# New molecular targeted therapies in thyroid cancer

Amalia Milano, Maria G. Chiofalo, Maria Basile, Antonella Salzano de Luna, Luciano Pezzullo and Francesco Caponigro

**Carcinoma of the thyroid gland is the most common malignancy of the endocrine system. Differentiated tumors are often curable with surgical resection and radioactive iodine. A small percentage of such patients, however, do not undergo remission and need new therapeutic approaches. Both anaplastic and medullary thyroid carcinomas exhibit aggressive behavior and are usually resistant to current therapeutic modalities. Thyroid carcinoma represents a fascinating model and a particularly promising paradigm for targeted therapy because some of the key oncogenic events are activating mutations of genes coding for tyrosine kinases, and these occur early in cancer development. A prototype is the RET proto-oncogene, a receptor tyrosine kinase, which is a key regulator of development and a 'hotspot' for oncogenic mutations. Mutations in the RET proto-oncogene have been identified as causative for papillary carcinoma and familial medullary thyroid carcinoma, making it an attractive target for selective inhibition in these subtypes. ZD 6474 has shown promising activity in preclinical models against RET kinase, and its contemporary inhibition of vascular endothelial growth factor and epidermal growth factor pathways renders it a very attractive drug for clinical trials in thyroid cancer. Activating point mutation of B-RAF can occur early in the development of papillary carcinoma. Moreover, papillary carcinomas with these mutations have more aggressive properties and are diagnosed more often at an advanced stage. Clinical evaluation of B-RAF-targeting drugs is undergoing and trials in thyroid cancer are planned. Agents that restore radioiodine uptake, such as histone deacetylase inhibitors and retinoids, represent another exciting field in new drug development in thyroid cancer. *Anti-Cancer Drugs* 17:869–879 © 2006 Lippincott Williams & Wilkins.**

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National Tumor Institute of Naples, 'Fondazione G. Pascale' Via M. Semmola, Napoli, Italy.

Correspondence to A. Milano, Istituto Nazionale Tumori 'Fondazione G. Pascale', Via M. Semmola, 80131 Napoli, Italy.
Tel: +39 081 5903362; fax +39 0815903822;
e-mail: ammilan@tin.it

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## Introduction

Carcinoma of the thyroid gland is an uncommon cancer, yet it is the most common malignancy of the endocrine system. Differentiated tumors, such as papillary or follicular, are often curable with surgical resection and radioactive iodine ($^{131}$I) ablation that can effectively treat tumor foci exhibiting $^{131}$I uptake. At least two-thirds of patients with recurrent neck disease and one-third of patients with distant metastases achieve a complete remission with the above therapeutic modalities [1], response being higher when lesions are small (less than 1 cm in diameter) [2]. Chemotherapy is indicated for patients with metastases that do not take up $^{131}$I [3,4]. Doxorubicin is the most widely used cytotoxic drug, but the response rate is discouragingly low, and ranges between 0 and 22%; furthermore, all responses are partial, their duration does not exceed a few months and no survival benefit occurs. Combination of doxorubicin with cisplatin has yielded similar response rates, but greater toxicity [3]. So, there is a percentage of patients with papillary or follicular tumors for whom new therapeutic modalities are needed [2]. Tall cell and Hurthle cell are two aggressive forms of differentiated follicular-derived thyroid carcinomas. These two histological subtypes are associated with a more aggressive biological behavior. The even more undifferentiated anaplastic thyroid carcinoma (ATC) shows rapid invasive growth to the surrounding tissues and has a high metastatic potential to distant organs. In such cases, surgery alone is unlikely to cure the patients, and a combination approach with chemotherapy and radiation is often carried out, yet the outcome of such intensive multimodal therapy is largely unsatisfactory. Medullary thyroid carcinoma (MTC), originating from the parafollicular C-cells of the neural crest, also exhibits aggressive behavior and is often resistant to current therapeutic modalities. Surgery represents the mainstay of treatment for MTC [5], while chemotherapy has been reported to induce only anecdotical responses. Doxorubicin is the most active agent and it induces responses in roughly 30% of patients [3]. The combination of cyclophosphamide, vincristine and dacarbazine has also shown some activity [6]. MTC occurs in a sporadic and a familial form. The latter may be associated with benign or malignant tumors of other endocrine organs and it is commonly referred to as the multiple endocrine neoplasia (MEN) syndrome, presenting as MEN2A (MTC, pheochromocytomas and parathyroid hyperplasia) or MEN2B (MTC,

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pheochromocytomas, ganglioneuromas, mucosal neuromas and marfanoid habitus).

