Anaplastic thyroid cancer: molecular pathogenesis and emerging therapies

Robert C Smallridge¹, Laura A Marlow² and John A Copland²

¹Division of Endocrinology, Department of Internal Medicine and ²Department of Cancer Biology, Mayo Clinic, 4500 San Pablo Road, Jacksonville, Florida 32224, USA

(Correspondence should be addressed to R C Smallridge; Email: smallridge.robert@mayo.edu)

Abstract

Anaplastic thyroid cancer (ATC) is a rare malignancy. While external beam radiation therapy has improved locoregional control, the median survival of ~ 4 months has not changed in more than half a century due to uncontrolled systemic metastases. The objective of this study was to review the literature in order to identify potential new strategies for treating this highly lethal cancer. PubMed searches were the principal source of articles reviewed. The molecular pathogenesis of ATC includes mutations in *BRAF*, *RAS*, catenin (cadherin-associated protein), beta 1, *PIK3CA*, *TP53*, *AXIN1*, *PTEN*, and *APC* genes, and chromosomal abnormalities are common. Several microarray studies have identified genes and pathways preferentially affected, and dysregulated microRNA profiles differ from differentiated thyroid cancers. Numerous proteins involving transcription factors, signaling pathways, mitosis, proliferation, cell cycle, apoptosis, adhesion, migration, epigenetics, and protein degradation are affected. A variety of agents have been successful in controlling ATC cell growth both *in vitro* and in nude mice xenografts. While many of these new compounds are in cancer clinical trials, there are few studies being conducted in ATC. With the recent increased knowledge of the many critical genes and proteins affected in ATC, and the extensive array of targeted therapies being developed for cancer patients, there are new opportunities to design clinical trials based upon tumor molecular profiling and preclinical studies of potentially synergistic combinatorial novel therapies.

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Incidence

Thyroid cancers, while uncommon, are increasing in prevalence in this country due, at least in part, to earlier detection from imaging. In 2008, there are estimated to be 37 340 new cases, with 1590 deaths (Jemal *et al.* 2008). Anaplastic thyroid carcinomas (ATCs) are estimated to comprise 1–2% of thyroid malignancies. Unfortunately, their rapid onset and fulminant course have not altered their detection or outcomes. This review will analyze their molecular pathogenesis, the results of preclinical studies and clinical management, and discuss possible new treatment strategies.

Molecular pathogenesis

Mutations

More than 90% of all thyroid cancers derive from the thyroid follicular cell, including papillary (PTC), follicular (FC), or Hurthle cell. ATC may derive

de novo or from pre-existing PTC or FC. A small number of gene mutations have been identified, and there appears to be a progression in mutations acquired during dedifferentiation. Several mutations occurring in PTC (e.g., *RAS* and *BRAF*) are also seen in ATC, suggesting these are early events (Nikiforov 2004). Late mutations include *TP53*, catenin (cadherin-associated protein), beta 1, and *PIK3CA*, suggesting that one or more of these mutations contribute to the extremely aggressive behavior of ATC. By contrast, the *RET/PTC* rearrangements found in childhood and radiation-induced PTCs, and the *PAX8/PPARG* fusion protein detected in follicular carcinoma, are not observed in poorly differentiated and ATCs (Nikiforov 2004; Table 1).

RAS

The *RAS* family of oncogenes regulate two important signaling pathways in thyroid cancer, the

Table 1 Mutations in anaplastic thyroid carcinomas

References	Ras	BRAF	TP53	Catenin ^a , beta 1	PIK3CA	Axin	APC	PTEN
Fagin <i>et al.</i> (1993)			5/6					
Donghi <i>et al.</i> (1993)			5/7					
Zou <i>et al.</i> (1993)			1/5					
Zedenius <i>et al.</i> (1996)			1/4					
Garcia-Rostan <i>et al.</i> (1999)				19/31				
Garcia-Rostan <i>et al.</i> (2003)	15/29							
Fukushima <i>et al.</i> (2003)	2/7	0/7						
Namba <i>et al.</i> (2003)		2/6						
Nikiforova <i>et al.</i> (2003)		3/29						
Soares <i>et al.</i> (2004)		6/17						
Begum <i>et al.</i> (2004)		8/16						
Kurihara <i>et al.</i> (2004)				1/22		18/22	2/22	
Quiros <i>et al.</i> (2005)	1/8	5/8						
Garcia-Rostan <i>et al.</i> (2005)					16/70			
Takano <i>et al.</i> (2007a)		4/20						
Mitsiades <i>et al.</i> (2007)		1/7						
Hou <i>et al.</i> (2007) and Liu <i>et al.</i> (2008)	4/50	14/49			6/50			8/48
Santarpia <i>et al.</i> (2008)	2/36	9/36			5/36			2/36
Costa <i>et al.</i> (2008)	13/36	9/36						
Total (%)	37/166 (22%)	61/231 (26%)	12/22 (55%)	20/53 (38%)	27/156 (17%)	18/22 (82%)	2/22 (9%)	10/84 (12%)

^a(cadherin associated protein)

RAS–RAF–MEK–ERK and the PI3K/AKT1 pathways. RAS mutations occur in both benign and malignant thyroid tumors (Fagin *et al.* 1993), with variable frequency in ATCs. Frequencies range from 6% up to half of cases (Fukushima *et al.* 2003, Garcia-Rostan *et al.* 2003, Nikiforov 2004, Quiros *et al.* 2005, Hou *et al.* 2007, Costa *et al.* 2008, Liu *et al.* 2008, Santarpia *et al.* 2008).

BRAF

BRAF is a serine/threonine kinase involved in cell proliferation. The most common mutation in papillary thyroid cancer is BRAF. It, too, is highly variable in ATC, ranging from 0 to 50%, due, in part, to small sample sizes and different laboratory methodologies. When ATCs with a papillary component were examined, this mutation was observed in both the differentiated and undifferentiated regions (Begum *et al.* 2004, Soares *et al.* 2004, Takano *et al.* 2007a)

TP53

The TP53 tumor suppressor gene (chromosome 17p) increases the cyclin kinase inhibitor, p21, promoting cell cycle arrest at G1/S, but is commonly mutated in cancer. Mutations impair TP53 transcriptional activity, and occur in 55% of ATCs (Donghi *et al.* 1993, Fagin *et al.* 1993, Zou *et al.* 1993, Zedenius *et al.* 1996,

Nikiforov 2004). Elevated levels can also be detected by immunohistochemistry (IHC; Lam *et al.* 2000, Quiros *et al.* 2005), which may reflect altered function without mutation (Malaguarnera *et al.* 2007, Wreesmann & Singh 2008). Boltze *et al.* (2002) examined a common polymorphism (codon 72, exon 4) of the TP53 gene. Homozygous proline was present in 100% of 22 ATC patient tumors, but in no benign nodules or differentiated thyroid cancers, suggesting this polymorphism may be a risk factor.

Wnt pathway genes

The catenin (cadherin-associated protein), beta 1 gene is involved in Wnt signaling and cell–cell adhesion. Both mutations and abnormal nuclear localization are seen in malignancies. Mutations were detected in 61% of 31 ATC cases (with nuclear localization in half with mutations) (Garcia-Rostan *et al.* 1999), while abnormalities were detected in 32% of poorly differentiated (Garcia-Rostan *et al.* 2001), but no papillary or follicular thyroid cancers (Garcia-Rostan *et al.* 2001, Miyake *et al.* 2001). By contrast, Rocha *et al.* (2003) evaluated 17 poorly differentiated (but no anaplastic) thyroid cancers. They found loss of cadherin 1, type 1, E-cadherin (epithelial) membrane expression, but no nuclear localization of catenin (cadherin-associated

protein), beta 1 and no mutation in either gene. Kurihara *et al.* (2004) found catenin (cadherin-associated protein), beta 1 mutations in only 1 out of the 22 patients, but nuclear and/or cytoplasmic staining was common. Axin 1 (chromosome 16p13.3) is a scaffold protein acting as a tumor suppressor in the Wnt pathway. Kurihara *et al.* (2004) found frequent abnormalities with 41 mutations in 82% of 22 ATC patient samples. By contrast, adenomatous polyposis coli (APC), which complexes with Axin 1, is mutated only infrequently (Kurihara *et al.* 2004).

PIK3CA

PI3K is a kinase that phosphorylates AKT1 isoforms. They, in turn, phosphorylate substrates involved in cell growth and survival. PIK3CA encodes a catalytic subunit of PI3K (Santarpia *et al.* 2008). PIK3CA (located on 3q26.3) was mutated in 12–23% of ATC cases (Garcia-Rostan *et al.* 2005, Hou *et al.* 2007, Liu *et al.* 2008, Santarpia *et al.* 2008) and copy gains occurred in 38–61% (Hou *et al.* 2007, Liu *et al.* 2008, Santarpia *et al.* 2008). Mutations were infrequently present in follicular (6–13%) or papillary (1–3%) tumors (Garcia-Rostan *et al.* 2005, Hou *et al.* 2007, Wang *et al.* 2007, Liu *et al.* 2008). AKT1 was activated in most ATC cases (85–93%) (Garcia-Rostan *et al.* 2005, Santarpia *et al.* 2008) and ERK was activated in 65% (Santarpia *et al.* 2008). Phosphatase and tensin homology encoding chromosome 10 (PTEN) negatively regulates the PI3K pathway was mutated in 17% of 48 cases (Liu *et al.* 2008).

RET

RET, a proto-oncogene that encodes a receptor tyrosine kinase, is mutated in familial medullary thyroid carcinoma, and RET/PTC rearrangements occur in PTC. RET was amplified in seven ATCs (Nakashima *et al.* 2007), and three had increased TP53 by IHC, suggesting genomic instability from aberrant TP53 may have led to the RET abnormality.

Cytogenetic analyses

Cytogenetic metaphase analysis of ATC was reported more than 20 years ago (Mark *et al.* 1987), while comparative genomic hybridization (CGH) has permitted detection of deletions or amplifications in chromosomal regions not identified by conventional cytogenetics or FISH analysis (Inazawa *et al.* 2004). Modification using array-CGH (a-CGH) has further improved genome-wide detection of regions that may harbor cancer associated genes (Inazawa *et al.* 2004). While the methodology does not identify specific genes,

it locates regions of chromosomal instability for more focused investigations (Pinkel & Albertson 2005).

The role of a-CGH in clinical practice, whether whole-genome screening or targeted arrays, is currently being discussed (Bejjani & Shaffer 2006, Done 2006, Veltman & de Vries 2006). While copy number variations are common and can potentially confound data interpretation (Gunn *et al.* 2007), the potential to integrate a-CGH results with histopathology and other molecular markers, and to relate the laboratory findings with patient diagnosis, prognosis, and therapy, will bring an individualized approach to cancer therapy in clinical practice (Climent *et al.* 2007).

