Novel treatment options for anaplastic thyroid cancer

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Abstract

Introduction: Several genetic alterations have been identified in different molecular pathways of anaplastic thyroid cancer (ATC) and associated with tumor aggressiveness and progression (BRAF, p53, RAS, EGFR, VEGFR1, VEGFR2, etc). New drugs targeting these molecular pathways have been recently evaluated in ATC.

Areas covered: We review the new targeted therapies of ATC.

Expert Opinion/Commentary: Interesting results have been reported with molecules targeting different pathways, as: a-BRAF (dabrafenib/trametinib, vemurafenib); b-angiogenesis (sorafenib, combretastatin, vandetanib, sunitinib, lenvatinib, CLM3, etc); c-EGFR (gefitinib); d- PPARγ agonists (rosiglitazone, pioglitazone, efatutazone). In patients with **ATC** treated with lenvatinib, a median overall survival of 10.6 (3.8–19.8) months was reported.

In order to bypass the resistance to the single drug, the capability of targeted drugs to synergize with radiation, or chemotherapy, or other targeted drugs is explored.

Moreover, new affordable individual genomic analysis and the opportunity to test these new treatments in primary cell cultures from every ATC patient *in vitro*, might permit to personalize the therapy, increasing the therapeutic effectiveness and avoiding the use of ineffective drugs.

The identification of new treatments is necessary, to extend life duration guaranteing a good quality of life.

Keywords: anaplastic thyroid cancer; molecular pathways; targeted drugs; *in vitro* studies; *in vivo* studies.

1. Introduction

Anaplastic thyroid cancer (ATC) represents less than 2% of thyroid carcinoma, but it is one of the most aggressive human neoplasms. It is associated with a rapid clinical course, and it accounts for 15–40% of thyroid carcinoma deaths [1, 2].

ATC is classified as Stage IV "thyroid cancer" (TC) (American Joint Committee on Cancer), regardless of tumor size or presence of lymph-node or distant metastasis [3], and it is commonly aggressive or metastatic at the diagnosis [4, 5].

It has been reported that the most efficacious therapy of ATC is multimodal treatment that includes debulking, hyperfractionated accelerated external beam radiotherapy, and chemotherapy (doxorubicin or cisplatin), (median survival of 10 months) [6]. In a paper by Foote et al. [7], evaluating 25 new ATC patients, 10 subjects (40%) had metastatic disease at the diagnosis and were treated with palliative treatment, 5 (20%) had regionally confined disease, and 10 consecutive patients (40%) had regionally confined ATC and were treated combining individualized surgery (if possible), intensity-modulated radiation therapy (IMRT), and radiosensitizing+adjuvant chemotherapy (4 cycles of docetaxel+doxorubicin). The overall survival (OS) at 1 and 2 years was 70% and 60%, with respect to <20% historical survival at 1 year in ATC patients earlier treated with surgery and conventional postoperative radiation. The combination of IMRT and radiosensitizing+adjuvant chemotherapy seems to improve outcomes, also in patients with stages IVA and IVB regionally confined ATC, even if the effectiveness in patients with stage IVC (metastatic) disease is still unclear. Onoda et al. [8] evaluated 6 patients who underwent external irradiation (45 to 60 Gy) in combination with concurrent low-dose weekly docetaxel administration at 10 mg/m^2. Survival was 86-1,901 days with additional systemic chemotherapy, and no toxicities over grade 3 were shown. The Authors concluded that chemoradiotherapy is useful

for locoregional control of ATC, with acceptable toxicity, lasting long enough to maintain patients' quality of life. A prospective clinical study [9] was conducted in 56 ATC patients to assess the feasibility and efficacy of weekly paclitaxel (80 mg/m^2) administration. The median OS was 6.7 months [confidence interval 4.4-9.0]. The 6-month survival was 54%. The objective response rate was 21%, and the clinical benefit rate was 73%. The median time to progression was 1.6 months. No adverse events occurred. The Authors concluded that the weekly paclitaxel administration in ATC patients could be effective in a neo-adjuvant setting.

ATA guidelines suggest that paclitaxel or docetaxel, doxorubicin, and also platins are effective in ATC, however, none of these drugs is able to extend survival in advanced ATC [10].

The rarity and aggressive nature of ATC makes it difficult to determine patient response to different treatments.

Various genetic mutations have been reported in different molecular pathways of ATC and associated with tumor progression [10, 11], and new drugs having these molecular pathways as targets have been recently evaluated in ATC [10].

Here we review the new targeted therapy of ATC.

2. Molecular targets of ATC

In ATC, the molecular and genetic alterations have been studied to identify genomic mutations specifically correlated with this neoplasm [10, 12].

Among the determinant genetic mutations in ATC carcinogenesis, BRAF V600E occurs in approximately 45% of papillary thyroid cancer (PTC), and 25% of ATCs [13, 14].

As a result, BRAF mutations kinase become active and phosphorylate downstream targets such as MEK and ERK [15].

Several studies have shown an association among BRAF V600E mutation with features linked to a poor prognosis, such as larger tumor, lymph node or extrathyroidal metastasis [16, 17].

Molecular testing should be performed as routine testing in patients with ATC, to evaluate targets of new treatments (for example BRAF mutations) [18-20].

RET/PTC rearrangements have been reported in 3 cases of ATC tissues [21], perhaps owing to the coexistence of ATC and PTC in the same tissue.

