



Tyrosine kinase inhibitors for the therapy of anaplastic thyroid cancer

Anaplastic thyroid cancer (ATC) is often incurable so new therapeutic approaches are needed. Tyrosine kinases inhibitors (such as imatinib, sunitinib or sorafenib) are under evaluation for the treatment of ATC. Other vascular disrupting agents, such as combretastatin A4 phosphate, and antiangiogenic agents, such as aplidin, PTK787/ZK222584 and human VEGF monoclonal antibodies (bevacizumab, cetuximab), have been evaluated. Small-molecule adenosine triphosphate competitive inhibitors directed intracellularly at EGFRs tyrosine kinase, such as erlotinib or gefitinib, are also studied. Furthermore, new molecules have been shown to be active against ATC, such as CLM94 and CLM3. However, more research is needed to finally identify therapies able to control and to cure this disease.

Keywords: anaplastic thyroid cancer • BRAFV600E • combination therapy and salvage therapy • EGF • PPAR- γ agonists • RAS • targeted therapies • tyrosine kinase inhibitors • VEGF

Anaplastic thyroid cancer (ATC) has a very poor prognosis due to its aggressive behavior and resistance to cancer treatments [1].

Surgery followed by chemotherapy and radiotherapy can significantly prolong the survival of patients carrying ATC intrathyroidal tumors, but this kind of presentation is very unusual. In fact, ATC is often advanced and metastatic at diagnosis [2,3]. Currently, the most effective treatment of ATC is multimodal treatment protocol that includes surgery, chemotherapy (doxorubicin and cisplatin) and hyperfractionated accelerated external beam radiotherapy with a median patient survival of 10 months [4].

During the last two decades, several somatic mutations in different molecular pathways of anaplastic thyroid carcinomas have been revealed and associated with progression of ATC [5,6].

Thus, clinical research targeting these pathways has been recently explored. There are a number of clinical trials for anaplastic thyroid carcinoma underway or being planned using different types of drugs, such as peroxisome proliferator-activated receptor

(PPAR- γ) agonists, and tyrosine kinase inhibitors [6].

Genetic study & molecular pathways in ATC

To date, several genetic mutations have been identified that play roles in the carcinogenesis of ATC. A well-studied mutation of thyroid cancer is BRAFV600E, occurring in approximately 26% of ATCs and 45% of papillary thyroid cancers (PTCs) [7,8].

The tumor suppressor gene p53 is frequently mutated in ATC [7] but uncommon in well-differentiated PTC and follicular thyroid cancer (FTC) (with a frequency ranging from 71 to 88% in ATC) [9,10]. Point mutations within RAS genes involve codons NRAS, HRAS and KRAS, with mutations of NRAS and HRAS at codon 61 and of KRAS at codon 12/13 being the most common. Mutant RAS proteins activate the MAPK and PI3K/AKT pathways. RAS mutations are found in ~10–15% PTCs, 40–50% of FTCs and in ~35% of poorly differentiated and ~50% of ATCs, where the presence of RAS mutations seems to

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correlate with a more aggressive tumor behavior and poor prognosis [11,12].

Mutations of PIK3CA are also common in ATC [7]. PIK3CA encodes the p110 α catalytic subunit of PI3K and PI3K, which regulates cell cycle progression, adhesion and motility [13]. Garcia-Rostan *et al.* [13] showed that the missense mutations of PIK3CA are frequently present in ATC.

VEGF-A is the major mediator of tumor angiogenesis, promoting the proliferation and survival of endothelial cells and increasing vascular permeability [14]. Differentiated thyroid cancer (DTC) has been found to express high levels of both VEGF, because of upregulation of its main receptor, VEGFR-2, with respect to normal thyroid [15]. Moreover, increased expression of VEGF in thyroid cancer has been associated with an increase in tumor size, distant metastasis and poor prognosis [16].

EGFR (ErbB-1; HER1 in humans) is the cell-surface receptor for members of the epidermal growth factor family (EGF-family) [17]. Mutations, amplifications or misregulations of EGFR or family members are implicated in about 30% of all epithelial cancers. EGFR is overexpressed in ATC, and is implicated in tumor progression and invasion in thyroid cancer [18,19].