Chromosomal aberrations in ATC

Mark *et al.* (1987) performed metaphase analyses on five ATC patients; three had 10q aberrations, and two had 3p25 abnormalities (Table 2). RET resides on 10q11.2 and PTEN on 10q23.3, while hypoploidy of 3p is common in follicular and Hürthle cell cancers. In two patients with complex clonal karyotypes, abnormalities were detected in 16 separate chromosomes, with both tumors having clones with losses of chromosome 8 and both sex chromosomes (Jenkins *et al.* 1990).

In the first report of CGH in ATC patients, DNA copy number changes were found in 11 out of the 13, gains were noted in 10 out of the 13, deletions in 3 out of the 13, both gains and deletions in two, and chromosome deletion in one. Overall, there were 27 gains and five deletions. Common gains included chromosomes 7p22-pter, 8q22-qter, and 9q34-qter, regions that harbor the *PDGR-α*, *myc*, and *ABL1* and *VAV2* genes respectively (Hemmer *et al.* 1999).

CGH studies in nine cases of ATC showed five had giant cell and four had spindle cell histology, with none having antecedent differentiated thyroid cancer (Wilkens *et al.* 2000). The most common gains or losses occurred in chromosomes 8p and 8q, and amplification of 5p. Fourteen additional chromosomes had imbalances in one or two of the tumors, and two cell lines had similar findings. The authors were uncertain of the role of chromosome 5 and 8 aberrations in ATC, but noted that p45^{skp2} and c-myc genes resided on 5p and 8q24 (Wilkens *et al.* 2000). Kitamura *et al.* (2000) detected allelic losses in 16 out of the 21 ATCs studied, including loss of 22q in 38%. CBX7, a putative tumor suppressor, resides on 22q13.1 and is markedly under expressed in ATC (Pallante *et al.* 2008). Loss of 22q has been shown by others (Table 2).

CGH was performed on 15 well differentiated, 12 poorly differentiated, and 15 ATC samples to look for changes associated with tumor progression

Table 2 Cytogenetic analyses in patients with anaplastic thyroid cancer

References	Patients (n)	Cell lines (n)	Gains	Losses	Both	Chromosomes
Jenkins et al. (1990)	2	–				Complex clonal karyotype; loss of eight (both); x; y –2, –4, –8, –9, +11, –13, +14, –14, +15, –17, –19, –20, –21, –22, –x, –y
Hemmer et al. (1999)	13	–	10/13	3/13	2/13	+7p (31%); +8q (23%); +9q (23%)
Wilkens et al. (2000)	9	2				Gains in 14 chromosomes; losses in eight chromosomes; most common: 5p amplified; 8p, 8q gains or losses
Kitamura et al. (2000)	21					Allelic losses 16/21; Losses: 1q (40%); 9p (58%) ; 11p (33%); 11q (33%); 17p (44%); 17q (43%); 19p (36%); 22q (38%)
Wreesmann et al. (2002)	15	–				Early Gains: 5, 8, 19; losses: 8, 22; intermediate: gains: 1, 6, 9, 17, 20; losses: 1, 2, 6, 13 late: gains: 3p, 11q; losses: 5q
Miura et al. (2003)	10	1	5/10	2/10		Gains: 1, 4, 5, 6, 7, 10, 14, 16, 19, 20, 21, x; losses: 3p; 9q
Rodrigues et al. (2004)	7	–				Gains in all chromosomes; losses less common; high amplification in: 3p, 5p, 6p, 9p, 12p, 14q, 18p
Rodrigues et al. (2007)	–	3				Amplifications: 3q24; 5p; 7p; 7q; 12p; 14q; 20
Lee et al. (2007)	–	8				Frequent: gains: 8q; 11q; 19; 20q; losses: 4q; amplification: 8q
Lee et al. (2008)	27					Gains: 1q21; 6p22-p21; 7q11.22-11.23; 11q13; 12q13; 16p11.2; 17q21; 19p13; 19q13. 1-q13.2; 20q11.2; 20q13.12; 22q11.21; 22q.13.1 Losses: 4q12-q13.1; 4q28.3; 13q21.2-q21.31

(Wreesmann et al. 2002). Both frequency and number of CGH abnormalities were identified in more undifferentiated tumors. All three tumor types had chromosomal gains at 5p15 (telomerase reverse transcriptase location), 5q11–13, 19p, 19q (the zinc finger TF ZN331 on 19q13.3, a frequent break point, is common in follicular adenomas and often involved in a translocation with 5q13), and losses at 8p. The poorly differentiated and anaplastic cancers had additional gains at 1p34–36 (stathmin location), 6p21 (location of p38 α - and δ MAP kinases), 9q34, 17q25 (survivin location), 20q, and losses at 1p11–31, 2q32–33, 4q11–13, 6q21, and 13q21–31. Unique to ATC were gains at 3p13–14 and 11q13 (CCND1 (cyclin D1) and FOSL1 location), and losses at 5q11–31. The authors cited a number of potentially affected genes identified in regions of chromosomes 1p, 3p, 5q, 11q, 13q, 17q, 20q including HTIF1, Wnt5A, APC, Wnt 11, LGALS3BP (galectin-3 binding protein; mediates cell–cell and cell–matrix interactions), SIRT8 (a proto-oncogene of unknown function), MMP9, MMP24, LAMBA5, and syndecan-4 (complexes with CXCR4).

Miura et al. (2003) performed CGH on ten ATC samples, six associated with papillary and two with

follicular thyroid cancer. Five patients had 24 cytogenetic abnormalities, 22 were gains and two were losses. The most frequent aberration was a gain in 1q (common in many cancers) in three out of the ten cases. The two losses were in 9q22.3–32 and 3p12.2–21.3, a region also detected by others (Wreesmann et al. 2002). The authors were unable to show any correlation of the CGH aberration with survival or other clinical parameters (Miura et al. 2003).

Rodrigues et al. (2004) examined seven ATCs; all had chromosomal imbalances. Three were gains at 20p and 20q, and losses of Xp in six cases, gains at 3q and 5p in five cases. 7q31 was often deleted. Important genes included *PI3K*, *BCL6* (codes Zn finger protein that represses transcription) and *SST* at 3q, the invasion and metastases genes (*MMP9* and *MMP24*), cell cycle and checkpoint gene (*STK15*) at 20q, and *Fra7G* at 7q.

Three recent reports examined ATC cell lines by array-CGH. Rodrigues et al. (2007) found high level amplification at 3q24, 5p, 7p (EGFR location), 7q (MET and BRAF location), 12p (K–RAS location), 14q, and 20. Lee et al. (2007) tested two novel and six established ATC cell lines. Gains and losses were

prevalent throughout with high level amplification at 1p, 8q (myc location), 9q, 11q, and 20q. FISH analysis showed gains in seven out of the eight cell lines for *UBE2C* (20q13.12), a gene overexpressed in ATC (Pallante *et al.* 2005). Lee *et al.* (2008) performed array-CGH to analyze for genome-wide copy number changes in 27 ATC tissues. An average of 44 copy number changes was detected in each tumor. Frequent gains were noted at 11q13 (site of *CCND1* (cyclin D1)), which was expressed in 18 out of the 27 tissues and also *FOSL1*, 16p11.2, 20q11.2, and 20q13.12 (*UBE2C* location). Loss of the *CDKN2A* locus on 9p21.3 was confirmed by finding undetectable p16 protein in 24 out of the 27 ATC tissues (Lee *et al.* 2008).

Liu *et al.* (2008) analyzed up to 51 ATCs for copy number gains in receptor tyrosine kinase genes and in *PI3K* and *BRAF* pathway genes. Copy number gains were detected in *EGFR* and *VEGFR1* (46%); *PDGFRB*, *PIK3Cα* and *PIK3Cβ* (38%); *PDGFRA* (24%); *KIT* (22%); *PDK1* (20%); *AKT1* (19%); *VEGFR2* (17%); and *MET* (12%). Overall, at least one copy number gain was present in 80.4% of cases (Liu *et al.* 2008).

These studies emphasize that ATCs have a high degree of numeric and structural genomic disarray. These abnormalities likely result in substantial gene expression changes in many genes. This high degree of disarray and the rarity of these tumors make it difficult to determine which targets of instability are selected for or convey growth advantages.

MicroRNA

A new level of the regulation of cell growth and survival has emerged with the recent discovery of microRNAs (Table 3). These molecules are small (~ 22 nucleotides), single stranded, non-coding RNAs. There are estimated 300–1000 microRNAs, each of which may bind to several hundred gene targets. Most often, they appear to repress gene expression post-transcriptionally (so that protein, not RNA, levels are reduced) (Esquela-Kerscher & Slack 2006), but they can also affect mRNA degradation (Volinia *et al.* 2006). MicroRNAs can act as oncogenes or tumor suppressor genes and, given their pleiotropic effects, can perform both functions in the same cell (Calin *et al.* 2004, Calin & Croce 2006, Esquela-Kerscher & Slack 2006).

MicroRNA (miR) expression can be altered in several ways, such as abnormalities in their large RNA precursors, their tendency to reside in cancer-associated genomic regions, or by epigenetic silencing (Calin *et al.* 2004, Calin & Croce 2006, Meng *et al.* 2006, Saito & Jones 2006, Zhang & Coukos 2006).

MicroRNAs play an important role in oncogenesis, and expression profiles or fingerprints may improve the specificity of diagnosis, therapy, and prognosis (Lu *et al.* 2005, Calin & Croce 2006, Volinia *et al.* 2006, Blower *et al.* 2008).

MicroRNAs may become therapeutic targets themselves (Jeyaseelan *et al.* 2007), and both overexpression and inhibition of their activity have influenced cellular growth and tumor responses to other chemotherapy agents (Meng *et al.* 2006, Blower *et al.* 2008). Downstream targets of these miRs are known in some instances, and the manipulation of miR expression can directly affect target genes. For example, miR-21 is overexpressed in cholangiocarcinoma, the miR-21 precursor reduces PTEN expression, and inhibition of miR-21 reduces the p85 subunit of PI3K (Meng *et al.* 2006). Similarly, miR-21 is overexpressed in glioblastomas, and its inhibition increases caspases and apoptosis (Chan *et al.* 2005).