Thyroid cancers represent an excellent model for the study of tumor-initiating genetic events. An outstanding example is the RET proto-oncogene, which is a receptor tyrosine kinase, a key regulator of development and a 'hotspot' for oncogenic mutations [7]. Mutations in the RET proto-oncogene have been identified as causative for papillary carcinoma and familial MTC. Chromosomal rearrangements linking the promoter and N-terminal domains of unrelated gene(s) to the C-terminal fragment of RET result in the aberrant production of a chimeric form of the receptor in thyroid cells that is constitutively active and qualifies as an attractive target for selective inhibition in both medullary and papillary thyroid cancers. Recently, in-vitro studies have investigated the expression of epidermal growth factor receptor (EGFR) in anaplastic thyroid cancer cell lines and have highlighted the potential of EGFR-targeting therapies as a new therapeutic approach [8]. Moreover, experimental evidence has been provided for a link between EGFR signaling and angiogenic mechanisms, through the up-regulation of vascular endothelial growth factor (VEGF), which is a key regulator of tumor-induced endothelial cell proliferation and vascular permeability. Various small-molecule tyrosine kinase inhibitors that block both the VEGFR and the EGFR tyrosine kinase activities are currently in clinical development.

Activating point mutations of the RAS genes are found with a similarly high frequency in thyroid adenomas and follicular carcinomas, suggesting that RAS mutations represent an early event in thyroid tumorigenesis [9]. Activating point mutation of B-RAF, predominant isoform of this serine–threonine kinase in thyroid follicular cells, can occur early in the development of papillary carcinoma [10]. Moreover, papillary carcinomas with these mutations have more aggressive properties, and present more often with extrathyroidal invasion and at a more advanced clinical stage. Inactivating point mutations of the p53 tumor-suppressor gene are rare in patients with differentiated thyroid carcinomas, but common in those with ATC, and the overexpression of genes that occurs in this setting can be exploited in the clinic through selective targeting [11,12]. Secondary lack of radioiodine uptake can be an additional reason for treatment failure and the evaluation of drugs aimed at restoring this uptake, such as histone deacetylase (HDAC) inhibitors and retinoids, is definitely worth pursuing [13–15]. Altogether these findings demonstrate that thyroid carcinomas represent a particularly promising paradigm for targeted therapy because some of the key oncogenic events are activating mutations of genes coding for tyrosine kinases and these occur early in cancer development.

In this paper, we discuss the rationale for the use of emerging targeted therapies in thyroid cancer, and analyze challenges and perspectives for the future. Targeted agents are described on the basis of their site of action.

## Drugs acting at the plasmatic membrane

### Vascular endothelial growth factor receptor, epidermal growth factor receptor and RET pathway inhibitors

#### *ZD6474*

ZD6474 is a novel oral heteroaromatic-substituted anilinoquinazoline that acts as a potent and reversible inhibitor of ATP binding to VEGF receptor (VEGFR) type 2 [16].

VEGF is one of the most potent stimulators of angiogenesis, inducing endothelial cell proliferation, protease expression, endothelial cell migration, capillary tube formation and endothelial cell survival signaling in newly formed blood vessels [17–19]. VEGF signaling occurs through the endothelial cell-associated tyrosine kinase receptors VEGFR-1 (Flt-1) and VEGFR-2 (KDR) [20]. Activation of VEGFR-2 alone, however, appears to be sufficient to induce the angiogenic and vascular permeabilization activity of VEGF [21]. Thus, the recognition of VEGF as a primary stimulus of angiogenesis has paved the way to many strategies to block VEGF activity, among which is the use of specific tyrosine kinase inhibitors. In addition, ZD6474 also inhibits EGFR tyrosine kinase activity. The EGFR autocrine signaling pathway is central to cancer progression and the overexpression of EGFR and/or its ligands, such as transforming growth factor-$\alpha$ and EGF, has been reported in many human tumors [22]. Moreover, activation of EGFR signaling can up-regulate the production of VEGF in human cancer cells [23,24]. In-vivo studies have also shown that the growth of ZD1839 (gefitinib)-resistant GEO tumor xenografts is blocked by ZD6474 [25]. This activity is most probably due to the inhibitory effect of ZD6474 on VEGF signaling in endothelial cells. ZD6474 has also demonstrated potent inhibition of ligand-dependent RET receptor and selective inhibition of RET-dependent thyroid tumor cell growth *in vitro* [26,27], providing a potential treatment option for subgroups of patients with papillary carcinoma and hereditary medullary carcinoma. In particular, the RET gene is activated by somatic rearrangements in papillary thyroid carcinoma (PTC), resulting in chimeric sequences called 'RET/PTC' [28,29]. RET rearrangements are found in 3–33% of papillary carcinomas unassociated with irradiation [30] and in 60–80% of those occurring after irradiation, such as in children exposed to the Chernobyl nuclear accident [31] or in patients who received external radiation treatment in childhood [32]. In addition to these findings, patients with hereditary MTC associated with MEN types 2A and 2B and familial MTC (FMTC) have point mutations in the RET proto-oncogene. In particular, MEN2A and

