

1 **Novel treatment options for**
2 **anaplastic thyroid cancer**

3
4 Fallahi P¹, Ruffilli I¹, Elia G¹, Ragusa F¹, Ulisse S², Baldini E²,
5 Miccoli M¹, Materazzi G³, Antonelli A¹, Ferrari SM¹.

6
7 ¹Department of Clinical and Experimental Medicine, University of Pisa, Via Savi, 10,
8 I-56126, Pisa, Italy; ²Department of Experimental Medicine, Sapienza University of
9 Rome, Viale dell'Università, 30, 00185, Rome, Italy; ³Department of Surgical,
10 Medical, Molecular Pathology and Critical Area, University of Pisa, Via Savi, 10, I-
11 56126, Pisa, Italy.

12
13 **E-mail addresses:**

14 Fallahi Poupak: poupak@int.med.unipi.it
15 Ruffilli Ilaria: ilaria.ruffilli@gmail.com
16 Elia Giusy: e.giusy_87@hotmail.it
17 Ragusa Francesca: francescaragusa86@gmail.com
18 Ulisse Salvatore: salvatore.ulisse@uniroma1.it
19 Baldini Enke: enke.baldini@uniroma1.it
20 Miccoli Mario: mario.miccoli@med.unipi.it
21 Materazzi Gabriele: g.materazzi@med.unipi.it
22 Antonelli Alessandro: alessandro.antonelli@med.unipi.it
23 Ferrari Silvia Martina: sm.ferrari@int.med.unipi.it
24

25 ***Corresponding author***

26 Alessandro Antonelli, Prof
27 Director: Immuno-Endocrine Section of Internal Medicine
28 Professor of Medicine
29 Head, Laboratory of Primary Human Cells
30 Department of Clinical and Experimental Medicine
31 University of Pisa, School of Medicine,
32 Honorary Editor, "Drugs" (IF=4.883)
33 Via Savi, 10, I-56126, Pisa, Italy
34 Phone: +39-050-992318
35 Fax: +39-050-993472
36 e-mail: alessandro.antonelli@med.unipi.it

1 **Abstract**

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

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

7 **Expert Opinion/Commentary:** Interesting results have been reported with molecules
8 targeting different pathways, as: a-BRAF (dabrafenib/trametinib, vemurafenib); b-
9 angiogenesis (sorafenib, combretastatin, vandetanib, sunitinib, lenvatinib, CLM3,
10 etc); c-EGFR (gefitinib); d- PPAR γ agonists (rosiglitazone, pioglitazone, efatutazone).
11 In patients with ATC treated with lenvatinib, a median overall survival of 10.6 (3.8–
12 19.8) months was reported.

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

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

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

21
22 **Keywords:** anaplastic thyroid cancer; molecular pathways; targeted drugs; *in vitro*
23 studies; *in vivo* studies.

24

1 **1. Introduction**

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

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

8 It has been reported that the most efficacious therapy of ATC is multimodal treatment
9 that includes debulking, hyperfractionated accelerated external beam radiotherapy,
10 and chemotherapy (doxorubicin or cisplatin), (median survival of 10 months) [6]. In a
11 paper by Foote et al. [7], evaluating 25 new ATC patients, 10 subjects (40%) had
12 metastatic disease at the diagnosis and were treated with palliative treatment, 5 (20%)
13 had regionally confined disease, and 10 consecutive patients (40%) had regionally
14 confined ATC and were treated combining individualized surgery (if possible),
15 intensity-modulated radiation therapy (IMRT), and radiosensitizing+adjuvant
16 chemotherapy (4 cycles of docetaxel+doxorubicin). The overall survival (OS) at 1 and
17 2 years was 70% and 60%, with respect to <20% historical survival at 1 year in ATC
18 patients earlier treated with surgery and conventional postoperative radiation. The
19 combination of IMRT and radiosensitizing+adjuvant chemotherapy seems to improve
20 outcomes, also in patients with stages IVA and IVB regionally confined ATC, even if
21 the effectiveness in patients with stage IVC (metastatic) disease is still unclear. Onoda
22 et al. [8] evaluated 6 patients who underwent external irradiation (45 to 60 Gy) in
23 combination with concurrent low-dose weekly docetaxel administration at 10 mg/m².
24 Survival was 86-1,901 days with additional systemic chemotherapy, and no toxicities
25 over grade 3 were shown. The Authors concluded that chemoradiotherapy is useful