Liu *et al.* [20] found frequent copy number gains in many receptor tyrosine kinase genes containing the EGFR, PDGFR- α , PDGFR- β , VEGFR1, VEGFR2, KIT, MET, PIK3Ca, PIK3Cb and PDK1 genes. Many of the genes play an important role in ATC tumorigenesis by aberrant activation of the oncogenic pathway. The prevalence of copy number gains was generally higher in ATC than in DTC [21]. It is suggested that the copy number variations should be important and involved in the progression and aggressiveness of ATC [22].

A major mechanism controlling cellular differentiation and biological behavior of cancer cells is histone acetylation that results in a more open chromatin configuration that increases the gene transcription rate. Cancer cells have been found to have dysregulated histone acetyltransferase or histone deacetylase activity [23,24].

It has been reported [20] RET/PTC rearrangement in three cases of ATC tissues, probably because some ATC tissues contained PTC tissues.

More recently, it has been suggested the upregulated expression of miR-20a in ATC counteracts thyroid cancer progression and may have therapeutic potential [25].

Many papers have recently reviewed the comprehensive genetic alterations in ATC to identify genomic alterations associated with ATC [6].

PPAR- γ agonists

PPAR- γ are members of a superfamily of nuclear hormone receptors [26]; activation of PPAR- γ isoforms elicits antineoplastic [27] effects in several types of cells. Recently, it has been shown *in vitro* that activating ligands of PPAR- γ : induce apoptosis and exert antiproliferative effects on human PTC cells [26]; prevent distant metastasis of BHP18–21 tumors in nude mice *in vivo* [26] and induce redifferentiation in thyroid cancer [28–30]. The expressions of the PPAR- γ are increased in human ATC cell lines [31], with respect to PTC, and PPAR- γ ligands inhibit proliferation and invasion, and induce apoptosis [31–33]. Rosiglitazone was able to increase the expression of thyroid-specific differentiation markers, suggesting that PPAR- γ agonists induce a partial reversion of the epithelial mesenchymal transition in ATC cells [32]. Furthermore, it has been recently shown in ‘primary cultured cells from human ATC’ (ANA), obtained from each patient [34,35], that PPAR- γ agonists rosiglitazone and pioglitazone are able to inhibit cell growth.

On the basis of the encouraging results of *in vitro* studies, recently an *in vivo* trial has been done.

Recently a novel high-affinity PPAR- γ agonist (RS5444), which is dependent upon PPAR- γ for its biological activity, has been shown to inhibit proliferation of (IC₅₀ ~ 0.8 nM) ATC cells, and of ATC tumor in nude mice [36].

It was also demonstrated that reactivation of suppressed RhoB is a critical step for the inhibition of ATC growth [33].

More recently, a Phase I study evaluated efatutazone and paclitaxel in ATC. Fifteen ATC patients received efatutazone (0.15, 0.3 or 0.5 mg) orally twice daily and then paclitaxel every 3 weeks. Median times to progression were 48 and 68 days in patients receiving 0.15 mg of efatutazone and 0.3 mg of efatutazone, respectively; corresponding median survival was 98 versus 138 days. Eight patients had ≥ 1 serious adverse event. The authors suggest that efatutazone and paclitaxel in combination were safe and tolerated and had biologic activity [37].

Targeted therapy for ATC

Raf kinase pathway

The orally active multikinase inhibitor (mKI) sorafenib, targeting VEGFR-1 and -2, B-Raf, RET and c-Kit, is a potentially effective agent for patients with thyroid cancer, due to its effects on RET, the B-Raf pathway and angiogenesis. Several Phase II clinical trials, and one Phase III trial, have evaluated the use of sorafenib in patients with metastatic iodine refractory thyroid carcinoma [38–41].

The results of these studies suggest that sorafenib is a new treatment option for patients with progressive radioactive iodine-refractory DTC [41].