While most studies in cancer have shown that miRs are underexpressed, overexpression may also contribute to oncogenesis or tumor progression, as in thyroid malignancies. Most thyroid cancers derive from the follicular cell. He *et al.* (2005) demonstrated that miRs are up-regulated in papillary thyroid carcinoma (PTC). The most highly expressed were miRs -221, -222, and -146. Other miRs overexpressed greater than twofold were -21, -220, -181, and -155. Underexpressed miRs included -138, -219, -26a, and -345. Pallante *et al.* (2005) identified five overexpressed miRs and suggested that -221, -222, and -181b could be a signature for PTC. The observation on miR-221 and -222 was extended by showing that forced overexpression would reduce p27^{kip1} protein and increase G1- to S-phase transition (Visone *et al.* 2007a). These observations suggest that miR-221 and -222 could be therapeutic targets in PTC. Four microRNAs (-197, -346, -192, and -328) were found overexpressed in FTC (Weber *et al.* 2006).

Four miRs (-30d, -125b, -26a, and 30a-5p) were underexpressed in ATC, but not in PTC (Visone *et al.* 2007b). Both miR-26a and -125b may target HMGA1 and HMGA2, two proteins involved in thyroid cell transformation (Berlingieri *et al.* 2002). These authors also showed that miR-138 was reduced, and miR-222 (increased in PTC) also was overexpressed (1.98 fold) in ATC.

Mitomo *et al.* (2008) showed that miRs-21, -146b, -221, and -222 were up-regulated in both ATC and PTC. MiR-21 targets E2F (involved in cell cycle and apoptosis) and inhibits PTEN. Down-regulated miRs included -26a, -138, -219, and -345. They also found that hTERT, a possible gene target for miR-138, was

Table 3 Some microRNAs altered in thyroid malignancies

Tumor	miR	Chromosome location	Functional effect		Targeted gene(s) studied
			Overexpression	Inhibition	
PTC	221 ↑	X Xp11.3	↑ colony formation	↓ cell growth	<i>KIT</i> , <i>p27^{kipl}</i> <i>p27^{kipl}</i>
	222 ↑				
	146 ↑				
	181b ↑				
FTC	197 ↑	1p13.3			
	346 ↑	10q23.2			
	192 ↑	11q13.1			
	328 ↑	16q22.1			
ATC	17–92 ↑	3p21 11q24.1 3p21 Xp11.3	↓ Cell growth		<i>HMGA1</i> <i>HMGA1</i>
	106a,b ↑				
	26a ↓		↓ Cell growth		
	125b ↓		↓ Cell growth		
	138 ↓				
	221 ↑				
	222 ↑				

References: (He *et al.* 2005, Mitomo *et al.* 2008, Pallante *et al.* 2006, Takakura *et al.* 2008, Visone *et al.* 2007a,b, Weber *et al.* 2006).
↑ = increased; ↓ = reduced

reduced and experimentally, hTERT protein could be reduced by overexpressing miR-138.

The miR-17–92 cluster of seven miRNAs as well as miR-106a and -106b were overexpressed in cell lines, and in three out of the six ATC patient samples, miR-17-3p and -17-5p were overexpressed (Takakura *et al.* 2008). Antisense inhibitors to miRs 17-3p, -17-5p, and -19a all inhibited cell growth, suggesting an oncogenic role for these miRs. Other miRs in the cluster (i.e., miR-19a and -19b) have PTEN as a target, and miR-106a and -106b have E2F1 as a target. Thus, there are multiple potential therapeutic targets in the miR-17–92 cluster (Takakura *et al.* 2008).

Although microRNA research is still young, it is gratifying that studies of miRs expression and function already exist for three principal types of thyroid cancer. The results suggest that each has several miRs distinct for that particular type of thyroid cancer. Thus, studies of miRs in thyroid oncogenesis hold promise to improve the evaluation and management of these tumors.

Functional genomics

Gene microarray

Gene expression microarrays have provided valuable insights into the molecular pathogenesis of thyroid cancer. Numerous studies have identified multiple over- and underexpressed genes involved in the regulation of critical cell functions (e.g., oncogenes, tumor suppressor genes, signaling pathways,

transcription factors, cell adhesion, thyroid functions, invasion, chemokines, etc. (Riesco-Eizaguirre & Santisteban 2007)). Various authors have employed this technology to identify differences amongst thyroid tumor types (Finley *et al.* 2004, Frattini *et al.* 2004, Mazzanti *et al.* 2004, Wreesmann *et al.* 2004, Giordano *et al.* 2005, Melillo *et al.* 2005, Eszlinger *et al.* 2007). In a meta-review of 21 studies, of 1785 genes differentially expressed, there were 39 felt to be most reliable amongst overlap comparisons. Only one of the studies involved anaplastic patients (Griffith *et al.* 2006).

Only a few studies have examined gene expression by microarray in ATC cell lines or patient samples. Onda *et al.* (2004b) utilized 11 ATC cell lines and ten patient samples. They found 31 genes over- and 56 genes underexpressed in cell lines. Functions affected pertinent to cancer cells include: Rab proteins localization (GD12), cell structure and endocytosis (destrin), microtubules (stathmin), and RaF inhibition (PBP; Onda *et al.* 2004b). Salvatore *et al.* (2007) did a microarray screen on five ATC samples, and validation on 22. They found 114 genes that distinguished ATC and PTC samples from normal thyroid, and 54 cell cycle progression and chromosome instability related genes up-regulated in ATC. Montero-Conde *et al.* (2008) examined seven ATC, six poorly differentiated, and 31 well-differentiated thyroid cancer patients by microarray. Pathways preferentially dysregulated in PDTC and ATC tissues were MAPK, cell cycle, focal adhesion, cytoskeleton, and TGFB1.

Pallante *et al.* (2008) detected a marked down-regulation of CBX7 expression by microarray and showed that mRNA for CDKN2A/p16, a tumor suppressor and target of CBX7, was highly overexpressed.

Proteins expression

While identifying altered expression of genes gives important clues, assessment of proteins expression yields more direct evidence of cellular functions that are dysregulated in cancer cells. Effects manifest themselves at the level of oncogene and tumor suppressor gene functions, receptors and signaling pathways, nuclear and organelle events, cell cycle, cell division, cellular death, adhesion, and migration. All of these functions have been studied to some degree in ATC, and these observations proffer diagnostic and therapeutic opportunities (Table 4).

Transcription factors

Thyroid transcription factors (*NKX2-1* and *FOXE1*) and paired box gene 8 (*Pax 8*) regulate expression of thyroid specific proteins including thyroglobulin, TSHR, thyroid peroxidase, and Na^+/I^- symporter. Their protein expression becomes reduced or absent as follicular cell dedifferentiation progresses. The mRNAs for *NKX2-1* (Ros *et al.* 1999, Takano *et al.* 2007b), *FOXE1* (Sequeira *et al.* 2001), and *Pax 8* (Ros *et al.* 1999) are also reduced or absent in ATCs.

Peroxisome proliferator-activated receptor gamma (PPARG) is a transcription factor affecting adipogenesis and differentiation. PPARG agonists have anti-tumor activity in a variety of malignancies (Copland *et al.* 2006b), and inhibition of PPARG function contributes to follicular thyroid cancer indicating tumor suppressive activity of PPARG (Kroll *et al.* 2000, Kato *et al.* 2006). ATC cell lines express functionally active nuclear PPARG protein (Hayashi *et al.* 2004, Aiello *et al.* 2006, Copland *et al.* 2006b) and the protein is present in at least 84% of patient ATC samples (unpublished observations).

Hepatocyte nuclear factor-1 α (HNF-1 α) plays a role in glucose metabolism, and may also influence carcinogenesis. Both HNF-1 α mRNA and protein were present in ATC cell lines. Transcripts were also present in most ATC but not PTC tissue samples, suggesting a role for HNF-1 α in more aggressive thyroid cancer (Xu *et al.* 2008).

The growth promoting transcriptional factor E2F1 is regulated by the tumor suppressor retinoblastoma gene, and its mRNA was up-regulated in 11 ATC cell lines, as well as most PTC lines (Onda *et al.* 2004a).

By contrast, transcription elongation factor A (S11) like 4 (TCEAL4) mRNA was down-regulated in both ATC cell lines and tissues (Akaishi *et al.* 2006). Id (inhibitor of DNA binding) proteins have a dominant negative effect on gene transcription and enhance cell proliferation. Up-regulation appears early and is detected in follicular adenomas, but the highest percentage was detected in ATC tissues (Kebebew *et al.* 2004).

The Y-box binding protein 1 (YBX1) interacts with inverted CCAAT nucleotide sequences, and was overexpressed in 27 out of the 28 ATC patients (Ito *et al.* 2003b). The CCAAT nucleotide sequence is present in several important cancer promoting genes including the *EGFR*, multi-drug resistance 1, proliferating cell nuclear antigen, and DNA topoisomerase 11 α (Ito *et al.* 2003b). The high mobility group I (Y) gene transcribes nuclear proteins, and is overexpressed in ATC cell lines (Scala *et al.* 2000). HMG1 proteins may suppress expression of CBX7, a protein that represses gene transcription and growth rate (Pallante *et al.* 2008). The FOSL1 protein, a transcription factor in the AP-1 complex that is involved in cell transformation, is expressed in thyroid adenomas and in differentiated as well as ATCs (Chiappetta *et al.* 2000), while c-myc is overexpressed in 59% of 22 ATC tissues (Kurihara *et al.* 2004).

Signaling pathways

The EGFR is a cell surface receptor which activates several intracellular tyrosine kinase regulated pathways (e.g., MAPK; PI3K) involved in proliferation, migration, etc., in many cancers. It is not detectable in normal thyroid tissues, and rarely in papillary thyroid carcinoma (Schiff *et al.* 2004, Elliott *et al.* 2008). However, it is detected in 58–87% of anaplastic tissues (Ensinger *et al.* 2004, Schiff *et al.* 2004, Wiseman *et al.* 2007b, Elliott *et al.* 2008) and in cell lines (Schiff *et al.* 2004).

Chemokine receptors also activate multiple downstream signaling pathways, including PI3K. CXCR4 is overexpressed in many cancers, and recently has been shown to be overexpressed in both ATC cell lines and patient samples (De Falco *et al.* 2007). A source of chemokines may be their paracrine secretion by tumor-associated macrophages, present in 19 out of the 20 ATC samples (Ryder *et al.* 2008).