1 for locoregional control of ATC, with acceptable toxicity, lasting long enough to
2 maintain patients' quality of life. A prospective clinical study [9] was conducted in 56
3 ATC patients to assess the feasibility and efficacy of weekly paclitaxel (80 mg/m²)
4 administration. The median OS was 6.7 months [confidence interval 4.4-9.0]. The 6-
5 month survival was 54%. The objective response rate was 21%, and the clinical
6 benefit rate was 73%. The median time to progression was 1.6 months. No adverse
7 events occurred. The Authors concluded that the weekly paclitaxel administration in
8 ATC patients could be effective in a neo-adjuvant setting.

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

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

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

17 Here we review the new targeted therapy of ATC.

18

19 **2. Molecular targets of ATC**

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

22 Among the determinant genetic mutations in ATC carcinogenesis, BRAF V600E
23 occurs in approximately 45% of papillary thyroid cancer (PTC), and 25% of ATCs
24 [13, 14].

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

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

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

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

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

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

21
22 Vascular endothelial growth factor (VEGF)-A is involved in the survival and
23 proliferation of endothelial cells [28]. In general, neoplastic cells expressing VEGF
24 are clinically aggressive, grow rapidly and metastasize to distant organs. Indeed,
25 VEGF is most strongly produced by highly malignant ATC [29]. Differentiated
26 thyroid cancers (DTC) express elevated levels of VEGF-A and VEGF-receptor

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

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

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

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

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

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

9 Programmed death-1 (PD-1) is an inhibitory receptor expressed on the surface of
10 activated T cells. T cell function is inhibited by the binding of PD-1 to its ligands, PD-
11 L1 and PD-L2, that are expressed within the tumor microenvironment, mediating the
12 inhibition of T cells, through a process called “adaptive resistance” [42]. PD-1 and
13 PD-L1 play a pivotal role in the ability of tumor cells to evade the host’s immune
14 system, and blocking their interactions enhances immune function *in vitro* and
15 mediates antitumor activity in preclinical models [43]. PD-L1 expression is
16 considered a potential biomarker for response of anti-PD-1 or anti-PD-L1 agents in
17 different tumors [44]. Four hundred and seven primary TCs with a median 13.7-year
18 of follow-up were evaluated for PD-L1 expression. Tumoral PD-L1 was expressed in
19 6.1% of PTC, 7.6% of FTC and 22.2% of ATC. The Authors concluded that PD-L1
20 was strongly expressed in patients with advanced TC, as FTC and ATC. Identification
21 of PD-L1 expression may have direct therapeutic relevance to patients with refractory
22 TC.

23

24 **3. Drugs that target BRAF in ATC**

25 *3.1 Dabrafenib and the combination dabrafenib/trametinib*

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

5 The effectiveness of inhibiting the activated RAS/RAF/MEK pathway in ATC cells
6 was investigated in 4 human ATC cell lines (ACT-1, OCUT-2, OCUT-4 and OCUT-
7 6) [46]. ACT-1 and OCUT-6 had wild-type BRAF and NRAS mutations, OCUT-4
8 had a BRAF mutation, and OCUT-2 had BRAF and PI3KCA mutations. Dabrafenib
9 inhibited the viability in BRAF mutated cells by G0/G1-arrest via the downregulation
10 of MEK/ERK phosphorylation. Upon treatment with dabrafenib, upregulated
11 phosphorylation of MEK was shown in RAS mutated cells leading to VEGF
12 upregulation. Trametinib inhibited the cellular viability downregulating ERK
13 phosphorylation. In the 4 ATC cell lines, dual blockade by both inhibitors showed
14 cytostatic effects.

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

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

1 11 months after the diagnosis owing to disease progression. The Authors concluded
2 that BRAF inhibitor monotherapy in ATC can get short clinical benefits. Murine
3 models of BRAF V600E-positive ATC have shown a significant extended survival in
4 mice administered with a combination of a BRAF inhibitor (as dabrafenib) and a
5 MEK inhibitor (as trametinib) with respect to those treated with a BRAF inhibitor
6 alone [48].