Patients with ATC who had failed up to previous therapies were enrolled in a multi-institutional Phase II trial of sorafenib. Twenty of them were treated with sorafenib 400 mg twice daily, and 2/20 (10%) patients had a partial response (PR) and 5/20 (25%) had stable disease (SD). The overall median progression-free survival (PFS) was 1.9 months, the median and a 1-year survival being 3.9 months and 20%, respectively. The authors conclude that sorafenib is active in ATC even if at a low frequency [42].

EGFR pathway

The small-molecule EGFR-TK inhibitor gefitinib inhibits cell growth in thyroid cancer lines and in RET-transfected cell lines at submicromolar concentrations [43]. The EGFR kinase inactivation induced by gefitinib potentiates the ionizing radiation-induced inhibition of cell proliferation on FTC and anaplastic cell lines [44].

A Phase II trial was conducted in patients with advanced or metastatic thyroid cancer (DTC = 18, medullary thyroid cancer [MTC] = 4, etc.) who received gefitinib (250 mg/daily), and reported tumor volume reductions in 32% of cases (none of them met criteria for PR); 48% of them attained SD at 3 months; the overall survival (OS) and median PFS were 17.5 months (70 weeks) and 3.7 months (14.8 weeks), respectively, suggesting that gefitinib could not have clinically significant activity as monotherapy [45].

In a patient with ATC treated with an intermittent high-dose gefitinib, and fixed-dose docetaxel, a PR was shown [46].

VEGF pathway

Vandetanib

The orally bioavailable mKI vandetanib targets EGFR, VEGFR-2 and -3, and RET kinases, and is approved for MTC treatment because of its effects on both RET activation and angiogenesis [47]. Two Phase II trials, and one Phase III trial, on vandetanib have been conducted in patients with MTC with positive results [47–49], and the US FDA and EMA-approved vandetanib [50] for the treatment of aggressive MTC.

A pretherapeutic drug evaluation by tumor xenografting in ATC has been recently published suggesting that vandetanib reduced the tumor volume (up to 61%) and tumor vascularity accompanied by decreased EGF-R/VEGF-R2 receptor activity [51].

Axitinib

The mKI axitinib targets PDGFR, VEGFR-1, -2 and -3 and c-Kit, has great selectivity against VEGFR-2,

and is considered the most potent VEGFR-2 inhibitor available. A strong activity of axitinib against thyroid cancer was evidenced in a Phase II trial on 60 patients with advanced, iodine-refractory thyroid cancer using axitinib 5 mg b.i.d. [52]. PR was shown in 18 patients (30%; eight patients with PTC, six FTC, two MTC and one ATC). Moreover, SD was also observed in other 23 patients (38%) and median PFS was 18.1 months (72.4 weeks).

Long-term outcomes were evaluated in a recent study in 60 patients with advanced thyroid cancer treated with axitinib. Objective response rate was 38% (23 patients had PR, and 18 had SD lasting ≥ 16 weeks). Responses occurred in all histologic subtypes. The median follow-up was of 34 months, median OS was 35 months, median PFS was 15 months and median duration of response was 21 months. These results suggest that axitinib is active and well tolerated in patients with advanced thyroid cancer demonstrating long OS [53].

Sunitinib

The mKI sunitinib targets c-Kit, VEGFR-2, PDGFR, RET, FLT-3 and CSF-1R [54]. Two Phase II trials with sunitinib in thyroid cancer have been published [55,56].

Recently, the results of a large open-label Phase II trial, which included 28 patients with progressive DTC and 7 patients with MTC [57] have been presented. They showed complete response in one patient, PR in 28% and SD in 46% of patients [57].

Recently a paper reported a case report showing clinical and visual activity using sunitinib as a salvage treatment in an ATC patient who was not fit to receive systemic chemotherapy treatment [58]. After the end of the second cycle, 12 weeks from the beginning of sunitinib treatment, a complete macroscopic response of the ATC in the neck was achieved. However, response in tumor size over neck mass did not correlate with lung metastasis shrinkage that were stable. Unfortunately, the patient died as a result of a massive upper gastrointestinal bleeding 5 months after the start of sunitinib treatment and while the patient was still on treatment [58].

Lenvatinib (E7080)

Lenvatinib (E7080) is an oral inhibitor of PDGFRb, VEGFR-1, -2, -3, RET, FGFR-1, -2, -3, -4 and c-KIT, it has been shown active in advanced DTC [59].

