 mg daily [Robinson *et al.* 2010]. A total of 19 patients were enrolled in the study. Documented disease progression prior to enrollment was not required. Confirmed PRs were seen in 3 patients (16%) and SD lasting at least 6 months was seen in an additional 10 patients (53%). Median PFS was not reached during follow up. Side effects were slightly less common than at the higher dose, with diarrhea (47%), fatigue (42%), rash (26%), and constipation (21%) being the most commonly reported adverse events. Dose reductions were performed in two patients (11%) and an additional two patients (11%) discontinued therapy for drug-related adverse events (1 renal insufficiency, 1 muscle weakness).

The results of a randomized, double-blind placebo-controlled phase III trial (ZETA) of vandetanib 300 mg daily in patients with locally advanced or metastatic MTC has been reported in abstract form [Wells *et al.* 2010]. A total of 331 patients were enrolled in the trial (hereditary MTC, 10%; sporadic MTC, 90%) with 231 patients randomized to the vandetanib arm and 100 patients to the placebo arm. The primary endpoint was PFS. At the time of progression, patients were unblinded with the option of receiving vandetanib as part of an open-label trial. After a median follow up of 24 months, PFS was significantly improved in the vandetanib arm (HR 0.45, CI 0.30–0.69). Median PFS was 19.8 months in the placebo arm and was not reached in the vandetanib arm. Statistically significant improvements were also noted in ORR, disease control rate, and biochemical response rate. Overall survival was not significantly different between the two groups (HR 0.89, CI 0.28–2.85). Adverse effects were similar to those reported in the phase II trials, with diarrhea (56% *versus* 26%), rash (45% *versus* 11%), nausea (33% *versus* 16%), hypertension (32% *versus* 5%), and headache (26% *versus* 9%) being more common in the vandetanib arm. Dose reductions were also more common in the vandetanib arm (35% *versus* 3%). After review of the phase III trial results, vandetanib was approved by the FDA in April 2011 for the treatment of advanced MTC that is not surgically

Table 1. Synopsis of recent clinical trials of targeted therapies in thyroid cancer.

Drug	Targets	Reference	Trial	Dosage	N	DTC (%)	MTC (%)	ATC (%)	ORR (%)	SD ≥ 6 months (%)	Median PFS	Comments
FDA-approved therapies Vandetanib	VEGFR2, EGFR, RET	Wells <i>et al.</i> [2010]	Phase III	300 mg daily	331		100%		45%	NR	NR	PFS significantly improved with vandetanib <i>versus</i> placebo (HR 0.45, 95% CI 0.30–0.69)
		Wells <i>et al.</i> [2010]	Phase II	300 mg daily	30		100%		20%	53%	27.9 months	Confirmed decreases $\geq 50\%$ in CTN and CEA seen in 80% and 53% of patients
		Robinson <i>et al.</i> [2010]	Phase II	100 mg daily	19		100%		16%	53%	NR	Confirmed decreases $\geq 50\%$ in CTN and CEA seen in 16% and 5% of patients
Investigational therapies VEGFR inhibitors	Axitinib	Cohen <i>et al.</i> [2008b]	Phase II	5 mg bid	60	77%	18%	3%	30%	NR	18.1 months	Treatment associated with increases in serum VEGF, decreases in soluble VEGFR
	Motesanib	Sherman <i>et al.</i> [2008]	Phase II	125 mg daily	93	100%			14%	35%	10 months	No significant difference in response between patients with ($n=10$) and without ($n=15$) <i>BRAF</i> V600E mutation
	Pazopanib	Bible <i>et al.</i> [2010]	Phase II	800 mg daily	37	100%	100%		49%	NR	11.7 months	Rates of PR + SD were 69% in <i>RET</i> mutant ($n=33$) versus 42% in <i>RET</i> wild type ($n=13$) Two deaths (5%) on study (acute MI, $n=1$; bowel perforation, $n=1$)
	Sorafenib	Gupta-Abramson <i>et al.</i> [2008] Kloos <i>et al.</i> [2009b]	Phase II	400 mg bid	56	90%	3%	7%	23%	NR	19.75 months	Median PFS in patients with DTC was 21 months Median PFS in treatment-naïve PTC, 16 months; in previously treated PTC, 10 months; in patients with either FTC or HTC, 4.5 months
Sunitinib	VEGFR1-2, PDGFR, KIT, RET, FLT3	Hofstijzer <i>et al.</i> [2009]	Phase II	400 mg bid	32	100%			25%	34%	14.5 months	Median PFS 17.25 months in patients without bone metastasis, 11.75 months in patients with bone metastasis
		Lam <i>et al.</i> [2010]	Phase II	400 mg bid	21		100%		21%	53%	NR	Median PFS in sporadic MTC, 17.9 months; median PFS not reached in sporadic MTC
		Carr <i>et al.</i> [2010]	Phase II	37.5 mg daily	35	80%	20%		31%	37%	12.8 months	4 patients had prior TKI therapy: there was 1 PR, 1 PD, and 2 withdrawals due to toxicity (including 1 patient with SD at 12 months)

(continued)

Table 1. Continued.

Drug	Targets	Reference	Trial	Dosage	N	DTC (%)	MTC (%)	ATC (%)	ORR (%)	SD ≥ 6 months (%)	Median PFS	Comments
XL184	VEGFR2, Met, KIT, FLT3, Tie-2	Cohen <i>et al.</i> [2008a]	Phase II	50 mg daily, 4 weeks every 6 weeks	37	100%			13%	NR	NR	Common side effects included fatigue (79%), diarrhea (56%), hand-foot syndrome (53%), neutropenia (49%)
		De Souza <i>et al.</i> [2010]	Phase II	50 mg daily, 4 weeks every 6 weeks	25		100%		32%	NR	NR	1-year PFS among patients with RET-activating mutations was 88%
		Ravaud <i>et al.</i> [2010]	Phase II	50 mg daily, 4 weeks every 6 weeks	15		100%		33%	NR	NR	Preliminary results of THYSU MTC cohort only
Ras-MAPK inhibitors Tiplifarnib (+ sorafenib)	VEGFR2, Met, KIT, FLT3, Tie-2	Kurzrock <i>et al.</i> [2010]	Phase I	MTD 175 mg daily	37		100%		27%	41%	NR	Randomized Phase III trial in MTC (EXAM) has completed accrual; results pending
			Phase II									
AZD6244	MEK	Hong <i>et al.</i> [2011]	Phase I/II	MTD 200 mg (+ 600 mg) daily	35	63%	37%		17%	34%	18 months	PR seen in 5 of 13 patients (38%) with MTC and 1 of 22 patients (4.5%) with DTC
		Lucas <i>et al.</i> [2010]	Phase II	100 mg bid	39	100%			3%	54%	13.4 months	Common side effects included rash (69%), fatigue (49%), diarrhea (49%), and peripheral edema (36%)
Other receptor kinase inhibitors Gefitinib	EGFR	Pennell <i>et al.</i> [2008]	Phase II	250 mg daily	27	67%	15%	19%	0%	22%	3.7 months	Median PFS in well DTC 3.9 months
		de Groot <i>et al.</i> [2007]	Phase II	600 mg daily	15		100%		0%	>27%	NR	No significant decreases in CTN or CEA levels were observed
Imatinib	Bcr-Abl, KIT, RET	Frank-Raue <i>et al.</i> [2007]	Phase II	600 mg daily	9		100%		0%	56%	NR	No grade ≥3 toxicities observed
		Ha <i>et al.</i> [2010]	Phase II	400 mg bid	11			100%	18%	NR	NR	All patients had overexpression of PDGFR by immunohistochemistry. Estimated 6-month PFS (27%), OS (46%)
Vasculature targeting agents Fosbretabulin	Tubulin	Mooney <i>et al.</i> [2009]	Phase II	45 mg/m ² IV, days 1, 8, and 15 every 28 days	26			100%	0%	NR	NR	Median OS, 4.7 months; 6-month OS, 34%; 12-month OS, 23%
Targeted radiotherapy [⁹⁰ Y-DOTA]-TOC	Somatostatin receptor	Iten <i>et al.</i> [2009]	Phase II	3.7 GBq/m ² per cycle	24	100%			NR	NR	NR	7 patients (29%) had decreases in serum Ig with treatment. Median OS 65.1 months in responders versus 28.9 months in non-responders.