7 Another study reported a case report of an 81-year-old woman with a growing neck
8 mass [49]. The initial diagnosis was of medullary thyroid cancer (MTC), thus she
9 underwent total thyroidectomy. Surgical pathology revealed a 9-cm ATC. Her tumor
10 harbored a BRAFV600E mutation (1799T>A p.V600E) and she was treated with
11 external beam radiation therapy. She was found to have lung metastases and
12 progression in the neck, after 4 months from the initial diagnosis. Then she received
13 an additional 24 Gy of external beam radiation to the neck, followed by pazopanib.
14 Her neck and lung masses progressed rapidly, and owing to the urgency to start
15 treatment, the liquid formulations of dabrafenib and trametinib were used. She started
16 full doses of both drugs (dabrafenib 150 mg twice a day and trametinib 2 mg daily),
17 and 2 weeks after she began to feel less pressure in her neck. Restaging CT 1 month
18 after starting dabrafenib and trametinib showed a remarkable treatment response in
19 the neck and lungs, and such response was sustained after 4 months of follow-up. The
20 patient experienced hypothyroidism while on treatment, fatigue, weakness and edema.
21 For this reason, dabrafenib and trametinib were dose reduced, but her edema did not
22 improve. After 6 months, she experienced progression, then stopped therapy and died
23 [49].

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

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

14

15 *3.2 Vemurafenib*

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

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

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

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

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

12

13 **4. Drugs that target angiogenesis in ATC**

14

15 *4.1 Vascular Disrupting Mechanism*

16 *4.1.1 Combretastatin*

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

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

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

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

9

10 **4.2 VEGF Pathway**

11 **4.2.1 Sorafenib**

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

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

19 Sorafenib has been tested in twenty patients with ATC (not succeeding to previous
20 therapies) in a multiinstitutional phase II trial. It was administered 400 mg twice
21 daily. Ten% of patients had a partial response (PR), while 25% had a SD. The median
22 PFS was 1.9 months, the median survival was 3.9 months, while 1 year survival was
23 20%. Sorafenib was not proved to be effective in patients with ATC [65].

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

1

2 4.2.2 Vandetanib

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

8 In ATC xenografts it has been reported vandetanib reduces the tumor mass (up to
9 60%), and the vascularization of the neoplasm, in association with a reduced receptor
10 activity of EGF-R/VEGF-R2 [71].

11 A randomised, double-blind, phase 2 trial enrolled adults with locally advanced or
12 metastatic DTC (PTC, FTC, or poorly differentiated) from 16 European medical
13 centres [72] (registered with ClinicalTrials.gov, number NCT00537095). Eligible
14 patients received vandetanib 300 mg per day (vandetanib group comprised 72
15 patients) or matched placebo (placebo group of 73 subjects). Patients belonging to the
16 vandetanib group had longer PFS than subjects administered with placebo (hazard
17 ratio [HR] 0.63, 60% CI 0.54-0.74; one-sided P = 0.008); median PFS was 11.1
18 months (95% CI 7.7-14.0) for patients in the vandetanib group and 5.9 months (4.0-
19 8.9) for subjects administered with placebo.

20

21 4.2.3 Sunitinib

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

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

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

1 a complete response was observed in 1 patient, with a PR in 28%, and 46% of SD
2 [76].

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

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

15

16 *4.2.4 Axitinib*

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

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

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

3

4 *4.2.5 Lenvatinib (E7080)*

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

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

13 A single-arm, open-label, phase II study was conducted in Japan in patients with
14 advanced TC treated with lenvatinib 24 mg/d in 28-d cycles until progressive disease
15 or development of unacceptable toxicity [83]. Primary endpoint was safety, and
16 secondary endpoint was efficacy, evaluated by PFS, OS, ORR, and disease control
17 rate. Fifty-one patients, including 25 subjects with ¹³¹I-refractory DTC, 9 with MTC,
18 and 17 with ATC were enrolled (ClinicalTrials.gov Identifier NCT01728623). The
19 most common any-grade treatment-related adverse events were hypertension (90%),
20 palmar-plantar erythrodysesthesia syndrome (77%), decreased appetite (78%),
21 proteinuria (61%), fatigue (73%), diarrhea (55%), and stomatitis (57%). Incidences of
22 grade 3 and 4 treatment-related adverse events were: 72% in ¹³¹I-refractory DTC;
23 100% in MTC; 88% in ATC. Only 1 patient discontinued treatment owing to
24 treatment-related adverse events. There were 4 fatal serious adverse events, all
25 considered unrelated to lenvatinib. Median duration of treatment was 5.5 months