The RAS–RAF–MEK–ERK and PI3K pathways are both involved in the pathogenesis of papillary and follicular thyroid cancers (Shinohara *et al.* 2007), and mutation in one of the genes regulating either pathway in ATC was identified in 50% of 36 cases (Santarpia

Table 4 Proteins expression in anaplastic thyroid carcinoma

Protein	Overexpressed		Underexpressed		References
	Cell line	Tumor	Cell line	Tumor	
Transcription					
PPARG	X	X			Hayashi <i>et al.</i> (2004), Aiello <i>et al.</i> (2006) and Copland <i>et al.</i> (2006b)
HNF-1 α	X	X			Xu <i>et al.</i> (2008)
NKX2-1				X	Bejarano <i>et al.</i> (2000), Miettinen & Franssila (2000) and Zhang <i>et al.</i> (2006)
FOXE1				X	Zhang <i>et al.</i> (2006)
Pax 8				X	Zhang <i>et al.</i> (2006)
CBX7			X	X	Pallante <i>et al.</i> (2008)
Id1		X			Kebebew <i>et al.</i> (2004)
YBX1		X			Ito <i>et al.</i> (2003b)
HMGI(Y)	X				Scala <i>et al.</i> (2000)
FOSL1		X			Chiappetta <i>et al.</i> (2000)
C-myc		X			Kurihara <i>et al.</i> (2004)
Signaling pathway					
EGFR	X	X			Ensinger <i>et al.</i> (2004), Schiff <i>et al.</i> (2004), Wiseman <i>et al.</i> (2007b) and Elliott <i>et al.</i> (2008)
CXCR4	X	X			De Falco <i>et al.</i> (2007)
pAKT1		X			Santarpia <i>et al.</i> (2008)
pERK		X			Santarpia <i>et al.</i> (2008)
Mitosis					
Aurora kinases	X	X			Sorrentino <i>et al.</i> (2005) and Ulisse <i>et al.</i> (2006)
TACC3			X		Ulisse <i>et al.</i> (2007)
K α 1 tubulin		X			Takano <i>et al.</i> (2001)
Topoisomerase-11		X			Wiseman <i>et al.</i> (2007a)
Proliferation					
MKI67		X			Tallini <i>et al.</i> (1999), Ito <i>et al.</i> (2003b), Saltman <i>et al.</i> (2006) and Wiseman <i>et al.</i> (2007a)
OEATC-1	X				Mizutani <i>et al.</i> (2005)
RBBP4	X	X			Pacifico <i>et al.</i> (2007)
Cell cycle					
Cyclin D1		X			Wang <i>et al.</i> (2000), Kurihara <i>et al.</i> (2004), Wiseman <i>et al.</i> (2007b) and Lee <i>et al.</i> (2008)
Cyclin D3	X	X			Baldassarre <i>et al.</i> (1999)
Cyclin E		X			Wiseman <i>et al.</i> (2007b)
p21				X	Saltman <i>et al.</i> (2006)
p27			X	X	Baldassarre <i>et al.</i> (1999) and Tallini <i>et al.</i> (1999)
Apoptosis					
IAPs	X	X			Ito <i>et al.</i> (2003a) and Tirro <i>et al.</i> (2006)
BCL2				X	Saltman <i>et al.</i> (2006) and Wiseman <i>et al.</i> (2007a)
DJ-1	X	X			Zhang <i>et al.</i> (2008)
NF- κ B	X				Starenki <i>et al.</i> (2004) and Meng <i>et al.</i> (2008)
α B-crystallin			X	X	Mineva <i>et al.</i> (2005)
LCN2	X	X			Iannetti <i>et al.</i> (2008)
Adhesion					
Cadherin 1, type 1, E-cadherin (epithelial)			X	X	Dahlman <i>et al.</i> (1998), Husmark <i>et al.</i> (1999), Motti <i>et al.</i> (2005) and Wiseman <i>et al.</i> (2006, 2007a)
Catenin (cadherin-associated protein), beta 1		X			Wiseman <i>et al.</i> (2007b)
ILK1	X	X			Younes <i>et al.</i> (2005)
FAK		X			Kim <i>et al.</i> (2004)

Table 4 continued

Protein	Overexpressed		Underexpressed		References
	Cell line	Tumor	Cell line	Tumor	
Tumor suppressor TP53		X			Saltman <i>et al.</i> (2006) and Wiseman <i>et al.</i> (2007a)
Rb			X		Motti <i>et al.</i> (2005)
p16				X	Lee <i>et al.</i> (2008)
PTEN				X	Hou <i>et al.</i> (2008)

et al. 2008). Both ERK and AKT1 proteins were phosphorylated and, thus, activated in 65–85% of samples, suggesting that these molecules may be important therapeutic targets (Santarpia *et al.* 2008).

Mitosis

Aurora kinases play an important role in cell division, and dysregulation may result in aneuploidy. Aurora B is overexpressed in ATC cell lines (Sorrentino *et al.* 2005, Ulisse *et al.* 2006), and patient samples had a marked increase when compared with normal thyroid tissue or PTCs (Ulisse *et al.* 2006). Like aurora B, aurora A is overexpressed at both the mRNA and protein levels in ATC cell lines, while Aurora C is increased only at the protein level (Ulisse *et al.* 2006). Aurora A interacts with RAS and TP53 (Ulisse *et al.* 2006), both of which are altered in ATC. Aurora A also phosphorylates TACC3 protein. Either over- or under-expression of TACC3 can alter mitosis. Its expression is reduced at the mRNA and protein levels in PTC, FTC, and ATC cell lines, but the lowest level was in anaplastic cells (Ulisse *et al.* 2007). Microtubules play an important role in mitosis, and overexpression of α 1 tubulin mRNA has been reported in ATC, but not other thyroid malignancies (Takano *et al.* 2001), and mitotic spindle assembly checkpoint genes mRNAs are also overexpressed (Wada *et al.* 2008). These observations provide some rationale for the use of microtubule inhibitors in ATC (Ain *et al.* 2000). Topoisomerase II- α is an enzyme necessary for chromatin segregation, and it was overexpressed in 92% of ATC samples, compared with 42% of differentiated thyroid cancers (Wiseman *et al.* 2007a).

Proliferation

The MKI67 nuclear antigen has been shown to reflect cellular proliferation rate, with a higher level detected in more aggressive tumors. A high-labeling index, as seen in ATC and poorly differentiated thyroid patients, correlated with persistent disease or death

(Tallini *et al.* 1999). MKI67 positive cells were detected in only 3.9% of 68 PTC patients but in 46.5% of 28 ATCs in one study (Ito *et al.* 2003b) and in 5% of well-differentiated PTC patients, but in 49% of poorly differentiated and 82% of anaplastic patients in another (Saltman *et al.* 2006). MIB1, which binds to MKI67, was detected in 100% of ATC samples but only 25% of differentiated thyroid cancers (Wiseman *et al.* 2007a). OEATC-1 and RbAp48 are two proteins overexpressed in ATC cells and which also influence proliferation (Mizutani *et al.* 2005, Pacifico *et al.* 2007).

Cell cycle

Cell cycle regulating genes and proteins (cyclins, cyclin-dependent kinases (CDKs), and CDK inhibitors) play a pivotal role in cell proliferation, with up-regulation of CDKs, down-regulation of CDKs, or both being frequent contributors to carcinogenesis. Immunohistochemistry performed on a tissue microarray of 31 ATC samples with 31 molecular markers showed the two most overexpressed proteins were cyclin D1 (100%) and cyclin E (93%) (Wiseman *et al.* 2007a), confirming a prior report of cyclin D1 overexpression in 77% of 13 ATCs (Wang *et al.* 2000). Kurihara *et al.* (2004) found increased cyclin D1 in only 27% of 22 tissues, but Lee *et al.* (2008) also found cyclin D1 protein in 18 out of the 27 (67%) ATC tumors. Cyclin D3 was also overexpressed in an ATC cell line (Baldassarre *et al.* 1999). These authors also showed that p27 protein expression was lost in one ATC cell line and reduced in six out of the eight ATC tissues (compared with approximately one-third of PTC and FTC tissues). Normally in the nucleus, p27 was mislocalized to and inactive in the cytoplasm, and correlated with cyclin D3 overexpression (Baldassarre *et al.* 1999). Tallini *et al.* (1999) studied well-differentiated, poorly-differentiated, and anaplastic tissues, and found a correlation of low p27^{KIP1} with larger tumor size, extrathyroidal extension and

survival. Saltman *et al.* (2006) noted a reduction in several CDKs in more undifferentiated tumors. P21 was detected in 40% of well-differentiated PTCs, only 7% of poorly differentiated thyroid cancers, and 0% of ATCs. For p27, the findings were 13, 8, and 5% respectively. In contrast to others, they observed cyclin D1 positivity in 15, 7, and 0% of cases as tumors became less differentiated.

Apoptosis

The inhibitors of apoptosis (IAPs) proteins prevent cells from undergoing apoptosis, and their presence may predict more resistant tumors. One of the IAPs, survivin, was present in 9 out of the 63 (14%) of well-differentiated, 11 out of the 29 (38%) poorly differentiated, and 17 out of the 19 (89%) anaplastic thyroid carcinomas (Ito *et al.* 2003a). ATC cell lines express several IAPs, including survivin, c-IAP1, c-IAP2, and XIAP (Tirro *et al.* 2006). By contrast, BCL2 is a proapoptotic protein. Its expression correlated inversely with differentiation (Saltman *et al.* 2006, Wiseman *et al.* 2007a). Another protein, DJ-1, protects cells from toxic stresses and higher levels promote insensitivity to TRAIL-induced apoptosis. DJ-1 is expressed in ATC cell lines, and also in more than 88% of follicular and anaplastic thyroid tissues (Zhang *et al.* 2008). NF- κ B, a protein that up-regulates antiapoptotic genes, is also expressed (Starenki *et al.* 2004, Meng *et al.* 2008). The small heat shock protein, α B-crystallin, is regulated by the CRYAB gene and is markedly reduced or absent in ATC tissues and cell line (Mineva *et al.* 2005). Moreover, the transcription factor, TFCP2L1, involved in CRYAB expression, is down-regulated in ATC (Mineva *et al.* 2005). By contrast, lipocalin 2 (LCN2), NGAL an NF- κ B target, is increased in ATC tissues (Iannetti *et al.* 2008).

Adhesion

Adhesion receptors include cadherins, integrins, selectins, and the immunoglobulin superfamily (Dahlman *et al.* 1998). Compared with normal thyroid and differentiated cancers, ATCs have reduced cadherin 1, type 1, E-cadherin (epithelial) (Dahlman *et al.* 1998, Husmark *et al.* 1999) and increased integrin α 2 β 1 subunit expression (Dahlman *et al.* 1998). Normal cells at high density become growth arrested at the G1 phase. This process is mediated by the adhesion molecule cadherin 1, type 1, E-cadherin (epithelial), which binds to catenin (cadherin-associated protein), beta 1 or junction plakoglobin and then up-regulates p27^{KIP1}. Motti *et al.* (2005) showed substantial differences in this coordinated response

in differentiated (DTC) versus anaplastic cell lines. High cell density promoted catenin (cadherin-associated protein), beta 1 expression in both DTCs and ATCs. By contrast, p27^{KIP1} mRNA was increased and protein degradation prolonged in DTC cells during confluence but not in ATC cells (Motti *et al.* 2005). Thus, reduced cadherin 1, type 1, E-cadherin (epithelial) expression contributes to the increased proliferation, the lack of contact inhibition and propensity to metastasize in ATC cells.