FMTC patients typically contain a mutation that alters one of six cysteines (codons 609, 611, 618, 620, 630 or 634) within the extracellular domain of RET, permitting disulfide bonding between receptor pairs and ligand-independent activation [7]. MEN2B usually contains a methionine to threonine substitution at position 918 (M918T) within the tyrosine kinase catalytic domain of RET. RET/PTC, RET/MEN2A, RET/MEN2B and RET/FMTC alleles induce transformed foci, anchorage-independent growth and tumorigenicity in NIH3T3 cells [28]. Carlomagno *et al.* [26] have shown that ZD6474 blocks the enzymatic activity of RET-derived oncoproteins in NIH3T3 cells, and the in-vivo phosphorylation and signaling of the RET/PTC3 and RET/MEN2B oncoproteins. Recently, ZD6474 was found to block the enzymatic activity of RET-derived oncoproteins in cultured cell lines. For this purpose, Vidal *et al.* [33] have developed a *Drosophila* model for MEN2A and MEN2B diseases by targeting oncogenic forms of RET to the developing *Drosophila* eye. The results indicate that ZD6474 can act as an in-vivo inhibitor of RET signaling with high efficacy and very low toxicity [33]. It is clear that a possible advantage of ZD6474 in RET-associated tumors is that it has the potential to act both as an antiangiogenic drug and as an anticancer drug. The simultaneous assault on both neoplastic (RET and EGFR pathways) and endothelial (VEGFR pathway) cells may offer a mechanism to circumvent the development of resistance. ZD6474 has recently undergone a phase I study in 77 patients with a variety of advanced cancers refractory to standard therapy who were treated with oral ZD6474 at six different doses (50, 100, 200, 300, 500 or 600 mg daily). Patients received a single oral dose followed by a 7-day observation period (cycle 0). At the end of this period, patients received once-daily treatment at the same dose level as in cycle 0 for a total of 28 days (cycle 1). Patients were treated until evidence of tumor progression or unacceptable toxicity. Adverse events were generally mild and the most common dose-limiting toxicities (DLTs) were diarrhea, hypertension and acneiform rash. In the 500-mg/day cohort, three of eight patients experienced DLTs and this dose was therefore considered to exceed the maximum tolerated dose. Once-daily oral dosing of ZD6474 at 300 mg/day is generally well tolerated in patients with advanced solid tumors and this dose is being investigated in phase II trials [34].

### *AMG706*

AMG 706 is a potent oral, multi-kinase inhibitor that targets VEGF, platelet-derived growth factor (PDGF), KIT and RET receptors, and has antiangiogenic and antitumor activity. In a preclinical study, AMG706 produced a statistically significant reduction in vascular blood flow in a human tumor xenograft [35]. Safety and pharmacokinetics of AMG706 have been recently evaluated in a phase I trial in advanced solid tumors. AMG706 was well tolerated up to the dose of 125 mg once daily using the intermittent (21 days of therapy followed by 7 days without dosing) and continuous dosing schedule. The most frequent adverse events were hypertension, fatigue and headache, but the treatment was generally well tolerated. Four percent of the 71 evaluable patients had a partial response, while 61% had stable disease. Notably, one of the seven patients with thyroid cancer had a partial response. Vascular changes were demonstrated by dynamic contrast-enhanced magnetic resonance imaging [36]. A phase II study of AMG706 in advanced thyroid cancer is ongoing.

### *PTK787/ZK222584*

A number of other antiangiogenic drugs are undergoing preclinical and clinical evaluation in various histotypes of thyroid cancer. PTK787/ZK222584 (vatalanib) is an orally active protein kinase inhibitor that potently and selectively blocks the VEGF/VEGFR system. Vatalanib has been studied in preclinical models of ATC and in human follicular thyroid tumor xenografts that were implanted into nude mice. In this study, after 4 weeks, the treatment with PTK787 induced a 41.4% reduction in tumor volumes, with a significant decrease in neoangiogenesis [37].

### *ZD1839 (gefitinib)*

EGFR is frequently overexpressed in several tumor types, including thyroid carcinomas [38,39], and this overexpression has been shown to correlate with poor prognosis in several studies across a wide range of tumors [40,41]. In preclinical studies, EGF has been shown to stimulate follicular cell proliferation, and to enhance the migration and invasiveness of PTC [42]. A few studies have examined the clinical implications of EGFR expression and location in thyroid cancer. In a recent report, cytoplasmatic immunopositivity was significantly associated with the extent of primary tumor infiltration in PTC, whereas membranous staining was not. Moreover, in a multivariate survival analysis, strong cytoplasmic EGFR staining of PTC was significantly associated with a decrease in recurrence-free survival [43]. Preclinical studies have demonstrated that EGFR is universally expressed in anaplastic cancer cell lines [8]. These findings have been confirmed by immunohistochemistry and EGFR assays [44,45]. The above data suggest that EGFR plays a role in determining the malignant potential of ATC.