The tumor suppressor gene p53 mutation is not common in follicular thyroid cancer (FTC) and PTC, while it is frequent in ATC (ranging from 70% to 88%) [22, 23].

Point mutations within RAS genes are found in approximately 15% PTCs, 40% of FTCs, and 50% of ATCs. RAS mutations involve codons HRAS, NRAS (in 61 codon), and KRAS (in codon 13/12). Mutant RAS activate PI3K/AKT and MAPK pathways, and are correlated to a poor prognosis and more aggressive behavior of ATC [24, 25]. In particular, some Authors suggest that a more accurate prediction of TC outcome is possible thanks to a more extensive genetic analysis, since some data suggest a more aggressive clinical course in those patients harboring tumors with combination of other mutations such as telomerase reverse transcriptase promoter (TERTp) and BRAF V600E or TERTp and RAS [26, 27].

Vascular endothelial growth factor (VEGF)-A is involved in the survival and proliferation of endothelial cells [28]. In general, neoplastic cells expressing VEGF are clinically aggressive, grow rapidly and metastasize to distant organs. Indeed, VEGF is most strongly produced by highly malignant ATC [29]. Differentiated thyroid cancers (DTC) express elevated levels of VEGF-A and VEGF-receptor

(VEGFR), mainly VEGFR-2, in comparison with normal thyroid tissue [30]. Furthermore, augmented VEGF expression in thyroid carcinoma was associated with poor prognosis, increased tumor size, and presence of metastases [31]. In a paper by Gulubova et al. [32], the expression of VEGF and microvessel density in TCs and the effect of VEGF expression in thyroid tumor cells on the dendritic cells were evaluated in 65 patients with different types of TCs: PTC, oncocytic (OTC), FTC and ATC. PTC expressed VEGF more significantly than ATC (92.3% versus 60.0%, P = 0.025). The microvessel density (identified by antibodies against CD31) in the tumor border of PTC was significantly higher with respect to FTC (P = 0.039), but not to ATC and OTC (P = 0.337 and 0.134). The Authors concluded VEGF expression in tumor cells of TC is able to induce neovascularization.

Amplifications, mutations or misregulations of epidermal growth factor receptor (EGFR) (the cell-surface receptor of members of the EGF family [33]) are involved in approximately 30% of epithelial carcinoma. EGFR was associated with tumor invasion and progression in TC [34, 35], and it is overexpressed in ATC.

A copy number gain has been observed in different receptor tyrosine kinase (RTK) genes (EGFR, VEGFR1, VEGFR2, PDGFRα, PDGFRβ, PIK3Ca, PIK3Cb, KIT, MET, and PDK1) [21] in DTC. However copy number gains were more prevalent in ATC, with respect to DTC [36]. Most of these genes are determinant in ATC carcinogenesis, for this reason it has been hypothesized that gene copy number variations is implicated in the aggressiveness and progression of this neoplasm [37].

Histone acetylation (resulting in an open chromatin configuration leading to increase in the gene transcription rate) is an important mechanism that controls biology of neoplastic cells, that have dysregulated histone deacetylase, or histone acetyltransferase activity [38, 39]. A paper by Zhang et al. [40] evaluated the first-in-

class dual inhibitor of EGFR, HER2 and histone deacetylase (HDAC)s, CUDC-101, in ATC. The CUDC-101 anticancer effect was associated with increased expression of p21 and E-cadherin, and reduced expression of survivin, XIAP, β-catenin, N-cadherin, and Vimentin. CUDC-101 inhibited tumor growth and metastases, and significantly prolonged survival, in an *in vivo* mouse model of metastatic ATC. The data provided a preclinical basis to evaluate CUDC-101 therapy in ATC.

Furthermore, the upregulated expression of miR-20a in ATC is supposed to counteract TC progression, having therapeutic implications [41].

Programmed death-1 (PD-1) is an inhibitory receptor expressed on the surface of activated T cells. T cell function is inhibited by the binding of PD-1 to its ligands, PD-L1 and PD-L2, that are expressed within the tumor microenvironment, mediating the inhibition of T cells, through a process called "adaptive resistance" [42]. PD-1 and PD-L1 play a pivotal role in the ability of tumor cells to evade the host's immune system, and blocking their interactions enhances immune function *in vitro* and mediates antitumor activity in preclinical models [43]. PD-L1 expression is considered a potential biomarker for response of anti-PD-1 or anti-PD-L1 agents in different tumors [44]. Four hundred and seven primary TCs with a median 13.7-year of follow-up were evaluated for PD-L1 expression. Tumoral PD-L1 was expressed in 6.1% of PTC, 7.6% of FTC and 22.2% of ATC. The Authors concluded that PD-L1 was strongly expressed in patients with advanced TC, as FTC and ATC. Identification of PD-L1 expression may have direct therapeutic relevance to patients with refractory TC.

3. Drugs that target BRAF in ATC

3.1 Dabrafenib and the combination dabrafenib/trametinib

Dabrafenib is a drug for the treatment of cancers with BRAF mutations. Different clinical trials showed that resistance to dabrafenib and other BRAF inhibitors occurs within 6 to 7 months, and to bypass this problem, the BRAF inhibitor dabrafenib was combined with the MEK inhibitor trametinib [45].