Cadherin 1, type 1, E-cadherin (epithelial) and catenin (cadherin-associated protein), beta 1 expression were examined by IHC in tissue micro-arrays in 12 cases of ATC that had associated DTC foci. cadherin 1, type 1, E-cadherin (epithelial) was expressed in 92% of the DTC foci, but in only 17% of the ATC tissues. Catenin (cadherin-associated protein), beta 1 was expressed in 67 and 50% respectively (Wiseman *et al.* 2006). In a 31 marker tissue array, the same authors found that catenin (cadherin-associated protein), beta 1 was one out of the five most strongly overexpressed proteins (Wiseman *et al.* 2007b). A focal adhesion protein, integrin-linked kinase (ILK), promotes anchorage-independent growth and invasion, and was elevated in ATC cell lines, and the majority of Hurthle cell and anaplastic carcinoma tissues (Younes *et al.* 2005). By contrast, no ILK expression was detected in ATC tissues (Wiseman *et al.* 2007b). More studies are necessary to determine its potential role in ATC. Another adhesion protein, focal adhesion kinase (FAK) associates with integrin receptors and is involved with cell migration and survival. FAK is overexpressed in all thyroid tumor types, including ATCs (Kim *et al.* 2004).

Tumor suppressors

The TP53 gene is commonly dysregulated in a variety of cancers. The gene is mutated in anaplastic but not well-differentiated thyroid malignancies, and the protein is also aberrantly overexpressed. TP53 was expressed in 0% of 41 well differentiated, 12% of 43 poorly differentiated, and 32% of 22 ATCs (Saltman *et al.* 2006). Wiseman *et al.* (2007b) reported TP53 expression in 61% of ATCs. The same authors examined 12 ATCs with differentiated foci, and detected TP53 in 83% of the anaplastic versus 17% of the differentiated components of the tumors (Wiseman *et al.* 2007a).

Retinoblastoma protein (pRb) binds to transcription factors and inhibits cell cycle progression when DNA is damaged. It is active in its hypophosphorylated state, and when differentiated thyroid cancer cells became

confluent, pRb became hypophosphorylated, but not in ATC cells (Motti *et al.* 2005). The tumor suppressor p16 was undetectable in 24 out of the 27 ATC tissues (Lee *et al.* 2008), and the *PTEN* gene was hypermethylated in 36 ATC patient samples (Hou *et al.* 2008).

Preclinical studies

In vitro studies

Transcription factors

PPARG initiates transcription as a heterodimer with RXR isoforms and, in multiple ATC cell lines, inhibits growth via apoptosis (Hayashi *et al.* 2004), anti-proliferation (Antonelli *et al.* 2008), p21 reduction of cell cycle progression (Aiello *et al.* 2006, Copland *et al.* 2006b), and inhibition of cyclin D1 (Aiello *et al.* 2006; Table 5). By contrast, the mRNA for retinoic acid receptor, alpha (RARA), involved in transcriptional regulation of 5'-deiodinase, was not induced by retinoic acid in ATC cell lines (Schmutzler *et al.* 2004). An adenovirus containing an antisense *HMGI(Y)* gene induced apoptosis and suppressed *HMGI(Y)* protein synthesis (Scala *et al.* 2000).

We have shown that a potent PPARG agonist transcriptionally increases PPARG which in turn increases RhoB. RhoB subsequently induces p21^{WAF1/CIP1} which inhibits cell growth (Copland *et al.* 2006a,b). In combination with paclitaxel, there is apoptotic synergy.

Signaling pathways

The frequent presence of EGFR in ATC tumors, and pleiotropy of its effects, makes it an attractive target for drug development. Gefitinib inhibits cell growth, although high doses are necessary (Nobuhara *et al.* 2005, Kurebayashi *et al.* 2006). The monoclonal antibody, cetuximab, also blocks the *EGFR* and subsequently *VEGF* gene expression and secretion, but evidence for inducing apoptosis is conflicting (Kurebayashi *et al.* 2006, Hoffmann *et al.* 2007). An interesting dual inhibitor of EGFR and VEGFR, AEE788, was shown in ATC cells to increase apoptosis and inhibit both cell proliferation and the phosphorylation of multiple proteins (Kim *et al.* 2005a, Hoffmann *et al.* 2007).

Imatinib, another tyrosine kinase inhibitor with multiple targets (e.g., BCR-ABL1; c-KIT; PDGFR), has variable activity in ATC cell lines. Imatinib inhibited phosphorylation of c-ABL1, increased p21, decreased several cyclins, and increased cells in S phase (Podtcheko *et al.* 2003) and also inhibited growth and induced apoptosis (Kurebayashi *et al.* 2006). Reduced

cell proliferation and the downstream Wnt/catenin (cadherin-associated protein), beta 1 pathway has been observed (Rao *et al.* 2006). However, the drug may not achieve its effects at clinically achievable concentrations (Dziba & Ain 2004).

Several oncogenes mutated in ATC cells signal through the MAPK-MAPK kinase (MEK)-ERK pathway. In 13 thyroid cell lines, including four ATC lines, two MEK inhibitors inhibited growth, with greater sensitivity in BRAF (+) cells. PD0325901 inhibited pERK, hypophosphorylated Rb, and arrested cells at G1 (Leboeuf *et al.* 2008). PD0325901 inhibited cell proliferation in RAS or BRAF mutant cells, and synergized with PI3K or NF- κ B inhibitors (Liu & Xing 2008).

Stromal cell-derived factor-1 (SDF-1), a ligand for the CXCR4-receptor, stimulated phosphorylation of two downstream kinases, ERK1/2, and AKT1, and subsequently, DNA synthesis in ATC cell lines (De Falco *et al.* 2007). Several agents, including siRNA CXCR4 and the antagonist, AMD3100, blocked entry of cells into S phase and cell proliferation (De Falco *et al.* 2007). AKT1 was also phosphorylated by IGF1 in ATC cells, an effect inhibited by thiazolidinediones and possibly PTEN up-regulation (Aiello *et al.* 2006). AKT1 inhibitor IV also inhibited AKT1 phosphorylation in five ATC cell lines (Hou *et al.* 2008). Signaling can occur also through c-Jun NH₂-terminal kinase (JNK) in ATC cells. The JNK inhibitor, SP600125, with or without ionizing radiation, inhibited cell growth (Bulgin *et al.* 2006).

Mitosis

Aurora kinases are critical in the process of cell division, and Aurora B phosphorylates histone H3. A small molecule Aurora kinase B quinazoline derivative inhibitor reduces phosphorylation and cell proliferation in ATC cell lines (Sorrentino *et al.* 2005). VX-680 effectively inhibited the cell cycle, reduced colony formation, and increased apoptosis of all Aurora kinases in multiple ATC cell lines (Arlot-Bonnemains *et al.* 2008).

Paclitaxel, a microtubule stabilizer, inhibited growth in multiple ATC cell lines (Ain *et al.* 1996, Dziba *et al.* 2002, Voigt *et al.* 2005). This agent has multiple effects, including cell cycle arrest, apoptosis, and phosphorylation of multiple signaling kinases (JNK, ERK, AKT1) (Pushkarev *et al.* 2004). The paclitaxel effects on apoptosis and tubulin acetylation are both enhanced by the histone deacetylase inhibitor, valproic acid (Catalano *et al.* 2007). Another tubulin-binding agent, combretastatin A4 phosphate, also increased cytotoxicity and, in several ATC cell lines, enhanced the paclitaxel effect (Dziba *et al.* 2002).

Table 5 Preclinical studies: *in vitro*

Targets	Agonists	Antagonists	References
Transcription PPARG	TZDs	GW9662; siRNA	Hayashi <i>et al.</i> (2004), Aiello <i>et al.</i> (2006), Copland <i>et al.</i> (2006b) and Antonelli <i>et al.</i> (2008)
HMGI(Y)		Adenoviral-antisense	Scala <i>et al.</i> (2000)
Signaling pathways EGFR		Gefitinib	Nobuhara <i>et al.</i> (2005) and Kurebayashi <i>et al.</i> (2006)
EGFR/VEGFR		AEE788	Kim <i>et al.</i> (2005a) and Hoffmann <i>et al.</i> (2007)
EGFR		Cetuximab	Hoffmann <i>et al.</i> (2007)
BCR–ABL1, c-KIT		Imatinib	Podtcheko <i>et al.</i> (2003), Dziba & Ain (2004), Kurebayashi <i>et al.</i> (2006) and Rao <i>et al.</i> (2006)
MAPKK(MEK)		PD0325901; AZD6244	Leboeuf <i>et al.</i> (2008) and Liu & Xing (2008)
CXCR4		siRNA; AMD3100	De Falco <i>et al.</i> (2007)
PTEN/AKT1	TZD (↑PTEN)	TZD (↓pAKT1)	Aiello <i>et al.</i> (2006)
JNK		AKT1 inhibitor IV	Hou <i>et al.</i> (2008)
Farnesyl transferase		SP600125	Bulgin <i>et al.</i> (2006)
Mitosis		Manumycin	Xu <i>et al.</i> (2001)
Aurora kinases		Aurora kinase inhibitor VX-680	Sorrentino <i>et al.</i> (2005) Arlot-Bonnemains <i>et al.</i> (2008)
Microtubules		Paclitaxel; combretastatin 4P; valproic acid	Ain <i>et al.</i> (1996), Dziba <i>et al.</i> (2002), Pushkarev <i>et al.</i> (2004), Voigt <i>et al.</i> (2005) and Catalano <i>et al.</i> (2007)
Proliferation			
OEATC-1		siRNA	Mizutani <i>et al.</i> (2005)
RBBP4		siRNA	Pacifico <i>et al.</i> (2007)
Oncolytic viruses		ONYX-015; ONYX-411; mutant vaccinia; adeno- virus HSV-TK and TP53- regulated	Nagayama <i>et al.</i> (2001), Kitazono <i>et al.</i> (2002), Portella <i>et al.</i> (2002), Libertini <i>et al.</i> (2007), Lin <i>et al.</i> (2007, 2008) and Reddi <i>et al.</i> (2008)
		Etoposide	Antonelli <i>et al.</i> (2008)
Cell cycle			
Cell cycle		Gemcitabine	Voigt <i>et al.</i> (2000)
Cyclins		CCND1 siRNA; Imatinib; plitidepsin	Podtcheko <i>et al.</i> (2003), Bravo <i>et al.</i> (2005) and Lee <i>et al.</i> (2008)
CDK inhibitors	TP53 adenovirus		Blagosklonny <i>et al.</i> (1998), Aiello <i>et al.</i> (2006) and Copland <i>et al.</i> (2006b)
	BMP7		Franzen & Heldin (2001)
	TZDs	siRNA; mithramycin	Podtcheko <i>et al.</i> (2003) and Bonofiglio <i>et al.</i> (2008)
Apoptosis			
IAPs		siRNA; Smac	Tirro <i>et al.</i> (2006)
NF-κB		DHMEQ	Meng <i>et al.</i> (2008)
IGF1		αIR3 antibody	Poulaki <i>et al.</i> (2002)
DJ-1		siRNA	Zhang <i>et al.</i> (2008)
PPARG	TZD		Aiello <i>et al.</i> (2006) and Copland <i>et al.</i> (2006b)
EGFR/VEGFR		AEE788	Kim <i>et al.</i> (2005a)
BCR–ABL1; c-KIT		Imatinib	Kurebayashi <i>et al.</i> (2006)
Aurora kinase		VX-680	Arlot-Bonnemains <i>et al.</i> (2008)
Integrin-linked kinase		QLT0267	Younes <i>et al.</i> (2005)
LCN2		siRNA	Iannetti <i>et al.</i> (2008)
Polo-like kinase 1		siRNA	Salvatore <i>et al.</i> (2007)
DUSP26		siRNA	Yu <i>et al.</i> (2007)
Migration			
Autotaxin	Autotaxin	IL1B, IL4, TGFB1	Kehlen <i>et al.</i> (2004)
VEGFR2		VEGFR2 inhibitor1	Balthasar <i>et al.</i> (2008)