Gefitinib, a synthetic anilinoquinazoline, is an orally active EGFR inhibitor that is highly selective, with minimal activity against other tyrosine kinases. Gefitinib has been shown to block EGF-stimulated EGFR autophosphorylation and EGFR-mediated downstream signal transduction [46]. Antitumor activity of ZD1839 has been shown in tumor cell lines and in experimental animal models [47,48]. Gefitinib is now approved in the US as single agent for the treatment of non-small cell

lung cancer after failure of both platinum-based and docetaxel chemotherapies [49,50]. ZD1839 was found to inhibit EGFR phosphorylation *in vitro* and *in vivo*, and to reduce the growth of anaplastic cells in a nude mouse model of thyroid carcinoma cells injected subcutaneously [51]. In another study, a marked growth inhibition of xenografts was found in gefitinib-treated mice. Moreover, inhibition of EGFR-transmitted growth stimulation by ZD1839 was clearly observed in ATC cell lines [8]. These results are in keeping with evidence in several malignancies and point to gefitinib as a potential candidate for clinical trials in ATC.

#### *Cetuximab*

Cetuximab, a human–murine chimeric monoclonal antibody to EGFR, has consistently shown synergism in combination with chemotherapeutic agents, such as irinotecan, both in preclinical models and in clinical studies [52,53]. When combined with irinotecan, cetuximab has been shown to potentiate its in-vitro antiproliferative and proapoptotic effect in ATC, using an orthotopic model of ATC in nude mice. Cetuximab, irinotecan and the combination of these two agents resulted in 77, 79 and 93% in-vivo inhibition of tumor growth, respectively. Furthermore, combination therapy with cetuximab and irinotecan was shown to be more effective and yet significantly less toxic in the orthotopic ATC xenografts than doxorubicin, which is used frequently for the treatment of anaplastic thyroid cancer [54].

#### *AEE788*

Preclinical studies have recently demonstrated the efficacy of AEE788, a dual inhibitor of EGFR and VEGFR tyrosine kinases, against ATC cells. In this study, AEE788 was able to inhibit the proliferation and induce apoptosis of ATC cell lines *in vitro*, and showed activity against ATC xenografts *in vivo* in nude mice. The administration of AEE788 alone or in combination with paclitaxel inhibited the growth of ATC xenografts by 44 and 69%, respectively, compared with the control group [55].

#### *PP1*

PP1 is a pyrazolopyrimidine with a strong activity toward RET kinase [56]. Vitagliano *et al.* [57] demonstrated that the treatment of a panel of thyroid carcinoma cell lines with both ZD6474 and PP1 inhibited the enzymatic activity and transforming effects of RET oncoproteins by interruption of mitogen-activated protein kinase (MAPK) phosphorylation, reduced levels of G1 cyclins, increasing levels of the cyclin-dependent kinase inhibitor p27Kip1, in RET/PTC-positive cancer cells. The use of antisense oligonucleotide confirmed that p27 plays a key role in growth arrest induced by ZD6474 and PP1.