The effectiveness of inhibiting the activated RAS/RAF/MEK pathway in ATC cells was investigated in 4 human ATC cell lines (ACT-1, OCUT-2, OCUT-4 and OCUT-6) [46]. ACT-1 and OCUT-6 had wild-type BRAF and NRAS mutations, OCUT-4 had a BRAF mutation, and OCUT-2 had BRAF and PI3KCA mutations. Dabrafenib inhibited the viability in BRAF mutated cells by G0/G1-arrest via the downregulation of MEK/ERK phosphorylation. Upon treatment with dabrafenib, upregulated phosphorylation of MEK was shown in RAS mutated cells leading to VEGF upregulation. Trametinib inhibited the cellular viability downregulating ERK phosphorylation. In the 4 ATC cell lines, dual blockade by both inhibitors showed cytostatic effects.

FDA approved the combination of dabrafenib and trametinib for BRAF V600E/K-mutant metastatic melanoma in 2014 [47].

Two cases of BRAF V600E-positive ATC administered with the BRAF inhibitor dabrafenib were reported by Lim et al. [48]. A 49-year-old woman with a T4bN1bM0 ATC, with symptomatic metastatic disease 8 weeks after radical chemoradiotherapy, was treated with dabrafenib. Upon 1 month from the beginning of the treatment, a complete symptomatic response was shown by FDG-PET scan. The therapy was stopped after 3 months because of disease progression, and the woman died 11 months after the diagnosis. The second patient was a 67-year-old man, who was administered with dabrafenib for a T4aN1bM0 ATC, halving the tumor size within 10 days of treatment. Stable disease (SD) was achieved for 11 weeks but the patient died

11 months after the diagnosis owing to disease progression. The Authors concluded that BRAF inhibitor monotherapy in ATC can get short clinical benefits. Murine models of BRAF V600E-positive ATC have shown a significant extended survival in mice administered with a combination of a BRAF inhibitor (as dabrafenib) and a MEK inhibitor (as trametinib) with respect to those treated with a BRAF inhibitor alone [48].

Another study reported a case report of an 81-year-old woman with a growing neck mass [49]. The initial diagnosis was of medullary thyroid cancer (MTC), thus she underwent total thyroidectomy. Surgical pathology revealed a 9-cm ATC. Her tumor harbored a BRAFV600E mutation (1799T>A p.V600E) and she was treated with external beam radiation therapy. She was found to have lung metastases and progression in the neck, after 4 months from the initial diagnosis. Then she received an additional 24 Gy of external beam radiation to the neck, followed by pazopanib. Her neck and lung masses progressed rapidly, and owing to the urgency to start treatment, the liquid formulations of dabrafenib and trametinib were used. She started full doses of both drugs (dabrafenib 150 mg twice a day and trametinib 2 mg daily), and 2 weeks after she began to feel less pressure in her neck. Restaging CT 1 month after starting dabrafenib and trametinib showed a remarkable treatment response in the neck and lungs, and such response was sustained after 4 months of follow-up. The patient experienced hypothyroidism while on treatment, fatigue, weakness and edema. For this reason, dabrafenib and trametinib were dose reduced, but her edema did not improve. After 6 months, she experienced progression, then stopped therapy and died [49].

A phase II, open-label study in patients with BRAF V600E-positive rare tumors (including ATC, biliary tract cancer, gastrointestinal stromal tumor, non-

seminomatous germ cell tumor/nongeminomatous germ cell tumor, hairy cell leukemia, World Health Organization (WHO) grade 1 or 2 glioma, WHO grade 3 or 4 (high-grade) glioma, multiple myeloma, and adenocarcinoma of the small intestine) evaluated the clinical efficacy and safety of the combination therapy of dabrafenib and trametinib (ClinicalTrials.gov Identifier NCT02034110) [50]. Patients receive dabrafenib (150 mg twice daily orally) and trametinib (2 mg once daily orally) on a continuous dosing schedule until unacceptable toxicity, disease progression, or death occurs. Responses are assessed every 8 weeks per tumor-specific response criteria. The primary study endpoint is overall response rate (ORR) by investigator assessment, and secondary objectives are duration of response, PFS, OS, and safety. Pharmacodynamic markers and quality of life will also be evaluated. This trial is still recruiting patients in the United States, Europe, Canada, and South Korea (verified on **May** 2017) [50].

3.2 Vemurafenib

Vemurafenib is a small molecule able to block BRAF, arresting MAPK pathway, that is approved by FDA in patients with metastatic melanomas harboring V600E mutation.

In a phase I study, three DTC patients were enrolled and treated with vemurafenib. One had a PR while the other two obtained a SD [51]. In a mouse model, vemurafenib suppressed growth of BRAF mutated human ATC [52]. A dramatic response to vemurafenib in a 51-year-old man with BRAF-mutated ATC has been described showing an almost complete clearing of metastatic disease by 8F-FDG–PET and computed tomography (CT) of the chest [53].

Another case report showed a sustained response to vemurafenib in a BRAFV600E-mutated ATC patient [54].

However a recent paper showed only a transient initial response in another ATC patient [55].

One hundred-twenty two patients with BRAF V600 mutation-positive cancer, including 7 with ATC, were evaluated by another paper [56]. Ninety-five patients received vemurafenib alone, and 27 with colorectal carcinoma were treated with vemurafenib and cetuximab in combination. Anecdotal responses were observed among patients with pleomorphic xanthoastrocytoma, ovarian cancer, ATC, cholangiocarcinoma, salivary-duct cancer, and clear-cell sarcoma and among patients with colorectal cancer receiving vemurafenib and cetuximab.