[continued]

Table 1. Continued.

Drug	Targets	Reference	Trial	Dosage	N	DTC (%)	MTC (%)	ATC (%)	ORR (%)	SD ≥ 6 months (%)	Median PFS	Comments
Redifferentiation agents												
Rosiglitazone	PPAR- γ	Kebebew <i>et al.</i> [2010]	Phase II	8 mg daily	21	100%			0%	NR	NR	5 patients had at least partial restoration of radioiodine uptake
Vorinostat	HDAC	Woyach <i>et al.</i> [2009]	Phase II	200 mg daily, 2 weeks every 3 weeks	19	84%	16%		0%	32%	NR	1 of 3 patients with PTC had increased radioiodine uptake following treatment
Romidepsin	HDAC	Sherman <i>et al.</i> [2009]	Phase II	13 mg/m ² IV, days 1, 8, and 15 every 28 days	20	100%			0%	NR	NR	2 patients (10%) with restoration of radioiodine uptake. One sudden death
Thalidomide and lenalidomide												
Thalidomide	TNF α , interleukins, interferons, bFGF	Ain <i>et al.</i> [2007]	Phase II	800 mg daily	36	81%	19%		14%	25%	NR	50% of responders discontinued study drug because of toxicity
Lenalidomide		Ain <i>et al.</i> [2008]	Phase II	25 mg daily	21	100%			19%	38%	NR	Primary grade ≥ 3 toxicities were hematologic (neutropenia, 44%; thrombocytopenia, 22%)

ATC, anaplastic thyroid cancer; Bcr-Abl; bFGF, basic fibroblast growth factor; bid; CEA; CI, confidence interval; CTN.; DTC, differentiated thyroid cancer; EGFR, epidermal growth factor receptor; EXAM, Efficacy of XL184 in advanced MTC study; FLT3; FTC, follicular thyroid cancer; HDAC, histone deacetylase; HR.; HTC, Hürthle cell thyroid cancer; IV, intravenously; KIT; MAPK; MEK; MI.; MTC, medullary thyroid cancer; NR.; ORR; OS.; PDGFR, platelet-derived growth factor receptor; PPAR- γ ; PFS, progression-free survival; PR, partial response; PTC, papillary thyroid cancer; Raf-1; RET.; SD, stable disease; Tie-2; Tg, thyroglobulin; THYU.; TNF α , tumor necrosis factor α ; TKI.; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; [[90Y-DOTA]-TOC.,

resectable and which is either progressive or symptomatic.

Other VEGFR inhibitors currently under investigation

Axitinib. Axitinib (AG-013736) is a potent oral inhibitor of VEGFR-1, -2, and -3, with less potent inhibition of platelet-derived growth factor receptor β (PDGFR- β) and c-KIT in *in vitro* assays [Hu-Lowe *et al.* 2008]. Direct cytotoxicity against thyroid cancer cell lines was not observed at pharmacologic doses [Braunstein *et al.* 2010], but the observed importance of neo-vascularization and VEGFR in thyroid cancer has prompted clinical evaluation in advanced thyroid cancers.

Cohen and colleagues reported a multicenter phase II trial of axitinib in patients with advanced thyroid cancer of all subtypes [Cohen *et al.* 2008b]. Subjects were eligible if they had radioiodine-resistant disease, at least one RECIST-defined target lesion that had not been externally radiated, and ECOG performance status of 0–1. The percentage of patients with progressive disease at study enrollment was not reported. Patients were initiated on axitinib at a dose of 5 mg twice daily with the option to titrate up to a dose of 10 mg twice daily as tolerated. The dose of axitinib could also be reduced to 2 mg twice daily in patients with dose-limiting, treatment-related adverse effects. Radiographic responses were assessed every 8 weeks using RECIST. A total of 60 patients were recruited to the study (30 papillary, 15 follicular/Hürthle cell, 11 medullary, 2 anaplastic, 2 other), of whom 45 were evaluable for response. Median follow up was 16.6 months. The overall response rate was 30% ($n=18$); all responding patients had a PR. An additional 23 patients (38%) had SD as the best response and 4 patients (7%) had PD. The rate of durable SD (≥ 6 months) was not reported. The median PFS was 18.1 months. Median overall survival was not reached. Common treatment-related adverse events included fatigue (50%), diarrhea (48%), nausea/anorexia/weight loss (33%, 30%, 25%), hypertension (28%), and headache (22%). Grade 3 or greater adverse events occurred in 19 patients (32%). Three patients experienced grade 4 toxicities that subsequently resolved: stroke ($n=1$), posterior reversible leukoencephalopathy ($n=1$), and proteinuria ($n=1$). An international phase II study of axitinib in patients with radioiodine-

refractory metastatic or unresectable locally advanced thyroid cancer is currently in progress [ClinicalTrials.gov identifier: NCT00 389441].

Motesanib. Motesanib (AMG-706) is another orally bioavailable inhibitor of VEGFR-1, -2, and -3, in addition to PDGFR and KIT [Polverino *et al.* 2006]. Direct cytotoxicity against thyroid cancer cell lines is minimal [Braunstein *et al.* 2010] but an initial phase I study of motesanib in advanced solid cancers did report activity in six of seven patients with advanced thyroid cancer (PR, $n=3$; SD, $n=3$) [Rosen *et al.* 2007].