Table 5 continued

Targets	Agonists	Antagonists	References
Protein degradation Proteasomes		Bortezomib	Mitsiades <i>et al.</i> (2006) and Conticello <i>et al.</i> (2007)
Epigenetic Histone acetylation		Valproic acid; depsipeptide	Kitazono <i>et al.</i> (2001), Catalano <i>et al.</i> (2006, 2007) and Xing (2007)
Methylation		5-aza-dCR	Husmark <i>et al.</i> (1999) and Schagdarsurengin <i>et al.</i> (2002, 2006)

Proliferation

OEATC-1, a gene overexpressed in ATCs, is an anonymous gene whose function is unclear. *OEATC-1* siRNA had a modest effect of inhibiting cell growth in an ATC cell line, but the mechanism is uncertain (Mizutani *et al.* 2005). *RBBP4* is a gene target of NF- κ B, and is overexpressed in ATC tissues and cell lines (Pacifico *et al.* 2007). Although its function is incompletely understood, an siRNA in FRO cells abolished colony formation in soft agar, and sensitized cell proliferation response to cisplatin and doxorubicin without affecting apoptosis (Pacifico *et al.* 2007).

Oncolytic viruses can infect cells and lyse them. Beneficial effects in ATC cell lines have been observed with an adenovirus HSV-TK construct and thyroglobulin promoter (Kitazono *et al.* 2002), with an adenovirus TP53-regulated Cre/loxP system in TP53 mutant cells (Nagayama *et al.* 2001), and with a mutant vaccinia virus (Lin *et al.* 2007, 2008), and *E1B* gene-defective adenovirus (ONYX-015) (Portella *et al.* 2002). The latter effect was enhanced by lovastatin (Libertini *et al.* 2007). The oncolytic adenovirus, ONYX-411, reduced cell viability in pRb dysfunctional ATC cell lines (Reddi *et al.* 2008). Abbosh *et al.* (2007) developed a conditionally replicative adenovirus therapy that drives *E1a* and *E1b* genes in ATC cell lines overpressing the Wnt/catenin (cadherin-associated protein), beta 1 pathway.

Cell cycle

Inhibition of cell cycle progression is an important strategy for reducing tumor growth. The cytotoxic drug gemcitabine in ATC cell lines induced G1/S arrest, and the effect was additive if followed by CDDP. Of interest, these drugs given in reverse order produced an antagonistic response as CDDP inhibits gemcitabine incorporation into DNA (Voigt *et al.* 2000). Imatinib increased cells in S phase. The CDK inhibitors, p21^{cip1/waf1}, and p27^{kip1}, were increased, and cyclins A and B1, and CDC2, were inhibited (Podtcheko *et al.* 2003). Bone morphogenetic protein 7

(BMP7), a member of the TGFB1 superfamily, caused growth inhibition in four ATC cell lines by up-regulating p21^{cip1} and p27^{kip1}, and by inhibiting CDK2 and CDK6 activity and Rb phosphorylation (Franzen & Heldin 2001). Other agents shown to have cytostatic effects on the cell cycle include the marine derived compound plitidepsin (Bravo *et al.* 2005), TP53-expressing adenovirus (Blagosklonny *et al.* 1998), and thiazolidinediones (TZDs) (Podtcheko *et al.* 2003, Aiello *et al.* 2006, Copland *et al.* 2006b, Bonofiglio *et al.* 2008). TZDs exert their growth inhibitory effect via PPARG transcriptional activation, and subsequent p21 activation (Aiello *et al.* 2006, Copland *et al.* 2006b) and involves Sp1 interaction, as it is inhibited by siRNAs to p21 or Sp1, and also by the Sp1 inhibitor, mithramycin (Bonofiglio *et al.* 2008). We have also shown that the PPARG activation of p21 is Rho B dependent and inhibited by Rho B siRNA (Copland *et al.* 2006a).

Cyclin D1 is overexpressed in HTH7 cells, and an siRNA for CCND1 inhibits both cyclin D1 mRNA and protein, but has minimal effect on cell growth (Lee *et al.* 2008).

Apoptosis

An important regulator of the life of cells is apoptosis, a process influenced by numerous pro- and antiapoptotic proteins and pathways. The IAPs, survivin, and C-IAP1, are increased, while the endogenous inhibitor, Smac, is reduced after chemotherapy; this response, thus, produces chemoresistance, a phenomenon seen in multiple tumors (Tirro *et al.* 2006). Several potential targets include the IAPs, as siRNA to c-IAP1, and survivin (Tirro *et al.* 2006) or inhibition of the IAPs by NF- κ B inhibition (Meng *et al.* 2008) both restore sensitivity to cytotoxic drugs. Overexpression of Smac is effective in enhancing cell death in resistant cells (Tirro *et al.* 2006), and inhibition of nuclear translocation of NF- κ B also potentiates taxane-induced apoptosis (Meng *et al.* 2008).

The growth factor, IGF1, protects FRO cells from TRAIL-induced apoptosis, an effect that can be abrogated by the IGF1 receptor antibody, aIR3 (Poulaki *et al.* 2002). Another anti-apoptotic protein, DJ-1, also renders cells less sensitive to TRAIL-induced apoptosis. Inhibition of DJ-1 by siRNA, or overexpression of DJ-1, can sensitize or make cells less sensitive to TRAIL-induced apoptosis (Zhang *et al.* 2008). AEE788, the dual inhibitor of EGFR and VEGFR, induced ~ 35% suppression in two ATC cell lines at the IC₅₀ drug concentration (Kim *et al.* 2005a). Other apoptotic drugs in ATC cell lines include imatinib (Kurebayashi *et al.* 2006), VX-680 (Arlot-Bonnemains *et al.* 2008), and an integrin-linked kinase inhibitor, QLT0267 (Younes *et al.* 2005). lipocalin 2 (LCN2) enhances survival by increasing iron-dependent nucleotide metabolism and reducing apoptosis (Iannetti *et al.* 2008).

The serine/threonine kinase, polo-like kinase 1 (PLK1), can be decreased when either TP53 or p21 are overexpressed, and apoptosis is increased by siRNA PLK1 (Salvatore *et al.* 2007). The *DUSP26* gene (coding for a MAPK phosphatase) is overexpressed in ATC cell lines, as is the protein in tumors (Yu *et al.* 2007). *DUSP26* dephosphorylates p38, a regulator of apoptosis upstream of caspase-3. A *DUSP26* siRNA was effective in activating caspase-3 and increasing apoptosis (Yu *et al.* 2007).

Migration

Little is known about motility in ATC cells. Autotaxin (ATX) is a motility promoting protein in a number of tumors. Higher levels of ATX mRNA were expressed in anaplastic versus other thyroid malignancies, and was increased in ATC cell lines also (Kehlen *et al.* 2004). In the 1736 cell line, IL1B, IL4, and TGFB1 suppressed, while EGF and bFGF stimulated ATX mRNA. Functionally, when ATX was transfected into UTC-1736 cells, migration was enhanced (Kehlen *et al.* 2004). Balthasar *et al.* (2008) showed that the VEGFR2 inhibitor 1 attenuated cell migration in FRO cells. Although they showed that sphingosine-1-phosphate stimulated VEGFA secretion, suggesting crosstalk between the G-protein coupled S1P receptor regulating migration and the VEGFR tyrosine kinase receptor, a high concentration of S1P was required (Balthasar *et al.* 2008). Thus, the mechanism of VEGFR affecting migration in FRO ATC cells is unclear.

Protein degradation

Proteins are degraded in the 26S proteasome, some of which play important roles in reducing cell growth.

Bortezomib is a proteasome inhibitor that prevented degradation of TP53, p21, and the inhibitor of NF-κB (i.e., IκB) cleaved caspases leading to apoptosis and synergized with doxorubicin in ATC cell lines (Mitsiades *et al.* 2006). Conticello *et al.* (2007) confirmed the p21 and apoptosis effects of bortezomib and showed that TRAIL-mediated cytotoxicity was increased.

Epigenetics

Epigenetic silencing of genes occurs through deacetylation or methylation. Kitazono *et al.* (2001) showed that deipeptide increased histone H3 acetylation and growth inhibition in SW-1736 cells. Several thyroid specific mRNAs, sodium iodide symporter and thyroglobulin, as well as ¹²⁵I uptake, were enhanced. Valproic acid (VPA), another histone deacetylase inhibitor, increased the acetylation of H4 histone, resulting in enhanced doxorubicin-mediated apoptosis and G2 cell cycle arrest (Catalano *et al.* 2006). The same authors showed that valproic acid, while having no independent effect on apoptosis, also enhanced the paclitaxel effect (Catalano *et al.* 2007); they also showed that VPA induced tubulin acetylation. Xing (2007) reviewed several studies showing that thyroid specific genes (e.g., Na⁺/I⁻ symporter, thyroid peroxidase, and thyroglobulin) as well as iodide uptake could be restored by deacetylase inhibition in poorly differentiated and ATCs.