4. Drugs that target angiogenesis in ATC

4.1 Vascular Disrupting Mechanism

4.1.1 Combretastatin

Combretastatin A4 phosphate (CA4P or fosbretabulin) is a microtubule depolymerizing agent that exerts its activity against tumor vascular networks, interrupting the blood flow in the tumor, causing necrosis [57]. A complete response was evidenced in 1 patient administered with combretastatin, still living 30 months after the therapy [58].

In ATC a randomized, controlled phase II/III study (FACT trial) evaluated carboplatin/paclitaxel, in association with CA4P (experimental group), or not (control group) [59]. Eighty patients were enrolled (55% had been submitted to a cancer-related operation, of whom 70% had near-total or total thyroidectomy). In the CA4P arm the median was 8.2 months with respect to 4.0 months in controls, with a hazard

ratio of 0.66 (P = 0.25) and a relative suggested reduction in risk of death of 35%. One-year survival was: 33.3%, in the CA4P arm; 7.7%, in the control arm. These results suggested that thyroidectomy followed by carboplatin/paclitaxel, in association with CA4P, shows a not significant trend toward improvement in survival in ATC patients [59].

More recently an open-label study in 80 patients with ATC, of carboplatin/paclitaxel chemotherapy, in association with/without fosbretabulin, has been conducted reporting no significant differences between the 2 arms in PFS [60].

4.2 VEGF Pathway

4.2.1 Sorafenib

Sorafenib is an orally active multikinase inhibitor (mKI) that targets BRAF, c-Kit, RET, and VEGFR-1 and -2, and exerts anti-neoplastic actions in patients with TC, owing to its effects on BRAF pathway, RET, and angiogenesis. Several phase I, II, and III trials have assessed the antineoplastic action of sorafenib in patients with aggressive TC [61-64].

A phase III study showed that sorafenib is an effective therapy for progressive radioactive iodine-refractory DTC [64].

Sorafenib has been tested in twenty patients with ATC (not suceeding to previous therapies) in a multiinstitutional phase II trial. It was administered 400 mg twice daily. Ten% of patients had a partial response (PR), while 25% had a SD. The median PFS was 1.9 months, the median survival was 3.9 months, while 1 year survival was 20%. Sorafenib was not proved to be effective in patients with ATC [65].

Recently a synergistic anti-proliferative effect of metformin and sorafenib on growth of ATC cells and their stem cells has been shown *in vitro* [66].

4.2.2 Vandetanib

Vandetanib is an oral available multiple TK inhibitor that targets VEGFR-2 and -3, EGFR, and RET kinases, and has anti-angiogenetic activity, and it is approved by FDA and EMA in aggressive MTC [67, 68]. Vandetanib was evaluated in 1 phase III trial and 2 phase II trials in patients with advanced MTC, showing a clinically important antineoplastic activity [67, 69, 70].

In ATC xenografts it has been reported vandetanib reduces the tumor mass (up to 60%), and the vascularization of the neoplasm, in association with a reduced receptor activity of EGF-R/VEGF-R2 [71].

A randomised, double-blind, phase 2 trial enrolled adults with locally advanced or metastatic DTC (PTC, FTC, or poorly differentiated) from 16 European medical centres [72] (registered with ClinicalTrials.gov, number NCT00537095). Eligible patients received vandetanib 300 mg per day (vandetanib group comprised 72 patients) or matched placebo (placebo group of 73 subjects). Patients belonging to the vandetanib group had longer PFS than subjects administered with placebo (hazard ratio [HR] 0.63, 60% CI 0.54-0.74; one-sided P = 0.008); median PFS was 11.1 months (95% CI 7.7-14.0) for patients in the vandetanib group and 5.9 months (4.0-8.9) for subjects administered with placebo.

4.2.3 Sunitinib

Sunitinib is a multitarget TK inhibitor against VEGFR-2, c-Kit, PDGFR, FLT-3, RET and CSF-1R [73].

Sunitinib has been evaluated in 2 different phase II trials in TC [74, 75].

In an open-label phase II trial in 28 DTC and 7 MTC patients with aggressive TC [76]

a complete response was observed in 1 patient, with a PR in 28%, and 46% of SD [76].

A case report recently investigated sunitinib salvage therapy in an ATC patient [77]. A complete response in the neck mass was observed in this patient 12 weeks from the start of sunitinib therapy (next to the end of the 2nd cycle). However, the disappearance of the neck mass was not associated with a response in lung metastases that remained stable during the treatment. The patient died because of a massive upper gastrointestinal bleeding while in treatment with sunitinib approximately 5 months from the start of the therapy [77].

A phase 2 trial enrolled 71 patients (45 with differentiated or anaplastic tumor: 21 PTC, 13 FTC, 4 ATC, 7 other; 26 with medullary TC) in 1st line anti-angiogenic therapy with sunitinib at 50 mg/d, 4/6w [78]. Median PFS and OS were 13.1 and 26.4 months in patients with advanced radioactive iodine resistant differentiated TC, 16.5 and 29.4 months in medullary TC patients.