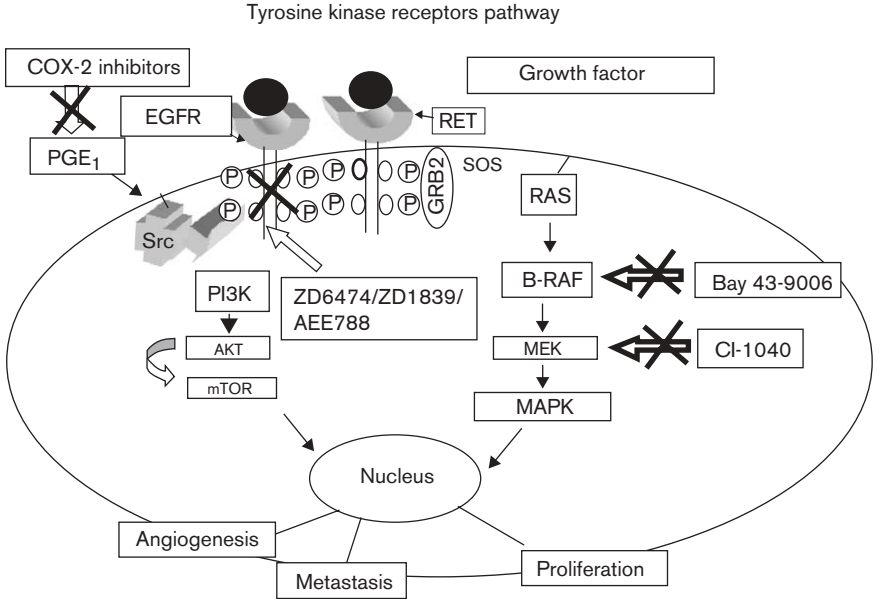
#### *Cyclooxygenase-2 inhibitors*

Cyclooxygenase-2 (COX-2) is an inducible enzyme that is involved in inflammation. Increasing evidence suggests a key role in promoting tumor cell growth and angiogenesis, probably through the activity of COX-2-derived prostaglandins, such as prostaglandin $E_2$ [58]. COX-2 overexpression has been observed in a variety of human cancers, including colon, cervix, lung, prostate and breast, and has been associated with poor prognosis [59–65]. In particular, recent studies have shown that COX-2 is expressed in thyroid lesions [66–69]. A recent study analyzed the mRNA expression levels of the enzymes COX-2 and thromboxane $A_2$ synthase, an enzyme located downstream from COX-2, in papillary carcinomas and matching normal tissues to determine the role of this enzyme in the development of PTC. Reverse-transcriptase polymerase chain reaction analysis showed significant increases of thromboxane $A_2$ mRNA levels in papillary carcinomas compared with normal thyroid tissues. This study also showed inhibition of tumor growth by the specific COX-2 inhibitor, NS-398 [70]. In addition, COX-2 stimulation of neoangiogenesis has been associated with the induction of VEGF in cancer cells [71]. EGFR activation induces COX-2 expression and prostaglandin $E_2$ production in cancer cells. In turn, prostaglandin $E_2$ can induce the activation of the phosphatidylinositol-3-kinase/Akt pathway through transactivation and phosphorylation of the EGFR [72]. In-vitro and in-vivo antitumor activity of ZD6474 and/or SC-236, a selective COX-2 inhibitor, in cancer cell lines with a functional EGFR autocrine pathway was investigated, and a prolonged growth inhibition with the combined treatment was demonstrated. This study provides a rationale for evaluating the simultaneous blockade of EGFR, COX-2 and VEGF signaling as cancer therapy in the clinic in a variety of cancers, among which are thyroid cancers [73]. Furthermore, the COX-2 pathway is believed to be involved in the modulation of multidrug resistance-1 in MTC, as P-glycoprotein expression and function in a rat model have been demonstrated to depend on COX-2 level. Zatelli *et al.* [74] demonstrated that rofecoxib, a selective COX-2 inhibitor, sensitizes human MTC cell lines expressing both multidrug resistance-1 and COX-2 to the cytotoxic effects of doxorubicin, reducing P-glycoprotein expression and function.

## Cytoplasm downstream effectors

Thyroid cell transformation to papillary cancers takes place through constitutive activation of effectors along the RET/PTC–RAS–B-RAF signaling pathway, one of which is required for tumor initiation or promotion. Constitutive activation of RET/PTC kinase activity promotes the interaction with Shc, an intermediate in the RAS pathway. RET-mediated transformation of NIH3T3 cells requires signaling via Shc and other docking proteins, such as Grb2–SOS complexes, leading to Ras-dependent activation of the MAPK pathway [75]. Activating point mutations in RAS small GTPases occur in about 10% of PTCs [76]. Point mutations in B-RAF are

**Fig. 1**




This figure schematically shows the tyrosine kinase receptors, such as EGFR and RET, and their downstream effectors. The two main signaling routes of the receptor tyrosine kinase are the RAS–RAF–MAPK pathway and the PI3K protein–serine/threonine kinase–AKT pathway. Once activated, RAF kinases can phosphorylate MEK, which in turn phosphorylates and activates ERKs that can phosphorylate several nuclear transcription factors and regulate cell cycle progression. Moreover, this figure shows the main drugs that act along this pathway. EGFR, epidermal growth factor receptor; MAPK, mitogen-activated protein kinase; PI3K, the phosphatidylinositol 3-kinase; ERK, extracellular signal-regulated kinase.

the most common genetic lesions found in thyroid carcinomas (up to 50% of cases) [77]. B-RAF belongs to the RAF family of serine/threonine kinases that includes C-RAF and A-RAF with different tissue distribution of expression [78]. Among the three forms of RAF kinases, B-RAF, with its gene located on chromosome 7, is the most potent activator of the MAPK pathway. B-RAF-activating missense point mutations in the kinase domain are clustered in exons 11 and 15 of the gene and T1799A, a transversion mutation, accounts for more than 80% of all B-RAF mutations [79].

RAF proteins are components of the RAF–MAPK–extracellular signal-regulated kinase (ERK) pathway, which is a highly conserved signaling module in eukaryotes. In addition, in human papillary carcinomas, RET, RAS and B-RAF genetic alterations are mutually exclusive, which suggests that mutations at more than one of these sites are unlikely to provide an additional biological advantage [77,80,81].

Once activated, RAF kinases can phosphorylate MAPK ERK (MEK), which in turn phosphorylates and activates ERKs that can phosphorylate several nuclear transcription factors and regulate cell cycle progression [82]. Therefore, PTCs are to be considered as initiated by a set of transforming events that target proteins that act along a linear signaling cascade. Activation of this signaling cascade results in upregulation of chemokines and their receptors that are relevant for sustained proliferation and motility of tumor cells (Fig. 1). Probably, these genes may act cooperatively to commit transformed thyroid cells to a malignant invasive phenotype [83]. The main drugs acting along this cascade are described below.