The antitumor activity of lenvatinib against human thyroid cancer was evaluated in xenograft models in nude mice (five DTC, five ATC and one MTC). Lenvatinib showed antiangiogenesis activity in DTC and in ATC xenografts [60].

CLM94, CLM3

Antonelli *et al.* demonstrated the antitumoral activity of CLM94, a novel cyclic amide with VEGFR-2 and antiangiogenic activity, in ANA cells both *in vitro* and *in vivo* [61].

CLM3 (a mKI that inhibits RET tyrosine kinase, EGFR, VEGFR2 and with antiangiogenic activity) [62,63] can inhibit *in vitro* the proliferation of ANA, inducing apoptosis. CLM3, significantly inhibited EGFR, AKT and ERK^{1/2} phosphorylation, and cyclin D1, and decreased the *VEGF-A* expression and microvessel density in ANA. The above-mentioned data showed the antitumor and antiangiogenic activity of CLM3 is very promising in ATC, opening a future avenue to clinical evaluation [63].

Vascular disrupting mechanism

Combretastatin

The microtubule depolymerizing agent combretastatin A4 phosphate (CA4P) exerts selectively its activity against established tumor vascular networks, producing interruption of tumor blood flow and necrosis of the tumor tissue [64]. A complete response was reported in a Phase I trial conducted on ATC, in one patient treated with combretastatin, and was alive 30 months after treatment [65].

The FACT trial was a randomized, controlled Phase II/III trial assessing the safety and efficacy of carboplatin/paclitaxel with CA4P (experimental arm) or without CA4P (control arm) in ATC [66]. A total of 80 patients were enrolled; 55% had undergone a cancer-related operation, of whom 70% had near-total/total thyroidectomy. Baseline characteristics for operative and nonoperative patients were not substantially different. Median survival for patients who had cancer-related operation was 8.2 months in the CA4P arm versus 4.0 months in the control arm. This suggests that thyroidectomy followed by CA4P combination regimen shows a nonsignificant trend toward improvement in patient survival [66].

An open-label study of doublet carboplatin/paclitaxel chemotherapy with or without fosbretabulin in patients with ATC has been recently conducted. Eighty patients were enrolled. There was no significant difference in PFS between the two arms [67].

Targeted therapies resistance

Clinical experience suggests that many of treatment-responsive patients experience relapse as a result of acquired resistance to targeted therapies [68]. This resistance is often associated with genomic changes (such as the amplification of a completely different cancer gene, or an additional point mutation within the gene encoding the protein to which the drug is targeted) originally

present in minimal subclones of cancer cells [69]. Thus, the production of second-generation drugs to combat resistance is clinically important. For example, in chronic myeloid leukemia patients resistance to imatinib therapy is associated with secondary mutations within the Abl kinase domain. Reduced drug sensitivity seems to be conferred by all of these mutations; second-generation Abl inhibitors (such as nilotinib and dasatinib) [70] can bypass the resistance of the imatinib-refractory Abl mutations, showing significant clinical activity.

The use of combination strategies that could minimize the possibilities of the resistant clones ever expanding have been evaluated, too.

For the above-mentioned reasons, the identification of new active compounds against aggressive DTC is needed [61].

Combination therapy & salvage therapy

Many clinical studies have indicated several limitations to the application of TKIs as a single agent in various types of cancers. For these reasons, the potential of sorafenib (or other TKIs) to synergize with other targeted agents, or chemotherapy, or radiation, has been widely explored with promising results [71–73].

In a clinical trial [74], sorafenib, in combination with tipifarnib (a farnesyltransferase inhibitor that inactivates Ras), was given to 35 patients with DTC and MTC in a Phase I trial. MTC PR rate was 38% (5 of 13), SD of at least 6 months was 31%. The DTC PR rate was 4.5%, and SD of at least 6 months was 36%. Median PFS for all 35 patients was 18 months. The authors concluded that inhibiting the Ras/Raf/MAPK kinase/ERK and RET kinase pathways with sorafenib and tipifarnib is well tolerated and active against thyroid cancer.