4.2.4 Axitinib

Axitinib is a multitarget TK inhibitor strongly selective for VEGFR-2, and targets VEGFR-1, -2, and -3, PDGFR, and c-Kit. In a phase trial 60 patients with iodine-refractory aggressive TC were treated with axitinib (5 mg b.i.d) [79]. Thirty% of patients showed PR (8 patients with PTC, 6 FTC, 2 MTC, and 1 ATC), while 38% had a SD; the median PFS was 18 months.

Another recent study evaluated the long-term outcomes in 60 patients with aggressive DTC (30 PTC, 15 FTC, 11 MTC, 2 ATC, 2 other) treated with axitinib. Thirty-eight% of patients had an objective response [PR in 23 patients, SD (≥16 weeks) in 18]. All histological subtypes responded to the treatment. The median OS was 35 months, with

a 15 months PFS, and a median duration of response of 21 months. This study showed axitinib is very effective and demonstrated long OS in DTC patients [80].

4.2.5 Lenvatinib (E7080)

The oral mKI lenvatinib is directed against VEGFR-1, -2, -3, PDGFRb, fibroblast growth factor receptors-1, -2, -3, -4, RET and c-KIT, and it has been demonstrated effective in aggressive DTC [81], for this reason it is actually approved by FDA and EMA for the treatment of advanced radioiodine–refractory DTC.

In vivo lenvatinib has shown antitumor activity against human TC in xenografts (in nude mice) of different histological types of TC (5 ATC, 5 DTC and 1 MTC). In these models lenvatinib has shown an important antiangiogenic activity both in DTC such as in ATC xenografts [82].

A single-arm, open-label, phase II study was conducted in Japan in patients with advanced TC treated with lenvatinib 24 mg/d in 28-d cycles until progressive disease or development of unacceptable toxicity [83]. Primary endpoint was safety, and secondary endpoint was efficacy, evaluated by PFS, OS, ORR, and disease control rate. Fifty-one patients, including 25 subjects with ^{131}I-refractory DTC, 9 with MTC, and 17 with ATC were enrolled (ClinicalTrials.gov Identifier NCT01728623). The most common any-grade treatment-related adverse events were hypertension (90%), palmar-plantar erythrodysaesthesia syndrome (77%), decreased appetite (78%), proteinuria (61%), fatigue (73%), diarrhea (55%), and stomatitis (57%). Incidences of grade 3 and 4 treatment-related adverse events were: 72% in ^{131}I-refractory DTC; 100% in MTC; 88% in ATC. Only 1 patient discontinued treatment owing to treatment-related adverse events. There were 4 fatal serious adverse events, all considered unrelated to lenvatinib. Median duration of treatment was 5.5 months

(range, 0.7–33.1) in ATC patients, and 8 received lenvatinib for more than 6 months. Lenvatinib showed tumor shrinkage in almost all subjects with advanced TC, including ATC patients. In ATC patients median OS (95% CI) was 10.6 (3.8–19.8) months. Toxicities were manageable with dose modifications [83].

4.2.6 CLM94, CLM3

The antitumoral activity of CLM94, a new cyclic amide with VEGFR-2 and antiangiogenic activity, has been recently demonstrated in ATC cells *in vitro* and *in vivo* in xenografts in the nude mice [84].

The antineoplastic activity of a pyrazolo [3,4-d]pyrimidine compound (CLM3) that is a multiple signal transduction inhibitor [including EGFR, the RET TK, and VEGFR-1 and with antiangiogenic activity] has been shown in primary ATC cells and in human ATC cell lines. CLM3 [85, 86] can inhibit the proliferation of "primary cultured cells from human ATC" (ANA) *in vitro*, and induces apoptosis, by reducing the phosphorylation of ERK1/2, EGFR, AKT, and cyclin D1, and decreasing the microvessel density in ANA. The results demonstrated the antiangiogenic and antitumor action of CLM3 is effective in ATC, opening the doors to next clinical evaluations [86].

More recently the antitumor activity of 2 new "pyrazolo[3,4-d]pyrimidine" compounds (CLM29 and CLM24) that inhibit several targets (including the RET tyrosine kinase, EGFR, VEGFR, with an antiangiogenic effect) in primary ATC cell cultures and in the human cell line 8305C was studied. The (V600E) BRAF mutation was observed in 3 ATCs; the results about the inhibition of proliferation by CLM29 and CLM24, obtained in ATC from tumors with (V600E) BRAF mutation were

similar to those from tumors without BRAF mutation. CLM29 inhibited too migration and invasion ($P < 0.01$) of primary ATC cells [87].

4.3 Inhibitors of EGFR Pathway

Gefitinib is an EGFR TK inhibitor with low molecular weight that reduces cell growth in TC cells [88]. Gefitinib inactivates the EGFR kinase and potentiates the inhibition induced by ionizing radiation of DTC and ATC cell proliferation [89].

A paper by Nobuhara et al. [90] investigated the expression of EGFR in ATC cell lines (OCUT-1, -2, TTA-1, KTC-1 and ACT-1), to assess the potential of therapies targeting EGFR as new therapeutic approaches. EGFR was expressed in all the ATC cell lines. Specific EGFR stimulation with epidermal growth factor showed significant phosphorylation of ERK1/2 and Akt, leading to growth stimulation in the ACT-1 cell line, that highly expressed EGFR, and this proliferation was inhibited by gefitinib. Furthermore, growth of xenografts inoculated in mice was inhibited dose-dependently with 25–50mg kg^{-1} of gefitinib administered orally. Inhibition of EGFR-transmitted growth stimulation by gefitinib was clearly observed in ATC cell lines.