Sherman and colleagues reported the results of an international, multicenter, open-label phase II study of motesanib in patients with metastatic, radioiodine-refractory DTC with radiographic evidence of disease progression by RECIST within the last 6 months prior to enrollment [Sherman *et al.* 2008]. ECOG performance status was 0–2. Patients received motesanib 125 mg orally daily for up to 48 weeks or until disease progression, with the option to extend treatment beyond 48 weeks if clinical benefit was observed. The dose of motesanib could be reduced as low as 75 mg daily in the event of treatment-related adverse event(s). Radiographic imaging for response was performed at 8-week intervals. A total of 93 patients were recruited to the study (57 papillary, 17 Hürthle cell, 15 follicular, 4 other). With a median follow up of 49 weeks, confirmed objective responses were seen in 13 patients (14%), all of whom had PRs. Stable disease as best overall response was seen in 62 patients (67%), with durable SD (≥ 6 months) seen in 33 patients (35%). Median PFS was 10 months; median overall survival was not reached, but 12-month survival was 73%. Adverse events were seen in 87 patients (94%), including diarrhea (59%), hypertension (56%), fatigue (46%), weight loss (40%), and abdominal pain (30%); 12 patients (13%) discontinued treatment due to adverse events. Grade 3 adverse events were common, seen in 51 patients (55%). Eight grade 4 adverse events were reported: hypocalcemia ($n=2$); hyperuricemia, hypokalemia, cerebral hemorrhage, confusion, agitation, and oliguria ($n=1$ each). Two deaths were reported, both related to pulmonary hemorrhage in patients with disease progression.

Schlumberger and colleagues reported the results of a companion study of motesanib in patients

with locally advanced or metastatic MTC with either symptomatic disease or evidence of radiographic disease progression within the 6 months prior to study enrollment [Schlumberger *et al.* 2009]. A total of 91 patients were enrolled, most of whom had sporadic MTC ($n=76$, 84%). Thirteen patients had familial MTC (14%) and disease status was unknown in the remaining two patients. No complete responses (CRs) were observed. Confirmed PRs were seen in 2 patients (2%), with SD seen in an additional 74 patients (81%). Durable SD was reported in 44 of these patients (48%). Median PFS was 12 months; median overall survival was not reached, but 12-month survival was reported as 75%. Adverse events were similar to those seen by Sherman and colleagues [Sherman *et al.* 2008], with 88% of patients experiencing at least one drug-related adverse event. Grade 3 adverse events were seen in 38% of patients, with three patients experiencing grade 4 events (asthenia, $n=2$; cholelithiasis, gallbladder toxicity, $n=1$). Seven deaths were observed during the course of the study, all of which were deemed to be unrelated to motesanib treatment but related to PD.

Retrospective analyses of these two trials suggested that increases in serum placental growth factor after 1 week of treatment and decreases in soluble VEGFR-2 after 3 weeks of treatment correlated with a favorable response to therapy [Bass *et al.* 2010].

Pazopanib. Pazopanib (GW786034, Votrient) is an orally bioavailable inhibitor of multiple receptor tyrosine kinases, including VEGFR-1, -2 and -3, PDGFR- α and - β , and KIT [Kumar *et al.* 2007]. As is also the case for axitinib and motesanib, direct cytotoxicity against thyroid cancer cell lines is rare [Braunstein *et al.* 2010]. Pazopanib is currently FDA approved for the treatment of advanced renal cell carcinoma.

Results of an NCI-sponsored multicenter, phase II trial of pazopanib in patients with locally advanced or metastatic, radioiodine-resistant DTC was recently reported [Bible *et al.* 2010]. All patients had evidence of radiographic disease progression according to RECIST in the 6 months prior to enrollment. A total of 37 patients were evaluable for clinical response (follicular, $n=11$; Hürthle cell, $n=11$; papillary, $n=15$). Patients were treated with pazopanib 800 mg daily administered continuously until disease

progression, drug intolerance, or both. The pazopanib dose could be reduced as low as 200 mg daily for drug-related adverse events. Response was evaluated radiographically at least every 8 weeks. No CRs were observed. Eighteen patients (49%) had confirmed PRs. Response rates among FTC (8/11, 73%), HTC (5/11, 45%), and PTC (5/15, 33%) were not statistically significant but the trial was underpowered to detect differences in response rates on the basis of tumor subtype. Responses were prolonged, 66% of patients having PRs lasting more than 1 year. Rates of SD were not reported. Median PFS was 11.7 months and 12-month survival was 81%. Adverse events were common and similar to those with other VEGFR-targeted tyrosine kinase inhibitors, and dose reductions were required in 16 patients (43%). The most common adverse events were fatigue (78%), skin and hair hypopigmentation (76%), diarrhea (73%), and nausea (73%). Hypertension was seen in 49% of patients and elevations in transaminases in 41%. Two deaths occurred during treatment, including one fatal myocardial infarction in a patient with underlying coronary artery disease and one patient who developed acute cholecystitis treated surgically, subsequently complicated by bowel volvulus and bowel perforation at a remote surgical anastomosis. Pharmacokinetics appear to play an important role in patient response to treatment, as the maximum plasma pazopanib concentration during cycle 1 was inversely correlated with the maximum change in tumor size ($p=0.021$) and was significantly higher in the patients who achieved a PR compared with those who did not ($p=0.009$). A randomized phase II study of paclitaxel and intensity-modulated radiation therapy plus either pazopanib or placebo in ATC [ClinicalTrials.gov identifier: NCT01236547] recently opened and is currently enrolling patients.

Sorafenib. Sorafenib (BAY 43-9006, Nexavar) is a multikinase inhibitor. In addition to activity against VEGFR-2 and -3, it also demonstrates activity against PDGFR- β , Flt-3, c-KIT, Raf-1, B-Raf, and RET kinases, which provides additional potential activity against aberrant signaling pathways in various cancers. Salvatore and colleagues showed antitumor activity of sorafenib in a panel of six *BRAF* V600E-positive thyroid carcinoma cell lines, including anaplastic thyroid cancer cell lines, and in nude mice bearing ARO cell xenografts [Salvatore *et al.* 2006].