## B-RAF/MEK inhibitors

### *Bay 43-9006 (sorafenib)*

Recent studies have been focused on the clinical significance of the B-RAF mutation, particularly on its diagnostic and prognostic values [10,84–86]. Nikiforova *et al.* [10] reported a correlation between B-RAF mutation and clinicopathological features of 104 papillary carcinomas (38 B-RAF-positive and 66 B-RAF-negative). A significant increase of B-RAF mutation was associated with extrathyroidal invasion, because 16 of 38 (42%) patients with B-RAF mutations had extrathyroidal invasion versus 13 of 66 patients (20%) without mutations ($P = 0.03$). Furthermore, B-RAF mutation was also associated with advanced stage because 10 of 38 (26%) patients with B-RAF mutations had stage III disease versus two of 66 (3%) patients without mutations ($P = 0.006$); seven of 38 (18%) patients with B-RAF mutation had stage IV disease versus three of 66 (4%) patients without mutation ($P = 0.03$) [10]. In parallel, in a Japanese series of 126 PTCs, Namba *et al.* [84] found a significant association of B-RAF with advanced stages of

the tumor and distant metastases, which were observed in seven of 38 patients (18%) with B-RAF mutations and in five of 88 patients (6%) without mutations ($P = 0.033$). On the other hand, in three other small-sized studies [85,87,88], no significant association of B-RAF mutation with any of the above parameters was revealed. These findings may suggest that strategies aimed at inhibition of B-RAF may be potentially effective for the treatment of papillary carcinoma and possibly of ATC, also in consideration of the promising in-vitro and in-vivo studies [89–91]. Among B-RAF inhibitors, sorafenib has been the first molecule to undergo clinical development. This compound is a potent competitive inhibitor of ATP binding in the catalytic domains of C-RAF, wild-type and mutant B-RAF [92,93]. Particularly, by binding with the kinase domain of B-RAF, sorafenib locks the kinase in an inactive state, inhibiting B-RAF-stimulated DNA synthesis and cell proliferation, and inducing apoptosis [92]. Sorafenib has also been shown to exert an antiangiogenic effect by targeting the receptor tyrosine kinases VEGFR-2 and PDGFR, and their associated signaling cascades [94]. Salvatore *et al.* [95] have evaluated the effect of sorafenib in a panel of six $^{V600E}$B-RAF-positive thyroid carcinoma cell lines and in nude mice bearing ARO cell xenografts. In the latter experiment, in particular, ARO cell tumor xenografts were significantly ($P < 0.0001$) smaller in nude mice treated with sorafenib than in control mice and this inhibition was associated with suppression of phospho-MAPK levels. These data lend further support to the idea that B-RAF provides signals crucial for proliferation of thyroid carcinoma cells spontaneously harboring the $^{V600E}$B-RAF mutation and, therefore, RAF suppression might have therapeutic potential in $^{V600E}$B-RAF-positive thyroid cancer [95].

A huge phase I program with sorafenib has been carried out, and diarrhea, skin rash and fatigue have qualified as DLTs [96,97]. There are hints that skin toxicity can be associated with treatment outcome [96]. The dose of 400 mg twice daily on a continuous basis has been selected for further drug evaluation. A phase III study is under way in patients with advanced renal carcinomas due to the activity shown in this chemoresistant disease. Among the studies in other tumors, a phase II trial in thyroid carcinoma is planned.

#### *CI-1040*

Several MAPK pathway inhibitors acting at different steps have also been developed [98]. The MEK inhibitor CI-1040 is a highly potent and selective inhibitor of both MEK isoforms (MEK-1 and MEK-2) [99]. Binding of CI-1040 in the hydrophobic binding pocket of MEKs induces a conformational change in unphosphorylated MEK that locks it into a closed but catalytically inactive form. A recent study demonstrated that treatment of cells with MEK inhibitors could restore the expression of thyroglobulin and sodium ($Na^+$)/iodine ($I^-$) symporter [100]. In this sense, the MAPK pathway inhibitors could be particularly useful in combination with radioiodine treatment for thyroid cancers that have decreased or lost radioiodine avidity. These hypotheses need to be tested.

Preclinical studies have demonstrated that CI-1040 inhibits the clonogenic growth of a panel of tumor cell lines, with tumors expressing constitutive phosphorylated ERK levels being more sensitive [99]. In a recent phase I study, CI-1040 was tested in multiple daily oral doses on a continuous schedule. Grade 3 asthenia qualified as a DLT at the level of 800 mg three times daily. Diarrhea, rash, nausea and vomiting were the most common toxicities. The maximum tolerated dose and recommended phase II oral dose was 800 mg orally administered twice daily on a continuous dosing schedule. One partial response was achieved in a patient with pancreatic cancer and 19 patients (28%) achieved stable disease lasting a median of 5.5 months; inhibition of tumor pERK was demonstrated in 10 patients. [101]. The following phase II study, however, failed to show drug activity in advanced colorectal, non-small lung cancer, breast and pancreatic cancer [102].

The therapeutic activity of CI-1040 in thyroid cancer is worth investigating.