Husmark *et al.* (1999) showed that 5-aza-2'-deoxycytidine (5-Aza), a demethylating agent, could up-regulate cadherin 1, type 1, E-cadherin (epithelial) and junction plakoglobin in ATC cell lines. Two genes frequently epigenetically silenced through CpG island promoter methylation are the RAS association domain family 1A gene (*RASSF1A*) and the CDK inhibitor, *p16^{INK4α}*. *RASSF1A*, located on chromosome 3p21, may also lose function from loss of heterozygosity. Schagdarsurengin *et al.* (2002) reported high levels of methylation of both genes in ATC tissues, and of *RASSF1A* in cell lines, and that treatment with the methylation inhibitor, 5-aza-2'-deoxycytidine, restored transcriptional activity. The same authors subsequently analyzed the methylation status of 17 gene promoters in nine ATC tissues and five ATC cell lines. Compared with other thyroid cancers, ATCs had a significant increase in methylation of five genes: *p16^{INK4α}* (cell cycle), death associated protein kinase (*DAPK*) and *UCHL1* (apoptosis), *MGMT* (DNA repair), and *TSHR* (Schagdarsurengin *et al.* 2006). 5-Aza successfully restored gene expression in all four ATC cell lines tested.

In vivo studies

Studies *in vivo* provide further confirmation of a drug or other agent’s effect, and are extremely important to perform prior to considering human clinical trials. The nude mouse xenograft model has been used almost exclusively for the small number of reports published in preclinical studies of ATC (Table 6). Imatinib and gefitinib both inhibit tumor growth (Podtcheko *et al.* 2003, Nobuhara *et al.* 2005, Kurebayashi *et al.* 2006). Kim *et al.* (2005a) showed that dual inhibition of EGFR and VEGFR, in combination with paclitaxel, is effective. Mechanistically, AEE788 reduced tumor cell autophosphorylation of EGFR and MAPK, and endothelial cell EGFR and VEGFR2 phosphorylation both singly and with paclitaxel. Microvessel density was decreased and apoptosis increased (Kim *et al.* 2005a). The MEK inhibitor, AZD6244, blunted tumor growth, and pERK (Leboeuf *et al.* 2008). The tubulin-binding agent, combretastatin A4P, was equally effective in growth inhibition (Dziba *et al.* 2002), while CXCR4 inhibition with AMD3100 inhibited tumor growth by more than 90% (De Falco *et al.* 2007).

The farnesyl transferase inhibitor, manumycin, was completely effective in inhibiting growth and, in combination with paclitaxel, caused tumor shrinkage. Manumycin, but not paclitaxel, inhibited angiogenesis in the animal model (Xu *et al.* 2001). An oncolytic vaccinia virus (GLV-1h68) produced almost complete inhibition of xenograft growth when injected

intratumorally (Lin *et al.* 2008), while the oncolytic virus, ONYX-015, produced a modest inhibition of tumor growth. The effect was more than doubled by lovastatin due, at least in part, to increased viral replication (Libertini *et al.* 2007). An LCN2 siRNA markedly inhibited growth of FRO cell tumors in nude mice, suggesting a possible NF-κB pathway target for therapeutics (Iannetti *et al.* 2008). DHMEQ, an inhibitor of NF-κB translocation, also inhibited tumor growth as a single agent, and synergized with docetaxel (Meng *et al.* 2008).

Future *in vivo* studies would benefit from an orthotopic model, which recreates the clinical presentation of local neck invasion and distant metastases (Kim *et al.* 2005a,b).

Clinical management

The rarity of anaplastic thyroid carcinoma limits the number of patients seen annually, even at referral centers. Thus, most series have accrued patients over several decades (Table 7). Comparisons across reported studies are confounded by the variety of treatments tried over these extended periods of time, and by the paucity of controlled clinical trials.

McIver *et al.* (2001) reviewed the 50-year experience with 134 patients at the Mayo Clinic. The primary tumor size averaged 7 cm (range: 2–16 cm), and 46% had distant metastases on presentation. Ninety-six (72%) of patients received some surgery (attempt at cure in 35 or debulking in the rest) and most received

Table 6 Preclinical *in vivo* xenograft studies in anaplastic thyroid carcinoma

References	Cell line	Agent	Growth inhibition (%)	Treatment (days)
Kurebayashi <i>et al.</i> (2006)	KTC-3	Imatinib	44%	14
		Gefitinib	53%	14
		Both	62%	14
Podtcheko <i>et al.</i> (2003)	FRO	Imatinib	~67%	14
Nobuhara <i>et al.</i> (2005)	ACT-1	Gefitinib	>90%	28
Kim <i>et al.</i> (2005a)	K4	AEE788	58%	42
		Paclitaxel	44%	
		Both	69%	
Leboeuf <i>et al.</i> (2008)	Cal62	AZD6244	~75%	27
Dziba <i>et al.</i> (2002)	BHT101	Combretastatin A4P	~60%	20
De Falco <i>et al.</i> (2007)	BHT101	AMD3100	92%	21
Xu <i>et al.</i> (2001)	Hth74	Manumycin	~100%	20
		Paclitaxel	~100%	
		Both	~100%	
Lin <i>et al.</i> (2008)	8505C	Mutant vaccinia	~96%	90
Libertini <i>et al.</i> (2007)	FRO	Lovastatin	–	48
		dl1520 (ONYX-015)	23%	
		Both	~55%	
Iannetti <i>et al.</i> (2008)	FRO	LCN2 siRNA	~97%	
Meng <i>et al.</i> (2008)	FRO	DHMEQ	>90%	14

Table 7 Clinical characteristics and survival in anaplastic thyroid carcinoma

	Years	Patient (M/F)	Age (yrs) (range)	Survival (mos)	Survival 1 year (%)
McIver <i>et al.</i> (2001)	1949–1999	54/80	67 (mean)	3	9.7%
Junor <i>et al.</i> (1992)	1961–1986	27/64	70 (median) (38–92)	5	~20%
Levendag <i>et al.</i> (1993)	1970–1986	17/34	67 (mean) (31–89)	2.7–3.4	6%
Voutilainen <i>et al.</i> (1999)	1967–1994	7/26	66 (mean) (36–89)	2.5	9.7%
Tan <i>et al.</i> (1995)	1968–1992	10/11	65 (mean) (38–83)	4.5	~23%
Pierie <i>et al.</i> (2002)	1969–1999	22/45	73 (median) (40–92)	~6	35%
Kim & Leeper (1983)	1979–1983	3/6	69 (mean) (54–79)	10	22%
Kobayashi <i>et al.</i> (1996)	1971–1993	9/28	68 (median) (34–82)	3	0–20%
Schlumberger <i>et al.</i> (1991)	1981–1991	9/11	61 (median) (27–82)	–	15%
Chemotherapy Committee. The Japanese Society of Thyroid Surgery (1995)	1992–1994	6/11	67 (median) (50–74)	–	–
Mitchell <i>et al.</i> (1999)	1991–1995	9/8	71 (median) (51–85)	2.5	0%
Tennvall <i>et al.</i> (2002)	1984–1999	17/38	76 (median) (46–94)	3.5	16%
Haigh <i>et al.</i> (2001)	1973–1998	13/20	69 (median) (47–80)	3–43	0–75%
Besic <i>et al.</i> (2005)	1972–2003	58/130	68 (median)	3	13%
De Crevoisier <i>et al.</i> (2004)	1990–2000	12/18	59 (mean) (40–79)	10	46%
Wang <i>et al.</i> (2006)	1983–2004	25/22	69 (median) (39–90)	5.6	–
Brignardello <i>et al.</i> (2007)	2000–2005	9/18	70 (mean) (46–89)	3.9	~25%
Ain <i>et al.</i> (2000)	1996–1999	13/6	58 (median) (47–86)	6	~30%

external beam radiotherapy (XRT) post-operatively. Surgery and XRT had a modest effect on survival compared with palliation alone (3.5 and 2.3 months, versus 3 weeks). Interestingly, the longest survivor (23 years) had surgery alone for a 6.1 cm primary tumor with 3 lymph nodes involved. Multimodal therapy (surgery, XRT, and radiosensitizing chemotherapy) was used in 13 patients; median survival was unaffected, although there was a trend toward an improved 1 year survival.

Junor *et al.* (1992) treated 91 patients in Glasgow, UK, from 1961 to 1986. Those who had surgery (5-total thyroidectomy; 28-partial thyroidectomy) had improved survival. Eighty-six patients received radiotherapy, and the dose did not alter survival; neither did chemotherapy improve survival.

Levendag *et al.* (1993) treated 51 patients from 1970 to 1986. Twenty-three had surgery ('total' resection in 15, with negative margins in one, and incisional biopsy in eight). Median survival was 3.4 months in the thyroidectomy group, and 2.7 months in the biopsy only patients. All received radiotherapy, but > 30 Gy in only 57%. Overall, survival at 1 year was 6%. Higher doses of radiation provided some advantage, with a 1 year survival of 10% in those receiving ≥ 30 Gy, and 20% if the extrapolated tolerance dose > 60 Gy.

In Helsinki, Finland, 33 patients had surgery over 27 years, although total thyroidectomy could be done in but 11 patients (Voutilainen *et al.* 1999). At diagnosis, 48% had distant metastases. Radiotherapy and

cytotoxic drugs were individualized, and neither the radiation schedule nor chemotherapeutics used were provided. Predictors of survival included: tumor resectability, radiotherapy, and distant metastases at diagnosis.

At the Roswell Cancer Institute, 21 patients were treated between 1968 and 1992 (Tan *et al.* 1995). Five had complete resection, 18 received XRT, and six were given adriamycin +/- one or more other agents. Overall, the median survival was 4.5 months and 2-year survival was 14%. Of the five who had complete resection, the 5-year survival was 60% (only three had XRT + chemo; one had XRT only, and one had only surgery). Twelve patients had incomplete resections; none lived for 2 years.

Pierie *et al.* (2002) treated 67 patients from 1969 to 1999. Complete resection was achieved in 12 (eight of whom had incidental ATC discovered at surgery). Survival was highest after complete resection and least with no resection (1 year: 93 and 4% respectively). Radiation doses > 45 Gy improved survival compared with lower doses, and chemotherapy had no significant effect (Pierie *et al.* 2002).