The role of salvage therapy was recently evaluated in 64 patients with metastatic DTC who received salvage therapy after their initial sorafenib failure. Salvage therapy included sunitinib (n = 4), pazopanib (n = 3), cabozantinib (n = 4), lenvatinib (n = 3) and vemurafenib (n = 3). Median OS of all 64 patients receiving first-line sorafenib was 37 months; median OS was significantly longer with salvage therapy compared with sorafenib alone (58 vs 28 months). Median PFS was 7.4 months with first-line sorafenib and 11.4 months with salvage therapy. These results suggest that other targeted agents are effective salvage treatments after sorafenib failure, despite similar mechanisms of action, and should be offered to patients who are able to receive salvage therapy [75].

Personalization of therapy

The advent of not expensive individual genomic analysis could lead to a new era of patient-specific, personalized care. Moreover, *in vitro* drug screening

in primary human tumor cells [76] can lead to a negative predictive value of 90%, and a positive predictive value of 60% [77] for the activity of clinical responses, allowing to avoid the administration of inactive chemotherapeutics to patients [78].

Until now, primary thyroid cancer cell cultures have been obtained from surgical biopsies. Fine-needle aspiration (FNA) cytology by passes the need of surgery. The possibility to obtain 'primary cell culture directly from FNA cytology samples of ATC' (FNA-ANA) paves the way to the use of FNA-ANA to test the sensitivity in each patient to different drugs. This could avoid unnecessary surgical procedures and the administration of inactive therapeutics [34,35,79,80].

Conclusion & future perspective

Different alterations of genes and molecular pathways have been shown in ATC (B-Raf, and VEGFR-2, EGFR, etc.). The development of novel compounds that target genes and molecular pathways playing a crucial role in the development of ATC has led to the introduction of new drugs that might overcome in the future the lack of effective therapies for ATC. However, until now, no significant amelioration of survival in ATC patients has been shown with targeted therapies. Furthermore, resistance and 'escape' to TKIs treatments have been described.

The potential of TKIs to synergize with other targeted agents, or chemotherapy, or radiation has been widely explored with promising results, to overcome the resistance to a single TKI agent. Furthermore, it has been shown that other targeted agents might be effective salvage treatments after a first-line TKI failure, despite similar mechanisms of action.

To reach the goals to extend life duration assuring a good quality of life, the identification of new compounds is needed. Furthermore, the advent of not expensive individual genomic analysis could lead to a new era of patient-specific, personalized care. Moreover, the personalization of the treatment could be achieved testing these novel drugs in primary ATC cells (obtained from each patient) *in vitro* to avoid the administration of inactive therapeutics.

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Executive summary

- Anaplastic thyroid cancer (ATC) has a very poor prognosis due to its aggressive behavior and resistance to cancer treatments.
- Currently, the most effective treatment of ATC is multimodal treatment protocol including surgery, chemotherapy (doxorubicin and cisplatin) and hyperfractionated accelerated external beam radiotherapy (median patient survival of 10 months).

Genetic study and molecular pathways in ATC

- Different alterations of genes and molecular pathways have been shown in ATC (B-Raf and VEGFR-2, EGFR, etc.).
- The development of novel compounds that target genes and molecular pathways playing a crucial role in the development of ATC has led to the introduction of new drugs that might overcome in the future the lack of effective therapies for ATC.

Targeted therapy for ATC

- Tyrosine kinases inhibitors (such as imatinib, sunitinib or sorafenib) are under evaluation for the treatment of ATC; antiangiogenic agents, vascular disrupting agents, such as combretastatin A4 phosphate, and small-molecule adenosine triphosphate competitive inhibitors directed intracellularly at EGFRs tyrosine kinase, such as gefitinib, have been evaluated.

Targeted therapy for ATC

- To reach the goals to extend life duration assuring a good quality of life, the identification of new compounds is needed.

Personalization of therapy

- The advent of not expensive individual genomic analysis could lead to a new era of patient-specific, personalized care.

Personalization of therapy

- The possibility to test these novel drugs in primary ATC cells (obtained from each patient) *in vitro* could help improve the personalization of the treatment, avoiding the administration of inactive therapeutics.

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