#### Imatinib mesylate

Preclinical studies have shown that c-ABL, a ubiquitous non-receptor tyrosine kinase that is located in both the nucleus and cytoplasm, and participates in regulation of the cell cycle and of the genotoxic stress response pathway [103–105], is overexpressed in p53-mutated/deficient ATC cell lines [106]. p53 gene mutation is very common in ATC, occurring in 70–85% of cases, but it is unusual in differentiated carcinoma (0–9%) [11,12]. The tyrosine kinase inhibitor imatinib, which selectively suppresses the activity of ABL, PDGFR and c-KIT [107,108], induced remarkable growth inhibition in p53-defective or mutant ATC cell lines. This effect is mediated by inhibition of c-ABL kinase. These findings suggest that selective suppression of c-ABL activity by imatinib may represent a potential anticancer strategy for p53-mutated undifferentiated thyroid carcinomas [106]. An ongoing phase II trial is being conducted to test the effect of imatinib in patients with anaplastic thyroid cancer.

## Nuclear-active drugs

### Histone deacetylase inhibitors

A major mechanism controlling cellular differentiation and biological behavior of cancer cells is the regulation of acetylation of conserved lysine residues on their amino-terminal tails of histones. Transcription in eukaryotic cells is influenced by the manner in which DNA is packaged.

In a transcriptionally inactive state, DNA is tightly compacted to prevent accessibility of transcription factors. DNA is packaged into chromatin, a highly organized and dynamic protein–DNA complex. The fundamental subunit of chromatin, the nucleosome, is composed of an octamer of four core histones. To activate transcription, the compact inactive nucleosome must undergo conformational relaxation, which may facilitate the binding of transcriptional factors. Chromatin relaxation is associated with histone acetylation by histone acetyltransferases. A variety of normal DNA-binding proteins or oncogene products can complex with other proteins, and these complexes recruit HDACs, which cleave acetyl groups from histones, thereby preventing chromatin relaxation and blocking gene transcription [109]. Importantly, dysregulated histone acetyltransferase or HDAC activity has been found in certain human cancers [14]. In this manner, tumor cells are unable to undergo the normal cellular differentiation programs, which contribute to their neoplastic transformation. HDAC inhibitors can increase acetylation of histones and various other proteins, and can induce programmed cell death preferentially in transformed cells, which make them promising anticancer agents [110]. Several compounds, such as butyrates, the anticonvulsant valproic acid and the antifungal agent trichostatin A, have been shown to act as HDAC inhibitors [111,112]. Depsipeptide (FR901228, NSC630176) is a novel anticancer agent isolated from the fermentation broth of *Chromobacterium violaceum* [113]. Laboratory studies have demonstrated that depsipeptide, like other HDAC inhibitors, induces expression of a specific subset of genes linked to inhibition of cell growth and induction of differentiation [114,115]. In particular, in thyroid cancer cells, low concentrations of depsipeptide and valproic acid have been shown to increase expression of a functional sodium ($Na^+$)/iodine ($I^-$) symporter in poorly differentiated thyroid carcinoma cells [13,14]. These findings suggest that HDAC inhibitors are a potential therapeutic strategy for resensitizing resistant thyroid cancer to radioiodine. In addition, preclinical studies demonstrated that depsipeptide enhances apoptotic killing by p53 gene therapy in thyroid carcinoma cells [116]. Depsipeptide has demonstrated potent cytotoxic activity against human tumor xenografts and murine tumors [117]. A phase I trial of depsipeptide has been run in 37 patients with refractory neoplasms, none of whom had thyroid carcinoma [118]. Fatigue, nausea, vomiting, transient thrombocytopenia and neutropenia qualified as DLT, while no evidence of cardiac toxicity was observed.

A phase II study for patients with recurrent and/or metastatic thyroid cancer that has not responded to radioactive iodine is ongoing.

A class of novel synthetic hybrid polar compounds includes hydroxamic acid-based suberoylanilide hydroxamic acid (SAHA), which causes accumulation of acetylated histones in cultured cells and induces differentiation and/or apoptosis of transformed cells in culture [119,120]. Tumor cells are much more sensitive to SAHA than normal cells [121]. Moreover, in a panel of thyroid carcinoma cell lines, including those with defects in the p53 pathway, SAHA induces accumulation of acetylated histones, early up-regulation of the cyclin-dependent kinase inhibitor p21 through p53-independent mechanism and a decrease in phosphorylation of the cyclin-dependent kinase substrate retinoblastoma, followed by growth arrest and apoptosis [122–124]. These findings suggest that SAHA is expected to be active even against tumors with p53 mutations, such as poorly differentiated and ATC [11], and provide the rationale for clinical studies of SAHA, either as a monotherapy or in combination, in ATC. The early experience generated from phase I clinical trials has confirmed SAHA biological activity, consisting in accumulation of acetylated histones *in vivo* for at least 4 h after infusion, with an acceptable safety profile [125]. Evidence of antitumor activity was also observed, including reduction in measurable disease in a refractory papillary thyroid cancer. Early studies of an oral SAHA formulation are currently ongoing.