Activity has also been demonstrated *in vitro* against the TT cell line (C634R *RET* mutation-positive MTC cell line) and xenograft models in MTC [Carlomagno *et al.* 2006]. Sorafenib is currently FDA approved for the treatment of unresectable hepatocellular carcinoma and advanced renal cell carcinoma. There have been four recently published phase II clinical trials of sorafenib in advanced thyroid cancer [Lam *et al.* 2010; Hoftijzer *et al.* 2009; Kloos *et al.* 2009b; Gupta-Abramson *et al.* 2008]. Initial dosing in all studies was at 400 mg orally twice daily, with subsequent dose adjustments as needed for toxicity. Gupta-Abramson and colleagues published the results of an initial trial in 30 patients with metastatic or unresectable thyroid carcinomas with predominantly differentiated histologies (PTC, $n=18$; FTC/HTC, $n=9$; MTC, $n=1$; ATC/poorly differentiated, $n=2$) [Gupta-Abramson *et al.* 2008]. Five patients withdrew prior to the first evaluation due to toxicity (17%) and 25 patients could be evaluated for response. Partial responses were seen in 7 patients (23%), with SD seen in an additional 16 patients (53%). Rates of durable SD were not reported. Both patients with ATC/poorly differentiated histologies had progressive disease as best response. Median PFS for all histologies was 19 months; median PFS in DTC only was 21 months. Overall survival was not reported.

Our group similarly evaluated sorafenib in an NCI-sponsored phase II trial in patients with predominantly differentiated thyroid cancers (PTC, $n=41$; FTC, $n=2$; HTC, $n=9$; ATC, $n=4$) [Kloos *et al.* 2009b]. Evidence of disease progression prior to enrollment was not required. Six patients could not be evaluated for response. The overall radiographic response rate in patients with differentiated histologies included PR of 12% (6/52), SD in an additional 65% (34/52), of whom 29 had durable SD lasting ≥ 6 months (56%), and PD in the remaining 12% (6/52). All patients who had a PR had PTC. Among four ATC patients, one had SD that lasted ≥ 6 months (1/4, 25%) and PD was seen in the remaining three (3/4, 75%). Median PFS was 16 months in chemotherapy-naïve patients with PTC ($n=33$), 10 months in patients with PTC who had received prior chemotherapy ($n=8$), and 4.5 months in patients with Hürthle cell or follicular histologies ($n=11$). Given the *in vitro* activity of sorafenib against Raf kinases, 22 patients with PTC had tissue analyzed for mutations of *BRAF*. Seventeen patients had

activating mutations, with 14 demonstrating the V600E mutation (64%), while three had a K601E mutation (14%). Statistical comparison of response between patients with and without *BRAF*-activating mutations could not be performed because of the small number of patients without activating mutations ($n=5$). Dynamic contrast-enhanced magnetic resonance imaging studies performed at prestudy and at 8 or 16 weeks on therapy showed a decrease in tumor perfusion in 10 of 14 patients, with PTC demonstrating an angiogenic effect of sorafenib.

Hoftijzer and colleagues evaluated sorafenib in a phase II trial exclusively in patients with radioiodine-refractory DTC (PTC, $n=13$; FTC, $n=15$; other, $n=4$) [Hoftijzer *et al.* 2009]. All patients had documented PD by RECIST within the year prior to enrollment. Of 32 patients initially enrolled, 22 could be evaluated for radiographic response at 26 weeks after enrollment; the remaining 10 patients withdrew because of disease progression ($n=4$), drug-related adverse events ($n=2$), other adverse events ($n=2$), or patient request ($n=2$). Partial responses were seen in 25% (8/32) of patients, with durable SD at 6.5 months in 34% (11/32). Progressive disease was seen in a total of seven patients by 6.5 months (7/32, 22%). Median PFS in all patients was 14.5 months. The investigators also evaluated patients for the effect of sorafenib on radioiodine uptake in 20 patients at 6.5 months. Three patients had uptake locally in the thyroid bed, but no patient demonstrated clinically significant uptake at known sites of metastatic disease, leading the authors to conclude that sorafenib did not have a clinically significant role in the reinduction of radioiodine uptake.

Finally, the results of an NCI-sponsored multicenter phase II trial of sorafenib in patients with advanced MTC were recently reported by our group [Lam *et al.* 2010]. A total of 21 patients were accrued (hereditary MTC, $n=5$; sporadic MTC, $n=16$). All patients with hereditary MTC had germline *RET* mutations; *RET* mutations were seen in 10 of 12 evaluable patients with sporadic MTC. Evidence of PD prior to enrollment was not required. Partial responses were seen in 2 patients (10%), SD in 18 patients (86%), and clinical evidence of disease progression in 1 patient (5%). Durable SD lasting ≥ 6 months was seen in 11 patients (53%), with SD ≥ 15 months seen in 9 patients (43%). Median PFS in patients with sporadic MTC was 17.9 months;

it was not reached in hereditary MTC. Median overall survival was not reached. In a *post hoc* analysis, 10 patients with sporadic MTC demonstrated PD within 12 months prior to study. Among these 10 patients, 1 PR of ≥ 21 months, 4 SD ≥ 15 months, 4 SD ≤ 6 months, and 1 clinical PD were seen.

Toxicities were similar in all four trials. Discontinuation for adverse events was reported in 0–20% of patients with dose reductions in an additional 47–76%. The most common adverse events were hand–foot syndrome (grade 1–2, 55–83%; grade 3–4, 7–14%), rash (grade 1–2, 67–75%; grade 3–4, 0–10%), fatigue (grade 1–2, 33–66%; grade 3–4, 0–16%), diarrhea (grade 1–2, 71–73%; grade 3–4, 4–10%), bloating (grade 1–2, 19–70%), musculoskeletal pain (grade 1–2, 52–57%), weight loss (grade 1–2, 48–76%; grade 3–4, 0–10%), and mucositis (grade 1–2, 14–48%; grade 3–4, 0–2%). Adjustment of thyroid medications was also common, 33–34% of patients requiring dose changes in thyroid replacement. Reported cardiovascular complications included hypertension (grade 1–2, 30–43%; grade 3–4, 4–13%), congestive heart failure (0–3%), myocardial infarction (0–3%), and pulmonary embolism (0–5%).

Sunitinib. Sunitinib (SU011248, Sutent) is an FDA-approved oral tyrosine kinase inhibitor for advanced renal cell carcinoma and gastrointestinal stromal tumors (GIST) that are resistant to imatinib, which has *in vitro* activity against VEGFR-1, -2, PDGFR, c-KIT, FLT3, and RET. Carr and colleagues reported the results of a phase II trial of sunitinib in patients with fluorodeoxyglucose (FDG)-avid, radioiodine-refractory DTC or metastatic MTC. Progressive disease at study entry was not required [Carr *et al.* 2010]. Sunitinib was administered at 37.5 mg daily with the option to reduce the dose because of adverse events to 25 mg daily. Treatment was continued until disease progression or unacceptable adverse events. A total of 35 patients were accrued to the study (PTC, $n=18$; FTC, $n=4$; HTC, $n=5$; MTC, $n=7$; other, $n=1$). After a median follow up of 15.5 months, there were 11 objective responses (8 of 29 patients with DTC, 3 of 6 patients with MTC) with 1 CR (3%) and 10 PR (28%) by RECIST criteria; an additional 13 patients had SD of at least 6 months duration (37%). There were four patients on study who had previously been treated with a tyrosine