Kim & Leeper (1983) introduced the strategy of low-dose adriamycin (10 mg/m² per week) with hyperfractionated radiotherapy (160 rads twice a day, 3 days a week: total dose = 5760 rads). Two patients died within 2 weeks; nine others had completed treatment, and seven had complete local responses. Median survival of the nine treated patients was

~ 10 months. Only one patient had complete thyroid resection, while five had partial resection and three biopsy only distant metastases at diagnosis.

In Japan, 16 patients were treated with conventional XRT from 1971 to 1983, and chemotherapy included adriamycin, mitomycin C, or cyclophosphamide. An additional 21 received hyperfractionated XRT with adriamycin and/or cisplatin from 1984 to 1993. Twenty-four had surgery. Of the 19 with complete resections, the 1 year survival was 20%, while no one with residual disease after surgery survived a year (Kobayashi *et al.* 1996).

One of few prospective clinical trials was conducted by the Eastern Cooperative Oncology Group (Shimaoka *et al.* 1985). From 1976 to 1982, 21 patients were randomized to doxorubicin versus 18 to doxorubicin plus cisplatin. The single agent group had no complete and one partial response while the two-drug regimen produced three complete, and three partial remissions (5% vs 33%; $p=0.03$). Median survival was 2.7 months, but two CRs were long-lasting at 41.3 and 34.7 months, with one patient treated with thyroidectomy and radiotherapy, and the other only a biopsy.

Schlumberger *et al.* (1991) reported their 10-year experience with a combination of chemotherapy and external beam radiotherapy. Patients ≤ 65 years ($n=12$) received doxorubicin and cisplatin every 4 weeks with XRT during the first four cycles. Two were disease-free after surgery, and one was alive at 34 months. Four had small neck disease at time of the therapy, and had CRs of 4, 6, 8, and 22 months. Six had initial distant metastases and were dead within 6 months. Eight patients ≥ 61 years received mitoxantrone every 4 weeks, with radiotherapy. Two had no detectable disease post-operatively and one had a 40-month remission. Three had initial neck disease only, but no surgery and three had distant metastases. The longest response was 8 months.

The Japanese Society of Thyroid Surgery performed a pilot study of intensive chemotherapy in 17 patients from 1992 to 1994 (Chemotherapy Committee The Japanese Society of Thyroid Surgery 1995). Therapy included cisplatin, adriamycin, etoposide, peplomycin, and granulocyte colony-stimulating factor every 3 weeks with local XRT after one cycle. Ten patients had advanced disease, only four received XRT, and two had PRs of 2 and 3 months. Seven patients had no residual disease after thyroidectomy, six received adjuvant chemotherapy, and five received XRT. Three were alive without recurrence at 3, 11, and 11 months.

Mitchell *et al.* (1999) treated 17 patients with aggressive radiation (twice daily, 5 days a week, 20–24 days, total dose = 60.8 Gy) to see whether they

could improve local control. Only one patient had prior thyroidectomy, six had debulking, seven had a biopsy, and three had fine needle aspiration. Clinical responses (partial or complete) were achieved in 59%, and stable disease in 29%, with local control maintained in 76%. However, all patients died within 8 months, and the authors concluded that toxicity was unacceptable.

Eighty-one patients from the Stockholm–Gotland region of Sweden were treated during a 27-year period (Nilsson *et al.* 1998). There were six separate time frames during which the type of chemotherapy and radiotherapy given were varied. As surgery was performed more frequently and radiation therapy was accelerated and hyperfractionated, local disease recurrence was substantially reduced. Unfortunately, overall survival was not improved and, in fact, was shortened. Results in these Swedish patients during the era of doxorubicin were summarized (Tennvall *et al.* 2002). Of 55 patients treated over 15 years, 40 had surgery initially. While local control occurred in 60%, median, and 1 year survival were similar to other reports.

Haigh *et al.* (2001) managed 33 patients and found that surgical resection with curative intent in eight resulted in a median survival of 43 months and 5-year survival of 50%. Most also received XRT, and a broad spectrum of chemotherapeutic agents was used. In 18 patients with palliative surgery and seven who received chemotherapy and XRT without surgery, the median survivals were 3 and 3.3 months. Thus, patients whose tumor could be visibly resected had a much better chance of treatment response.

In Slovenia, Besic *et al.* (2005) treated 188 patients between 1972 and 2003. Factors favoring survival were age (<70 years), good performance status, slow tumor growth, and lack of extrathyroidal extension or distant metastases. In an earlier report of 162 patients (Besic *et al.* 2001), survival was similar in those who had primary surgery versus primary radiotherapy and/or chemotherapy. However, median survival was best in patients who had primary chemo- and radiotherapy and in whom surgery could then be performed (Besic *et al.* 2001).

De Crevoisier *et al.* (2004) treated 30 patients over 10 years in a prospective protocol combining surgery, chemotherapy, and radiotherapy. Their patients differed in that only 6 out of the 30 had distant metastases at presentation. Surgery was followed by chemotherapy (doxorubicin plus cisplatin) every 4 weeks starting 3 weeks after surgery, and hyperfractionated accelerated XRT after cycle two. Nine patients had visibly complete resection, and seven had complete remission (none had initial tracheal extension). Of the nine with complete resection, six had mixed ATC, and three had

<20% ATC. The median survival of all patients was 10 months, while one and 3-year survivals were 46 and 27% respectively.

Wang *et al.* (2006) treated 47 patients with radiotherapy from 1983 to 2004. Twenty-three patients with good performance status and no distant metastases received radical radiotherapy (>40 Gy), while 24 with poor performance status or distant metastases received palliative therapy (<40 Gy). Overall, median survival was 5.6 months (11.1 and 3.2 months respectively).

In Turin, Italy, 27 patients were treated in a 5-year period (Brignardello *et al.* 2007). Surgery was performed initially (maximum debulking in 11 and palliative in six). Chemotherapy (doxorubicin + cisplatin) was given during and after radiation. The approach in five patients was neo-adjuvant chemo/XRT followed when possible by surgery, while five others had paclitaxel therapy alone. As in other series, surgical resection was the major contributor to patient responses. While median survival was only 3.9 months, those with maximal debulking had a 6-month survival of 58%; those with palliative or no surgery, only 10%.

Twelve population-based cancer registries in the NCI's Surveillance, Epidemiology, and End Results (SEER) database from 1971 to 2000 were reviewed (Kebebew *et al.* 2005). There were 516 patients (171 men, 345 women) with a mean age of 71.3 years at diagnosis of ATC. The 1 year survival was 19.3%, and favorable predictors were age <60 years, intrathyroidal disease, and combined use of surgery and radiotherapy.

Based on preclinical data (Ain *et al.* 1996), an open-label phase 2 clinical trial of paclitaxel as a 96-hour infusion was conducted (Ain *et al.* 2000). Prior surgery included thyroidectomy, lobectomy, or biopsy in 8, 3, and 8 patients respectively. Disease progression occurred in 42%, while stable disease (5%), partial regression (47%) or complete regression (5%) was observed in the remainder. Overall, survival was 6 months, with a median survival of 32 weeks in the ten responders, and only 7 weeks in eight non-responders.

New therapies

Thyroid trials

Three studies of novel targeted agents have included a limited number of ATC patients. Three ATC patients in a phase 1 study received combretastatin A-4 phosphate, one of whom had a complete remission and was alive at 30 months (Dowlati *et al.* 2002). In a phase 1 trial of intermittent high-dose gefitinib and

docetaxel, one patient had ATC, and experienced a 4-month partial remission (Fury *et al.* 2007). Two ATC patients enrolled in a phase 2 trial with axitinib; one had a partial remission (duration unknown), and one progressive disease (Cohen *et al.* 2008).

A review of www.clinicaltrials.gov on June 30, 2008 showed the following trials listed for ATC. Phase 2 thyroid specific trials included four completed (two with combretastatin, one with intensified combination hydroxyurea/paclitaxel and hyperfractionated XRT, and one with irofulven/capecitabine); two were active/not recruiting (imatinib, gefitinib); and four recruiting (sorafenib, pazopanib, sunitinib, and combination PPARG agonist, CS-7017/paclitaxel).

There were five phase 1 trials listed that included ATC patients as eligible. Two trials were completed (SU5416/paclitaxel and tipifarnib/trastuzumab), two were active/not recruiting (IL-12/trastuzumab, NGR-TNF (European study)), and one was recruiting (sorafenib/bevacizumab).

Results from these studies will hopefully provide insight into which newer agents might be tested further.

Potential agents

It should be mentioned that several ATC cell lines, including DRO, ARO, and KAT4, may be derived from cancers other than anaplastic thyroid tumors (Schweppe *et al.* 2008). Given this concern, we have not cited results that used only these cell lines. However, it would be worthwhile repeating these studies with other confirmed ATC cell lines, as there are some interesting additional potential therapeutic targets.

There are many potential therapeutic targets for this deadly disease, as listed in Tables 1, 3–7. A number of articles have reviewed the many pathways that have applicability to ATC, and have discussed a variety of agents being developed. The reader is referred to the following recent reviews for details: chromosomal abnormalities (Frohling & Dohner 2008), oncogenes (Croce 2008), RET, and RAS-MAPK pathways (Santoro & Carlomagno 2006, Sebolt-Leopold 2008, Williams & Smallridge 2004), AKT1 (Shinohara *et al.* 2007), c-MET (Salgia 2008), angiogenesis (Kerbel 2008), EGFR (Ciardiello & Tortora 2008), apoptosis (Ferreira *et al.* 2002, Altieri 2008, Vucic 2008), mitosis (Strebhardt & Ullrich 2006, Bhat & Setaluri 2007, Gautschi *et al.* 2008), Hsp 90 (Bishop *et al.* 2007), epigenetics (Xing 2007, Esteller 2008), farnesyl transferases (Appels *et al.* 2005), proteasomes (Orlowski & Kuhn 2008), tumor microenvironment

(Blansfield *et al.* 2008, Parsons *et al.* 2008), and microRNAs (Jeyaseelan *et al.* 2007).

Future directions

Survival of patients with ATC has changed little in the past 50 years, despite more aggressive systemic and radiotherapies. Much has been learned about the genes, RNAs, and proteins dysregulated. Given the genomic instability of ATC, effective therapies may benefit from comprehensive microarray analysis (Shai 2006) or genome-wide screening (Sultan *et al.* 2008) to develop an individualized therapeutic regimen that maximally inhibits major pathways at multiple genetic and epigenetic levels. In addition, to more fully characterize the complex molecular profile, subsequent rapid *in vitro* screening assays of combinations of targeted therapies may further optimize a patient's treatment plan. Finally, refinement may derive from evolving drug delivery systems such as nanoparticles (Cho *et al.* 2008).

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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