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

5

6 4.2.6 CLM94, CLM3

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

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

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

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

3

4 ***4.3 Inhibitors of EGFR Pathway***

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

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

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

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

24

25 ***4.4 Anticancer immunotherapy targeting PD-1 and PD-L1***

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

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

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

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

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

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

8

9 **5. Targeting PPAR γ**

10 PPAR γ are nuclear hormone receptors [97] and their activation induces antineoplastic
11 [98] effects in different cancer cells. PPAR γ activatory ligands have been shown: 1- to
12 have antiproliferative action on PTC cells, inducing apoptosis [97]; 2- to prevent in
13 nude mice distant metastasis of BHP18–21 tumors [97]; 3- to induce redifferentiation
14 of dedifferentiated TC cells [99-101]. PPAR γ is overexpressed in human ATC cells
15 [102], with respect to DTC, and PPAR γ activation inhibits invasion and proliferation,
16 inducing also apoptosis [102-104]. Rosiglitazone, a PPAR γ agonist, increased the
17 expression of thyroid specific differentiation markers in ATC cells [103].
18 Furthermore, in ANA [105, 106], rosiglitazone or pioglitazone inhibited ATC cell
19 growth.

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

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

3

4 **6. Cancer stem cell (CSC)-targeted therapies**

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

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

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

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

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

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

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

13

14 **7. Resistance to targeted treatments**

15 Owing to acquired resistance, many patients initially responsive to targeted therapies
16 may experience relapse of the disease or progression in the clinical setting [114].
17 Genomic changes, originally present in small sub-clones of cancer cells (such as: a-
18 point mutation in gene encoding for the protein that is the target of the drug; b- the
19 amplification of other different cancer genes), are associated with the appearance of
20 this resistance [115]. For this reason other second- or third-generation targeted drugs
21 against resistance are clinically determinant. As an example, resistance to imatinib in
22 patients with chronic myeloid leukemia, is due to secondary mutations into the Abl
23 kinase domain. Second-generation inhibitors of Abl (such as dasatinib, or nilotinib)
24 [116] can show significant clinical activity, bypassing the resistance of the imatinib-
25 Abl mutations in these patients.

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

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

6

7 **8. Personalization of targeted therapy**

8 New affordable individual genomic analysis permits patient-specific, personalized
9 therapies. Furthermore, the *in vitro* screening with primary cancer cells from each
10 patient [120] of targeted drugs can suggest an *in vivo* non-responsivity (with a 90%
11 negative predictive value), and a 60% positive predictive value of clinical response
12 [121]. This can avoid the administration of ineffective, and potentially harmful, drugs
13 to cancer patients [122].

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

19

20 **9. Conclusion & Future Perspective**

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

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

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

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

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

18

1 **10. Expert Commentary**

2 Anaplastic thyroid cancer (ATC) represents less than 2% of thyroid carcinoma, but it
3 is one of the most aggressive human neoplasms, associated with a rapid clinical
4 course, and accounting for 15–40% of thyroid carcinoma deaths (median survival of
5 10 months). ATC is classified as Stage IV TC (American Joint Committee on
6 Cancer), regardless of tumor size or presence of lymph-node or distant metastasis.

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

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

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

16 Among the determinant genetic mutations in ATC carcinogenesis, BRAFV600E
17 occurs in approximately 45% of papillary thyroid cancer (PTC), and 25% of ATCs,
18 and an association among BRAF V600E mutation with features linked to a poor
19 prognosis, such as larger tumor, lymph node or extrathyroidal metastasis has been
20 shown. 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
22 suppressor gene p53 mutation is frequent in ATC (ranging from 70% to 88%). Point
23 mutations within RAS genes are found in approximately 15% PTCs, 40% of FTCs,
24 and 50% of ATCs. RAS mutations involve codons HRAS, NRAS (at 61 codon), and
25 KRAS (at codon 13/12). VEGF-A is involved in the survival and proliferation of