kinase inhibitor. Two patients had received prior motesanib, one of whom had a PR on sunitinib and the other developed PD; two patients had received prior sorafenib, both of whom discontinued treatment because of toxicities, but one of whom had a RECIST response of a 29% reduction in tumor while on treatment. One death occurred on study secondary to a gastrointestinal bleed in a patient on full-dose anticoagulation for a history of venous thromboembolic disease; two other patients, including one patient on warfarin for atrial fibrillation, also required hospital admission for gastrointestinal bleeding, leading to study amendment and exclusion of patients on full-dose anticoagulation. The most common side effects were cytopenias (grade ≥ 3 , 46%), fatigue (all cases, 26%; grade ≥ 3 , 11%), hand–foot syndrome (all cases, 26%; grade ≥ 3 , 17%), diarrhea (all cases, 26%; grade ≥ 3 , 17%), and gastrointestinal bleeding (all cases, 14%; grade ≥ 3 , 6%; grade 5, 3%). Dose reductions were needed in 60% of patients, with four patients (11%) discontinuing treatment because of toxicity.

Preliminary results of several ongoing phase II trials of sunitinib in advanced thyroid cancer have been reported in abstract form. Cohen and colleagues and De Souza and colleagues summarized the results of an NCI-sponsored phase II study of sunitinib in patients with unresectable radioiodine-refractory DTC or unresectable MTC [De Souza *et al.* 2010; Cohen *et al.* 2008a]. Sunitinib was administered in 6-week cycles with sunitinib 50 mg given daily for 4 weeks followed by a 2-week break. Among 37 patients with DTC enrolled at the first interim analysis, 31 could be evaluated for response at 3 months, with PR reported in 13%, SD in 68%, and PD in 10%. A total of 25 patients with MTC were enrolled in the study by the second analysis. After a median follow up of 11 months, 23 patients could be evaluated for a response, of whom 8 had a PR (32%) and 13 had SD (52%). The most common reported adverse events were fatigue, lymphopenia, nausea, diarrhea, mucositis, and hand–foot syndrome.

Ravaud and colleagues summarized the results of a subset of patients with MTC enrolled in a phase II study of sunitinib in locally advanced or metastatic ATC or DTC (THYSU) [Ravaud *et al.* 2010]. All 15 patients accrued at the interim analysis had metastatic disease. Patients were treated with sunitinib 50 mg daily for 4 weeks followed by a 2-week drug-free period. After a

mean follow up of 6.2 months, five patients had either a confirmed or unconfirmed PR (33%) and four had SD lasting >12 weeks (27%). Full reporting on toxicities was not provided, but cardiac side effects were observed, leading to study amendment to include monitoring of left ventricular ejection fraction every two cycles and serum brain natriuretic peptide prior to every cycle. Full reporting is awaited at study conclusion.

VEGFR-targeted agents currently in development.

Cabozantinib (XL184) is a potent, orally bioavailable receptor tyrosine kinase inhibitor with activity against Met, VEGFR-2, KIT, Flt3, and Tie-2. Kurzrock and colleagues reported on their phase I experience with XL184 in 85 patients with advanced malignancies, including a subset of 37 patients with advanced MTC [Kurzrock *et al.* 2010]. Dose-limiting grade ≥ 3 adverse events included hand-foot syndrome (10%), fatigue (10%), elevated lipase (9%), diarrhea (7%), elevated amylase (5%), weight loss (3%), and elevated AST and ALT (3%). Among patients with advanced MTC, 34 could be assessed for response. Ten patients had a PR (27%), with an additional 15 patients (41%) having SD ≥ 6 months. EXAM (Efficacy of XL184 in advanced MTC) is a multicenter international phase III placebo-controlled study of XL184 that recently completed enrolling patients with advanced MTC [ClinicalTrials.gov identifier: NCT00704730]. Results have not yet been reported.

Cediranib (AZD2171, Recentin) is a potent oral inhibitor of all three VEGF receptor kinases (VEGFR-1, -2, -3). The most common adverse events in a phase I trial were fatigue (36%), nausea (36%), diarrhea (28%), and vomiting (28%) [Dreys *et al.* 2005]. Maximum tolerated dose was 45 mg daily. Of note, three of eight patients receiving 60 mg daily experienced grade 3 or higher adverse effects, with one patient having a grade 4 intracranial hemorrhage and a second patient with grade 4 hypoglycemia. Cediranib is currently under investigation in combination with the thalidomide analogue lenalidomide in patients with advanced radioiodine refractory DTC [ClinicalTrials.gov identifier: NCT01208051].

E7080 is a potent inhibitor of the split kinase family of transmembrane growth factor receptors, including VEGFR-1 and -2, FGFR-1 and PDGFR- β . Maximum tolerated dose in a phase

I study of 59 patients was 25 mg daily, the most common reasons for dose reduction being grade 2 or worse proteinuria ($n=5$) and hypertension ($n=5$) [Glen *et al.* 2008]. Two other grade 3/4 toxicities included hemorrhage and thrombosis ($n=1$) and tachycardia ($n=1$). Enrollment to a multicenter phase II trial of E7080 in medullary and radioiodine refractory DTC [ClinicalTrials.gov identifier: NCT00784303] has recently been completed.

RAS-MAPK and PI3K/AKT/mTOR pathway inhibitors

Farnesyltransferase inhibition. Farnesylation of the Ras family of proteins is an important post-translational modification that is required for anchoring of these proteins to the inner cell membrane. Unfarnesylated Ras proteins are unable to transmit signals from the cell surface, and inhibition of farnesyltransferase, which is the key enzyme involved in farnesylation, is associated with modulation of signal transduction, inhibition of cell growth, and induction of apoptosis [Reuter *et al.* 2000]. Tipifarnib (R115777) is a potent oral and intravenous inhibitor of farnesyltransferase. Hong and colleagues reported on their experience with tipifarnib in combination with sorafenib in 35 patients with advanced thyroid cancer (DTC, $n=22$; MTC, $n=13$) enrolled in a phase I trial [Hong *et al.* 2011]. The maximum tolerated dose was sorafenib 600 mg and tipifarnib 200 mg, each administered daily for 21 days out of a 28-day cycle. A total of 25 patients (DTC, $n=15$; MTC, $n=10$) were evaluable for response. Among the 22 patients with DTC, a PR was seen in one patient (4.5%) and eight patients (36%) had durable SD lasting ≥ 6 months. For the 13 patients with MTC, five patients had a PR (38%) and four (31%) had durable SD lasting ≥ 6 months. Median PFS among all patients was 18 months; median overall survival (OS) was not reached after a median follow up of 24 months. The most common grade ≥ 3 toxicities were rash, elevated amylase/lipase, and fatigue. Further clinical trials with this combination have yet to open.