### Vitamin A-derived retinoic acids

Retinoids are vitamin A derivatives that activate retinoic acid (RA) receptors (RARs) and retinoid X receptors (RXRs) [126]. Retinoids are key regulators of vertebrate morphogenesis, proliferation and differentiation [127]. Moreover, retinoids have been shown to inhibit cellular growth and to induce redifferentiation in some poorly differentiated thyroid cancer cell lines [128,129]. These receptors are members of the steroid hormone receptor gene superfamily, bind to the naturally occurring all-*trans*-RA (RARs) and 9-*cis*-RA (RXRs), and are essential for the differentiation and maintenance of normal epithelium. In general, these receptors function as ligand-dependent transcription factors and modulate the expression of RA-responsive genes by interaction with additional protein cofactors [130]. The prototype for retinoid-based therapies comes from the experience with using all-*trans*-RA in combination with chemotherapy for the treatment of acute promyelocytic leukaemia [126]. Additionally, Schmutzler *et al.* [15] investigated the ability of all-*trans*-RA to induce redifferentiation in human thyroid carcinoma cell lines (including follicular and anaplastic) by its influence on iodide metabolism, 5′-deiodinase induction and proliferation reduction. In a clinical trial, 20 patients with advanced thyroid carcinoma lacking radioiodide accumulation were selected for treatment with 13-*cis*-RA at a dose of 1.5 mg/kg/day over 5 weeks. Iodine uptake increased in eight patients, while thyroglobulin increased in 12 patients. These data support a possible effect of retinoids on differentiation status of thyroid carcinoma [128].

A phase I clinical trial of LGD1550, a potent activator of gene expression that binds all three RAR isoforms, accrued 27 patients, seven of whom had thyroid carcinoma. Patients received oral LGD1550 at daily doses ranging between 20 and 400 $\mu g/m^2$. Four of seven patients with thyroid cancer had stable disease that lasted between 20 and 56 weeks [131]. Skin toxicity was the DLT at 400 $\mu g/m^2$; other frequent toxicities were nausea and headache. The assumption that RA causes growth reduction of tumors, and increased expression and functionality of a thyroid differentiation marker warrants additional clinical studies.

### Peroxisome proliferator-activated receptor-γ expression ligands

Another class of nuclear hormone receptors is the peroxisome proliferator-activated receptors (PPARs), of which three isotypes exist (α, β and γ). Like other nuclear hormone receptors, PPAR-γ contains a DNA-binding domain and COOH-terminal ligand-binding domain that mediates dimerization and transactivation functions [132]. The ligands for PPAR-γ include naturally occurring prostaglandins and most notably the thiazolidinediones, a class of antidiabetic drugs [133]. The effects of liganded PPAR-γ on thyroid carcinoma cell lines have been studied as well. Martelli *et al.* [134] studied several human thyroid carcinoma cell lines with regard to PPAR-γ expression. In this study, five of six carcinoma cell lines expressed PPAR-γ, and had decreased cellular growth at 48 h, increased $G_1$ cell cycle arrest and increased apoptosis when treated with a thiazolidinedione [134]. In another study, Ohta *et al.* [135] used troglitazone to study its effects on papillary carcinoma cell lines that differentially expressed PPAR-γ receptors. Troglitazone caused a significant decrease in cell number in culture in PPAR-γ-positive papillary carcinomas compared with those that were PPAR-γ-negative at 72 h [135]. Furthermore, it is worth noting that PPAR-γ and RXRs preferentially form heterodimers that interact with peroxisome proliferator response elements regulating the expression of target genes [136,137]. Some studies suggest that the combination of thiazolidinediones and retinoids may redifferentiate tumors by a synergistic or additive mechanism [138]. In fact, a preclinical study showed that DRO-90 cell lines that express both RXR-γ and PPAR-γ mRNA and protein had a more substantial decrease in cellular proliferation with either RXR-γ or PPAR-γ ligand than other cell lines that expressed only one nuclear hormone receptor type [139]. Moreover, the level of apoptosis is synergistically enhanced when DRO-90 cells are treated with the combination of retinoids and thiazolidinediones. In summary, expression of RXR-γ and/or PPAR-γ seems to predict response to ligand treatment and is necessary for inhibition of thyroid carcinoma growth with appropriate ligands.

### Conclusion

From a clinical and biological standpoint, thyroid cancers represent a broad spectrum of neoplastic disorders that include histological subtypes, such as poorly differentiated, anaplastic and MTCs, which are refractory to most conventional systemic therapeutic strategies. Although differentiated thyroid cancers are frequently curable with current standard modalities, even a substantial fraction of these patients need new approaches. Thyroid carcinoma represents a fascinating model, in which the initiating event, through a variety of genetic alterations, may be found and plays a pivotal role in tumor genesis and progression. Efforts targeted at inhibiting activated pathways may lead to development of novel effective therapies for thyroid cancer, which could substantially modify its outcome, especially in the poor prognosis subsets.

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