A phase II trial was carried on in metastatic patients with aggressive TC (among whom 18 DTC) with (250 mg/daily) gefitinib. The results showed reduction of the tumor volume in 32% of patients (with no PR), SD at 3 months in 48% of patients, the OS was 17.5 months, and median PFS was 3.7 months. The Authors suggested that gefitinib has no significant effect in monotherapy [91].

However, in a case report of an ATC patient, administered with fixed-dose docetaxel and intermittent high-dose gefitinib, a PR was reported [92].

4.4 Anticancer immunotherapy targeting PD-1 and PD-L1

Two clinical trials of monoclonal antibodies targeting PD-1 and PD-L1 showed promising results as new anticancer immunotherapy.

An anti-PD-L1 antibody was administered intravenously (at escalating doses ranging from 0.3 to 10 mg/kilogram of body weight) to 207 patients with selected advanced cancers (75 with non–small-cell lung cancer, 55 with melanoma, 18 with colorectal cancer, 17 with renal-cell cancer, 17 with ovarian cancer, 14 with pancreatic cancer, 7 with gastric cancer, and 4 with breast cancer), every 14 days in 6-week cycles for up to 16 cycles or until the patient had a complete response or confirmed disease progression. Lasting tumor regression (objective response rate of 6 to 17%) and prolonged stabilization of disease (rates of 12 to 41% at 24 weeks) were obtained in patients with advanced cancers [93].

In a cohort of patients with advanced melanoma, non-small-cell lung cancer, castration-resistant prostate cancer, or renal-cell or colorectal cancer, those with tumors that resulted positive for PD-L1 expression, received an anti-PD-1 antibody and showed response rates of 36% in the anti-PD1 study [94].

BRAF, KRAS, EGFR mutations and protein overexpression of C-KIT and PD-L1 were assessed in ATC. Among the 13 ATC patients, 3 (23%) had BRAF V600E mutation, and 1 (8 %) patient had C-KIT overexpression. PD-L1 expression was reported in 3 (23%) patients. KRAS codon 12/13 and EGFR exon 18, 19, 20 and 21 were all wild type in our patients. The Authors concluded that protein kinase inhibitors and immunotherapy could be useful adjuvant therapies for ATC [95].

A paper evaluated the role of PD-L1 in TC and the effect of anti-PD-L1 antibody immunotherapy on tumor regression and intra-tumoral immune response alone or in combination with a BRAF inhibitor. TC cell lines and tumor samples from patients with BRAF V600E-positive tumors have higher levels of PD-L1 than either BRAF

WT tumors or matched normal tissues. Immunocompetent mice (B6129SF1/J) implanted with syngeneic 3747 BRAF V600E/WT P53-/- murine tumor cells were randomized to control, PLX4720, anti PD-L1 antibody and their combination. The combination of PD-L1 antibody and the BRAF inhibitor PLX4720 had a strong synergistic improvement in tumor shrinkage and an increase in tumor infiltrating lymphocytes. Clinical trials of this therapeutic combination could be useful in ATC patients [96].

5. Targeting PPARγ

PPARγ are nuclear hormone receptors [97] and their activation induces antineoplastic [98] effects in different cancer cells. PPARγ activatory ligands have been shown: 1- to have antiproliferative action on PTC cells, inducing apoptosis [97]; 2- to prevent in nude mice distant metastasis of BHP18–21 tumors [97]; 3- to induce redifferentiation of dedifferentiated TC cells [99-101]. PPARγ is overexpressed in human ATC cells [102], with respect to DTC, and PPARγ activation inhibits invasion and proliferation, inducing also apoptosis [102-104]. Rosiglitazone, a PPARγ agonist, increased the expression of thyroid specific differentiation markers in ATC cells [103]. Furthermore, in ANA [105, 106], rosiglitazone or pioglitazone inhibited ATC cell growth.

The activity of the PPARγ agonist efatutazone, and paclitaxel, was assessed in 15 ATC patients, administered orally with efatutazone (0.15, 0.3, 0.5 mg) two times per day, then with paclitaxel (every 3 weeks). The median progression time was 48 days in patients treated with 0.15 mg efatutazone, and 68 days in those treated with 0.3 mg efatutazone; the corresponding median survival were 98, versus 138 days,

respectively. The authors suggested that paclitaxel in association with efatutazone were tolerated and biologically active [107].

6. Cancer stem cell (CSC)-targeted therapies

The CSC model suggests the presence of a small, biologically distinct subpopulation of cancer cells (namely CSCs) in each tumor with a slow cycling rate and existing in a "stem cell niche" that regulates self-renewal and multi-lineage potential, explaining recurrence, metastasis, and therapy-resistance.

CSCs can grow *in vitro* as spheres (thyrospheres in the case of thyroid), sometimes exhibit radio/chemo-resistance, and have molecular likeness with embryonic and/or adult SCs.

CSC-targeted therapies are developed targeting CSC-specific cell surface markers or signal transduction pathways, that control CSC initiation and growth [108].