MEK inhibition. AZD6244 (ARRY-142886, selumetinib) is a potent inhibitor of MEK, an important cell-signaling molecule known to be involved in growth-promoting pathways in many advanced malignancies. Lucas and colleagues reported on an NCI-sponsored phase II study of AZD6244 in patients with iodine-refractory PTC or PTC with

follicular elements who had demonstrated disease progression in the last 12 months [Lucas *et al.* 2010]. AZD6244 was administered continuously at a dose of 100 mg orally twice daily. Of a total of 39 individuals enrolled in the trial, best response was evaluable in 32 patients, of whom 1 had a PR (3%) and 21 had SD (54%). Median PFS was 8 months. The most common reported adverse events included rash (all grades, 69%; grade ≥ 3 , 18%), fatigue (all grades, 49%; grade ≥ 3 , 8%), diarrhea (all grades, 49%; grade ≥ 3 , 5%), and peripheral edema (all grades, 36%; grade ≥ 3 , 5%).

A clinical trial of AZD6244 as a radioiodine-sensitizing agent is currently enrolling patients with radioiodine-refractory DTC [ClinicalTrials.gov identifier: NCT00970359]. A secondary endpoint is to determine whether *BRAF* mutational status affects the response to AZD6244 and the reacquisition of radioiodine sensitivity.

mTOR inhibition. Everolimus (RAD001, Afinitor) is a potent oral inhibitor of the mammalian target of rapamycin (mTOR) and is currently FDA approved for the treatment of patients with advanced renal cell carcinoma that is refractory to sunitinib and sorafenib, progressive metastatic pancreatic neuroendocrine carcinoma, and subependymal giant cell astrocytoma associated with tuberous sclerosis in patients who are not surgical candidates. Everolimus has activity against selected DTC and ATC cell lines [Behlendorf *et al.* 2009; Papewalis *et al.* 2009]. There are currently three phase II clinical trials open investigating everolimus alone in patients with either locally advanced or metastatic ATC or MTC or radioiodine-refractory DTC [ClinicalTrials.gov identifiers: NCT01118065, NCT00936858, and NCT01164176]. Preliminary results are not available. Combination therapy with everolimus has also been actively investigated. There are two phase II studies currently enrolling patients with locally advanced thyroid cancer to a therapeutic trial of sorafenib plus everolimus, one in sorafenib-naïve and mTOR inhibitor-naïve patients [ClinicalTrials.gov identifier: NCT01141309] and a second in patients who have progressed while on treatment with sorafenib [ClinicalTrials.gov identifier: NCT01263951]. A third phase II study is enrolling patients to a combination of everolimus and the somatostatin analogue paserotide (SOM230).

Temsirolimus (CCI-779, Torisel) is a parenterally administered mTOR inhibitor currently FDA approved for the treatment of advanced renal cell carcinoma. Preclinical studies have demonstrated inhibition of cell proliferation in thyroid cancer cell lines with PI3K/Akt-activating mutations [Liu *et al.* 2009]. There is currently one phase II trial investigating the combination of sorafenib and temsirolimus in radioiodine-refractory DTC [ClinicalTrials.gov identifier: NCT01025453].

Raf inhibition. XL281 is an orally available, small-molecule Raf kinase inhibitor. A phase I study in solid tumors established the maximum tolerated dose as 150 mg daily, the most common adverse events being fatigue (48%), diarrhea (35%), nausea (35%), vomiting (35%), and anorexia (30%) [Schwartz *et al.* 2009]. Durable SD was seen in two patients with radioiodine-refractory PTC harboring *BRAF* V600E mutations (≥ 15 months and ≥ 17 months). The maximum tolerated dose was expanded to include a cohort of 10 patients with PTC, but full results in this cohort have not been reported.

Other receptor tyrosine kinase inhibitors

EGFR inhibition. Gefitinib (Iressa, ZD1839) is a small-molecule inhibitor of EGFR that is approved in Europe for the treatment of locally advanced or metastatic non-small cell lung cancer with activating mutations of EGFR. Given that EGFR is expressed at high levels in normal and malignant thyroid tissue [Mitsiades *et al.* 2006] and that gefitinib has activity against ATC cell lines [Nobuhara *et al.* 2005], Pennell and colleagues investigated the efficacy of gefitinib administered at a dose of 250 mg daily in 27 patients with locally advanced or metastatic thyroid cancer (DTC, $n=18$; MTC, $n=4$; ATC, $n=5$) [Pennell *et al.* 2008]. No objective responses were observed, but six patients (22%) had SD of at least 6 months duration. The median PFS was 3.7 months, with a median OS of 17.5 months; among patients with well differentiated DTC, the median PFS was 3.9 months, with a median OS of 27.4 months. Therapy was generally well tolerated, the only reported grade ≥ 3 side effects being rash (7%) and diarrhea (4%).

Imatinib. Imatinib (Gleevec, Glivec) is a tyrosine kinase inhibitor with activity against several targets, including Bcr-Abl, PDGFR- α/β , c-KIT, and

RET [Savage *et al.* 2002]. It is currently approved for the treatment of chronic myeloid leukemia, gastrointestinal stromal tumors, and dermatofibrosarcoma protuberans. Two small phase II studies have investigated the efficacy of imatinib in patients with advanced MTC [De Groot *et al.* 2007; Frank-Raue *et al.* 2007]. De Groot and colleagues enrolled a total of 15 patients who were treated with imatinib 600 mg daily with the option to increase to 800 mg daily if there was evidence of disease progression [De Groot *et al.* 2007]. No objective radiographic responses were observed. Among five patients treated for 12 months, one patient had PD and four had SD; all patients with SD had SD at study entry. Serious adverse events occurred in two patients (13%) with grade 4 laryngeal mucosal swelling requiring emergent tracheostomy within 1 week of starting imatinib. Hypothyroidism was also common (all grades, 60%; grade 3, 20%). Frank-Raue and colleagues enrolled nine patients with unresectable, progressive MTC to a trial of therapy with imatinib 600 mg daily [Frank-Raue *et al.* 2007]. No objective responses were observed; five patients (56%) had SD for ≥ 6 months and one patient had SD for ≥ 12 months. Treatment was generally well tolerated in this cohort, with no grade ≥ 3 toxicities reported. One patient did discontinue treatment after 2 weeks because of worsening of pre-existing diarrhea. Common toxicities included rash (22%), vomiting and abdominal pain (33%), diarrhea (22%), and edema (33%).

Imatinib has also been investigated in a phase II study in patients with advanced ATC (2010) [Ha *et al.* 2010]. A total of 11 patients were enrolled into the study, all of whom had overexpression of PDGFR as determined by initial immunohistochemical screening of tumor samples. Patients were treated with imatinib 400 mg bid, with the option to reduce the dose as low as 300 mg daily. Among eight patients who were evaluable for response, two had a PR (18%) at 8 weeks and SD was seen in four (36%). Overall PFS at 6 months was estimated at 27%, with a 6-month OS of 46%. The most common grade 3 toxicities reported were lymphopenia (44%), edema (27%), anemia (18%), nausea/emesis (18%), myalgia/arthralgias (18%), electrolyte abnormalities (18%), and syncope (18%). No grade 4 or 5 toxicities were reported.

Vascular targeting agents

Fosbretabulin. Fosbretabulin (Zybrestat, combretastatin A4 phosphate, CA4P) is a novel

tubulin inhibitor derived from the bark of the African bushwillow tree, *Combretum caffrum*. Xenograft mouse models have suggested that fosbretabulin targets the tumor vasculature, likely via direct toxicity to vascular endothelial cells [Chaplin *et al.* 1999; Grosios *et al.* 1999]. Tumor vasculature typically lacks the organized smooth muscle and pericyte support of mature vessels, making the drug more toxic to these vessels. Early phase I and II studies have demonstrated reduced tumor blood flow, particularly in highly vascular tumors such as thyroid cancers [Akerley *et al.* 2007; Stevenson *et al.* 2003]. Mooney and colleagues reported on a phase II trial of fosbretabulin in ATC, which recruited 26 patients with locally advanced or metastatic disease [Mooney *et al.* 2009]. Patients were eligible if they had local disease progression after initial combined modality therapy, usually including systemic chemotherapy and radiation, or if they had metastatic disease with progression after no more than a single systemic chemotherapy. Exclusion criteria included active brain metastases, symptomatic peripheral vascular disease, history of angina or myocardial infarction, heart failure, and uncontrolled hypertension. Treatment consisted of fosbretabulin at a dose of 45 mg/m² by intravenous infusion on days 1, 8, and 15 of a 28-day cycle. At study conclusion, no objective radiographic responses (CR, PR) were observed; seven patients (27%) had SD after two cycles of therapy. Median survival among patients treated with fosbretabulin was 4.7 months and overall survival was 23% at 12 months. The study did not achieve its primary endpoint, which was a doubling of overall survival from the historical norm of 4–6 months. Treatment was generally well tolerated, the most common reported grade ≥ 3 toxicities being myelosuppression (grade 3, 35%; grade 4, 4%) and tumor pain (grade 3, 12%; grade 4, 4%). QTc prolongation ≥ 450 ms was seen in four patients (15%), including one patient who discontinued the study. No ventricular arrhythmia or cardiac ischemia was reported in this selected population of patients. A study of fosbretabulin in combination with paclitaxel and carboplatin in the treatment of ATC is ongoing [ClinicalTrials.gov identifier: NCT00507429].

Targeted radiotherapy

[[90]Yttrium-DOTA]-TOC. Octreotide is a long-acting somatostatin analog that can suppress

thyroid cell growth *in vitro*, but has demonstrated limited activity when used in patients with advanced thyroid cancer [Zlock *et al.* 1994]. Iten and colleagues recently reported a single-center phase II experience with [(90)yttrium-DOTA]-TOC, which is a radioconjugated somatostatin analog, in patients with octreotide-scan positive, metastatic, radioiodine-refractory DTC (PTC, $n=5$; FTC, $n=17$; unknown, $n=2$) [Iten *et al.* 2009]. Patients received 3.7 GBq/m² per cycle, with the option to receive additional treatments if there was a decrease in serum thyroglobulin or if the patient reported a significant improvement in quality of life after treatment. Seven patients (29%) had a decrease in serum thyroglobulin following treatment; the median decrease among responders was 49%, whereas the median increase among non-responders was 37%. Patients who responded to therapy had a significantly longer median survival than nonresponders (42.4 months compared with 13.7 months, $p=0.04$). Common toxicities included cytopenias (33%), nausea (17%), and renal toxicity (17%). One patient developed grade 4 renal toxicity following treatment. Radiographic responses to treatment were not reported.

Redifferentiation agents

Thiazolidinediones. The thiazolidinediones are a class of PPAR- γ agonists originally investigated and approved as insulin-sensitizing agents. Interest in using these drugs as an adjunct to the treatment of radioiodine refractory thyroid cancer has stemmed largely from preclinical studies that have shown upregulation of the sodium-iodide symporter and apoptosis in FTC cell lines [Frohlich *et al.* 2005] as well as inhibition of cell growth in the ATC cell line DRO [Shimazaki *et al.* 2008]. Additionally, PPAR- γ expression is known to be dysregulated in FTC cells; a chromosomal rearrangement of the PPAR- γ gene with PAX8 (PAX8/PPAR- γ) has been observed in approximately 40% of FTC cells, which results in the loss of tumor suppressor function [McIver *et al.* 2004].

Kebebew and colleagues recruited 21 patients to a study of rosiglitazone as a redifferentiating agent in patients with histologically confirmed DTC with either unresectable disease or an increasing serum thyroglobulin level in the absence of a positive radioiodine scan [Kebebew *et al.* 2009]. Rosiglitazone was started at 4 mg

daily for 1 week and increased to 8 mg daily for an additional 7 weeks. I-131 scans were performed at the end of the 8 weeks. Significant dose-limiting toxicities were not observed. No patient had an objective radiographic response at 3 months, but five patients did demonstrate either increased radioiodine uptake or a decreased thyroglobulin level. The authors concluded that rosiglitazone may increase radioiodine uptake in a selected group of individuals, but they were unable to demonstrate that this resulted in any overall clinical benefit.

Inolitazone (CS-7017) is an orally bioavailable thiazolidinedione related to rosiglitazone that has shown synergistic activity against ATC cell lines when given in combination with paclitaxel [Copland *et al.* 2006]. This combination is currently under investigation in a phase I/II study of patients with advanced ATC [ClinicalTrials.gov identifier: NCT00603941].

Histone deacetylase inhibition. Several inhibitors of histone deacetylases (HDACs) are currently under development. Vorinostat (L-001079038, SAHA, Zolinza) is a second-generation oral molecular inhibitor that binds to the catalytic domain of HDACs and is currently FDA approved in patients with cutaneous T-cell lymphoma with persistent disease following at least two prior systemic therapies. Vorinostat alone has been shown to induce growth arrest in thyroid cancer cell lines [Mitsiades *et al.* 2005] and may also sensitize tumor cells to doxorubicin mediated cytotoxicity. Our group reported on a phase II trial of vorinostat in patients with histologically confirmed radioiodine refractory DTC or MTC who had received no more than two prior systemic therapies for their disease [Woyach *et al.* 2009]. Subjects were eligible if they had at least one RECIST-defined target lesion, had not received systemic treatment or radiation treatment in the last 4 weeks, and had not received radioiodine within the last 24 weeks. Patients were treated in 21-day cycles with vorinostat 200 mg orally twice daily for 2 weeks followed by 1 week off. Response to treatment was assessed every 12 weeks by computed tomography or magnetic resonance imaging, with objective responses defined according to RECIST. A total of 19 patients were recruited to the study (16 DTC, 3 MTC), of whom 16 were evaluable for response. No objective radiographic responses were observed in any patient,

and nine patients had SD as best overall response. Ten patients underwent dose escalation to 300 mg twice daily, but all patients had to be de-escalated at some point. Common reasons for dose reduction were thrombocytopenia (42%), hyperglycemia (11%), diarrhea (5%), hypocalcemia (5%), weakness (5%), nausea (5%), and elevated international normalized ratio (5%). Grade ≥ 3 toxicities included hyperglycemia and hypertension (5%) and aortic thrombus (5%). All patients who had SD withdrew from the trial because of adverse events or concerns about quality of life.