Different intracellular signal transduction pathways are determinant mediators of thyroid CSC biology: 1. PTC-spheres express insulin-like growth factor (IGF)-I/II and IGF-IR, and stimulation of this signaling pathway increases the number and size of spheres [109]; 2. the sonic hedgehog (Shh) pathway is activated in some ATC cell lines (as SW1736, BCPAP, and KAT-18), and pathway inhibitors and shRNA-mediated suppression of Shh signaling molecules inhibit ALDH activity and thyrosphere formation [110]; 3. the STAT3 signaling cascade, activated in ATC-CD133+ cells, and the suppressive effect of a JAK–STAT inhibitor cucurbitacin I on CSC characteristics has been shown [111].

Until now, the molecular pathogenesis of TC is still not clear, in particular little is known regarding the development of ATC. Conventional therapies target mature cancer cells, not eradicating thyroid CSCs. CSCs efficiently repair DNA damage after

the exposure to cytotoxic injury, being capable of reconstituting the original tumor. For these reasons, it is important to identify novel therapeutic approaches that target thyroid CSCs [112].

Possible strategies to destroy thyroid CSCs and bypass radio/chemo-resistance may involve the following: increasing sensitization of CSCs directly with agents able to kill specifically CSCs or promote their differentiation; targeting and blocking important CSCs signaling pathway components [as STAT3, c-Met, SOX2, RET, CD44, ABC sub-family G member (ABCG)2 and ABCB1]; and destroying CSC niches. Further studies evaluating the molecular pathways responsible for thyroid CSC survival and expansion are necessary to increase the understanding of thyroid CSCs, to identify efficacious therapeutic targets, and to achieve the complete TC eradication [113].

7. Resistance to targeted treatments

Owing to acquired resistance, many patients initially responsive to targeted therapies may experience relapse of the disease or progression in the clinical setting [114]. Genomic changes, originally present in small sub-clones of cancer cells (such as: a- point mutation in gene encoding for the protein that is the target of the drug; b- the amplification of other different cancer genes), are associated with the appearance of this resistance [115]. For this reason other second- or third-generation targeted drugs against resistance are clinically determinant. As an example, resistance to imatinib in patients with chronic myeloid leukemia, is due to secondary mutations into the Abl kinase domain. Second-generation inhibitors of Abl (such as dasatinib, or nilotinib) [116] can show significant clinical activity, bypassing the resistance of the imatinib-Abl mutations in these patients.

Also combining treatments minimizing the risk of the appearance of resistant clones have been assessed. In fact, the possibility to synergize sorafenib (or other targeted drugs) with chemotherapy, or radiation, or other targeted agents has been evaluated with good results [117-119].

The identification of new targeted drugs active in aggressive DTC will be necessary.

8. Personalization of targeted therapy

New affordable individual genomic analysis permits patient-specific, personalized therapies. Furthermore, the *in vitro* screening with primary cancer cells from each patient [120] of targeted drugs can suggest an *in vivo* non-responsivity (with a 90% negative predictive value), and a 60% positive predictive value of clinical response [121]. This can avoid the administration of ineffective, and potentially harmful, drugs to cancer patients [122].

The use of primary TC cells from patients has been complex until now because of their establishment from surgical biopsies. However recently FNAC bypasses the necessity of surgery. In fact, “primary cells” obtained from FNAC of ATC can be used to test the sensitivity in every subject to various therapies. This can avoid not needed biopsies, and the use of ineffective drugs [105, 106, 123, 124].

9. Conclusion & Future Perspective

Multimodal treatment that includes debulking, hyperfractionated accelerated external beam radiotherapy, and chemotherapy (doxorubicin or cisplatin) are actually the most effective treatments in ATC.

New drugs targeting the molecular pathways identified to be associated with aggressiveness and progression of ATC (BRAF, RET/PTC, p53, RAS, EGFR,

VEGFR1, VEGFR2, PDGFRα, PDGFRβ, PIK3Ca, PIK3Cb, KIT, MET, and PDK1, etc) are under evaluation, as dabrafenib/trametinib, vemurafenib, sorafenib, combretastatin, vandetanib, sunitinib, lenvatinib, CLM3, gefitinib, and PPARγ agonists. An improvement in survival has been reported, for example, in patients with advanced TC treated with lenvatinib, who showed a median OS of 10.6 (3.8–19.8) months [83].

Furthermore, recent not expensive individual genomic analysis and the possibility to test these new drugs in primary cells *in vitro* established from every ATC patient, could lead to the personalization of the therapy, increasing the therapeutic effectiveness and avoiding the use of ineffective treatments.

Furthermore, recently a great attention is given to the epigenetic alterations underlying thyroid carcinogenesis, including those that drive poorly differentiated TC and ATC. Dysregulated epigenetic candidates are the Aurora group, KMT2D, PTEN, RASSF1A, multiple non-coding RNAs (ncRNA), and the SWI/SNF chromatin-remodeling complex. Better knowledge of the signaling pathways affected by epigenetic dysregulation may improve prognostic testing and support the advancement of thyroid-specific epigenetic therapy [125].

10. Expert Commentary

Anaplastic thyroid cancer (ATC) represents less than 2% of thyroid carcinoma, but it is one of the most aggressive human neoplasms, associated with a rapid clinical course, and accounting for 15–40% of thyroid carcinoma deaths (median survival of 10 months). ATC is classified as Stage IV TC (American Joint Committee on Cancer), regardless of tumor size or presence of lymph-node or distant metastasis.

It has been reported that the most efficacious therapy of ATC is multimodal treatment including debulking, hyperfractionated accelerated external beam radiotherapy, and chemotherapy (doxorubicin or cisplatin).

ATA guidelines suggest that paclitaxel or docetaxel, doxorubicin, and also platins are effective in ATC, however, none of these drugs is able to extend survival in advanced ATC.

Various genetic mutations have been reported in different molecular pathways of ATC and associated with tumor progression, and new drugs that have these molecular pathways as targets have been recently evaluated in ATC.

Among the determinant genetic mutations in ATC carcinogenesis, BRAFV600E occurrs in approximately 45% of papillary thyroid cancer (PTC), and 25% of ATCs, and an association among BRAF V600E mutation with features linked to a poor prognosis, such as larger tumor, lymph node or extrathyroidal metastasis has been shown. RET/PTC rearrangements have been reported in 3 cases of ATC tissues, perhaps owing to the coexistence of ATC and PTC in the same tissue. The tumor suppressor gene p53 mutation is frequent in ATC (ranging from 70% to 88%). Point mutations within RAS genes are found in approximately 15% PTCs, 40% of FTCs, and 50% of ATCs. RAS mutations involve codons HRAS, NRAS (at 61 codon), and KRAS (at codon 13/12). VEGF-A is involved in the survival and proliferation of

endothelial cells. Furthermore, increased expression of VEGF in thyroid carcinoma has been associated with poor prognosis, an increased tumor size, and the presence of metastases. Amplifications, mutations or misregulations of EGFR are involved in approximately 30% of epithelial cancers. EGFR is associated with tumor invasion and progression in TC, and it is overexpressed in ATC. A copy number gain has been observed in different receptor tyrosine kinase (RTK) genes (EGFR, VEGFR1, VEGFR2, PDGFRα, PDGFRβ, PIK3Ca, PIK3Cb, KIT, MET, and PDK1) in DTC. However copy number gains were more prevalent in ATC with respect to DTC. Most of these genes are determinant in ATC carcinogenesis, for this reason it has been hypothesized that gene copy number variations is implicated in the aggressiveness and progression of this neoplasm. Histone acetylation (resulting in an open chromatin configuration leading to increase in the gene transcription rate) is an important mechanism that controls the biology of cancer cells, that have dysregulated histone deacetylase, or histone acetyltransferase activity. The upregulated expression of miR-20a in ATC is supposed to counteract TC progression, having therapeutic implications.

Interesting results have been reported with molecules targeting these different pathways, as: a-BRAF (dabrafenib/trametinib, vemurafenib); b-angiogenesis (sorafenib, combretastatin, vandetanib, sunitinib, lenvatinib, CLM3, etc); c-EGFR (gefitinib); d- PPARγ agonists (rosiglitazone, pioglitazone, efatutazone).

In order to bypass the resistance to a single drug, the capability of targeted drugs to synergize with radiation, or chemotherapy, or other targeted drugs is explored.

Moreover, new affordable individual genomic analysis and the opportunity to test these novel treatments in primary cell cultures from every ATC patient *in vitro*, might permit to personalize the therapy, increasing the therapeutic effectiveness and

avoiding the use of ineffective drugs.

11. Five-year view

Researchers are going on to evaluate the long-term efficacy and tolerability of these novel treatment options in patients with ATC.

To bypass the resistance to these targeted therapies, their capability to synergize with radiation, or chemotherapy, or other targeted drugs is explored.

Moreover, new affordable individual genomic analysis and the opportunity to test these novel treatments in primary cell cultures from each ATC patient *in vitro*, might permit to personalize the therapy, increasing the therapeutic effectiveness and avoiding the use of ineffective drugs.

In order to improve survival and the quality of life of these patients, the identification of new treatments is necessary.

Key-issues

- Several genetic alterations have been identified in different molecular pathways of anaplastic thyroid cancer (ATC) and associated with tumor aggressiveness and progression (BRAF, p53, RAS, EGFR, VEGFR1, VEGFR2, etc).
- New drugs targeting these molecular pathways have been recently evaluated in ATC, as the identification of new treatments is necessary in order to extend life duration guaranteing a good quality of life.
- Interesting results have been reported with molecules targeting different pathways, as: a-BRAF (dabrafenib/trametinib, vemurafenib); b-angiogenesis (sorafenib, combretastatin, vandetanib, sunitinib, lenvatinib, CLM3, etc); c-EGFR (gefitinib); d- PPARγ agonists (rosiglitazone, pioglitazone, efatutazone).

- To bypass the resistance to a single drug, the capability of targeted drugs to synergize with radiation, or chemotherapy, or other targeted drugs is explored.
- New affordable individual genomic analysis and the opportunity to test these novel treatments in primary cell cultures from each ATC patient *in vitro*, might permit to personalize the therapy, increasing the therapeutic effectiveness and avoiding the use of ineffective drugs.

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Abbreviations

ANA: primary cultured cells from human ATC
ATC: anaplastic thyroid cancer
CA4P: Combretastatin A4 phosphate
CLM3: pyrazolo [3,4-d]pyrimidine compound
CT: computed tomography
DTC: Differentiated thyroid cancers
EGFR: epidermal growth factor receptor
FNA: fine-needle aspiration
FTC: follicular thyroid cancer
mKI: multikinase inhibitor
MTC: medullary thyroid cancer
OS: overall survival
PFS: progression free-survival
PR: partial response
PTC: papillary thyroid cancer
RTK: receptor tyrosine kinase
SD: stable disease
VEGF: Vascular endothelial growth factor

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