Romidepsin (desipeptide) is a potent HDAC inhibitor that has demonstrated activity in thyroid cancer cell lines [Furuya *et al.* 2004]. It was studied in 20 patients with radioiodine-refractory DTC (PTC, $n=8$; FTC, $n=1$; HTC, $n=11$) in a phase II study administered at a dose of 13 mg/m² intravenously on days 1, 8, and 15 every 28 days. Two patients (10%) demonstrated restoration of radioiodine reuptake [Sherman *et al.* 2009]. No radiographic objective responses were documented. Two grade 4/5 adverse events that were possibly related to romidepsin therapy were reported, including one patient with sudden death and one patient with a grade 4 pulmonary embolus. The study was closed early because of poor patient accrual following the sudden death of that one patient.

DNA demethylating agents. Epigenetic modulation via DNA methylation is well described in thyroid cancer. Azacitidine (Vidaza) is a parenterally administered nucleoside analogue of cytidine that is incorporated into DNA, where it irreversibly binds and inhibits DNA methyltransferase, resulting in demethylation and potentiating the expression of genes that have been aberrantly repressed in tumor cells. Azacitidine is perhaps best studied in hematologic malignancies, where it is approved for the treatment of myelodysplastic syndromes and chronic myelomonocytic leukemia. Preclinical work with selected thyroid carcinoma cell lines that have reduced expression of sodium-iodine symporter mRNA and TSH receptor mRNA has shown that treatment with azacitidine can be associated with restoration of mRNA transcription and iodide transport [Xing *et al.* 2003; Venkataraman *et al.* 1999]. A phase I clinical trial to investigate the effect of 4 weeks of azacitidine on I-131 uptake is currently enrolling [ClinicalTrials.gov identifier: NCT00004062].

Decitabine (Dacogen) belongs to the same class of pharmacologic agents as azacitidine and is also incorporated into DNA as a cytidine analogue, where it inhibits DNA methyltransferase. It is currently approved for the treatment of myelodysplastic syndromes. A phase II study of decitabine in radioiodine refractory patients with metastatic PTC or FTC has completed accrual and results are pending [ClinicalTrials.gov identifier: NCT00085293]. The primary aim of the study is to determine the effect of decitabine in restoring radioiodine uptake and has the secondary aim of determining the efficacy of radioiodine therapy in patients with restored radioiodine uptake.

Thalidomide and lenalidomide

Thalidomide. Thalidomide (Synovir, Thalomid) is a synthetic derivative of glutamic acid with teratogenic, immunomodulatory, and anti-angiogenic properties. Thalidomide functions as a prodrug and is metabolized into its active forms in a species-specific and likely tissue-specific manner. Observed drug effects include inhibition of the effects of tumor necrosis factor α , interleukins, and interferons, and inhibition of VEGF and basic fibroblast growth factor (bFGF). It is currently FDA approved for the treatment of multiple myeloma.

Given the vascular nature of thyroid cancer, Ain and colleagues reported the results of a phase II study of thalidomide in 36 patients with radioiodine-refractory DTC or MTC and distant metastases, with evidence of tumor growth $>30\%$ over the previous 1 year [Ain *et al.* 2007]. Thalidomide was started at a dose of 200 mg orally daily and gradually increased to a maximum of 800 mg daily as tolerated. Response to therapy was assessed by computed tomography scan every 2 months while on therapy. PRs were seen in five patients (14%), with SD seen in an additional nine patients (25%). The median duration of a PR was 4 months and the median duration of SD was 6 months. Fatigue was the most common reported side effect (grade 1/2, 69%; grade ≥ 3 , 8%), followed by peripheral neuropathy (grade 1/2, 53%; grade ≥ 3 , 6%). Other serious adverse events included grade ≥ 3 infections ($n=4$), pericardial effusion ($n=1$), and pulmonary embolus ($n=1$). Median survival among patients who responded to therapy was 23.5 months compared with 11 months among

nonresponders, though this difference was not statistically significant.

Lenalidomide. Lenalidomide (Revlimid) is an analog of thalidomide, currently FDA approved for the treatment of multiple myeloma. It has been associated with an improved toxicity profile and has a reduced incidence of somnolence, fatigue, and peripheral neuropathy when compared with thalidomide. A phase II study of lenalidomide administered at 25 mg daily in radioiodine-refractory PTC and FTC has recently completed accrual [ClinicalTrials.gov identifier: NCT00287287]. Preliminary results for patients with distantly metastatic, radioiodine-refractory DTC were reported in abstract form by Ain and colleagues [Ain *et al.* 2008]. All patients had radiographic evidence of disease progression in the 12 months prior to study enrollment. Among 21 patients enrolled by December 2007, 18 had been treated for more than 6 months and were evaluable for response. PRs were seen in four patients (19%), with SD seen in an additional eight patients (38%). The most common grade ≥ 3 toxicities were hematological (neutropenia, 38%; thrombocytopenia, 19%) and responded to dose reduction.

Conclusion

Significant progress has been made in the last few years with respect to the development of new and targeted therapies for thyroid cancer on the basis of the underlying molecular pathophysiology. Vandetanib represents the first of these new targeted agents to receive FDA approval, and is now a viable treatment option for patients with advanced, unresectable, progressive or symptomatic MTC. It is strongly recommended that patients with advanced, radioiodine-refractory DTC or ATC be offered enrollment in a clinical trial. However, not all patients are either able or willing to be enrolled in a clinical trial. Both the American Thyroid Association (ATA) and the National Cancer Centers Network (NCCN) have endorsed the consideration of sunitinib, sorafenib, and pazopanib for the treatment of patients with DTC or MTC, given the promising results from early phase II clinical trials [Tuttle *et al.* 2011; Cooper *et al.* 2009; Kloos *et al.* 2009b]. Clinicians will need to familiarize themselves with the potential toxicities of these agents and the management of these toxicities. The coming years promise further developments in the treatment of thyroid cancer and the expansion of the armamentarium available to the practicing clinician.

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Conflict of interest statement

The authors declare no conflicts of interest in preparing this article.

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