1 endothelial cells. Furthermore, increased expression of VEGF in thyroid carcinoma
2 has been associated with poor prognosis, an increased tumor size, and the presence of
3 metastases. Amplifications, mutations or misregulations of EGFR are involved in
4 approximately 30% of epithelial cancers. EGFR is associated with tumor invasion and
5 progression in TC, and it is overexpressed in ATC. A copy number gain has been
6 observed in different receptor tyrosine kinase (RTK) genes (EGFR, VEGFR1,
7 VEGFR2, PDGFR α , PDGFR β , PIK3Ca, PIK3Cb, KIT, MET, and PDK1) in DTC.
8 However copy number gains were more prevalent in ATC with respect to DTC. Most
9 of these genes are determinant in ATC carcinogenesis, for this reason it has been
10 hypothesized that gene copy number variations is implicated in the aggressiveness and
11 progression of this neoplasm. Histone acetylation (resulting in an open chromatin
12 configuration leading to increase in the gene transcription rate) is an important
13 mechanism that controls the biology of cancer cells, that have dysregulated histone
14 deacetylase, or histone acetyltransferase activity. The upregulated expression of miR-
15 20a in ATC is supposed to counteract TC progression, having therapeutic
16 implications.

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

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

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

1 avoiding the use of ineffective drugs.

2

3 **11. Five-year view**

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

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

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

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

14

15 **Key-issues**

16 • Several genetic alterations have been identified in different molecular pathways
17 of anaplastic thyroid cancer (ATC) and associated with tumor aggressiveness and
18 progression (BRAF, p53, RAS, EGFR, VEGFR1, VEGFR2, etc).

19 • New drugs targeting these molecular pathways have been recently evaluated in
20 ATC, as the identification of new treatments is necessary in order to extend life
21 duration guaranteeing a good quality of life.

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

1 • To bypass the resistance to a single drug, the capability of targeted drugs to
2 synergize with radiation, or chemotherapy, or other targeted drugs is explored.

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

7

8

9

10

1 **Financial and competing interests disclosure:**

2 The authors report no conflicts of interest

3

4 **Abbreviations**

5

6 ANA: primary cultured cells from human ATC

7 ATC: anaplastic thyroid cancer

8 CA4P: Combretastatin A4 phosphate

9 CLM3: pyrazolo [3,4-d]pyrimidine compound

10 CT: computed tomography

11 DTC: Differentiated thyroid cancers

12 EGFR: epidermal growth factor receptor

13 FNA: fine-needle aspiration

14 FTC: follicular thyroid cancer

15 mKI: multikinase inhibitor

16 MTC: medullary thyroid cancer

17 OS: overall survival

18 PFS: progression free-survival

19 PR: partial response

20 PTC: papillary thyroid cancer

21 RTK: receptor tyrosine kinase

22 SD: stable disease

23 VEGF: Vascular endothelial growth factor

24

1 **References:**

2

3 1. Hundahl SA, Fleming ID, Fremgen AM, et al. A National Cancer Data Base
4 report on 53,856 cases of thyroid carcinoma treated in the U.S., 1985–1995
5 [see comments]. *Cancer*. 1998;83:2638–2648.

6 2. Kitamura Y, Shimizu K, Nagahama M, et al. Immediate causes of death in
7 thyroid carcinoma: clinicopathological analysis of 161 fatal cases. *J Clin*
8 *Endocrinol Metab*. 1999;84:4043–4049.

9 3. Greene FL, Page DL, Fleming ID, et al. (editors). *AJCC cancer staging*
10 *manual*. 6th edition. Chicago: Springer 2002.

11 4. Miccoli P, Materazzi G, Antonelli A, et al. New trends in the treatment of
12 undifferentiated carcinomas of the thyroid. *Langenbecks Arch Surg*.
13 2007;392:397–404.

14 5. Kebebew E. Anaplastic thyroid cancer: rare, fatal, and neglected. *Surgery*.
15 2012;152:1088–1089.

16 6. De Crevoisier R, Baudin E, Bachelot A, et al. Combined treatment of
17 anaplastic thyroid carcinoma with surgery, chemotherapy, and
18 hyperfractionated accelerated external radiotherapy. *Int J Radiat Oncol Biol*
19 *Phys*. 2004;60:1137–1143.

20 7. Foote RL, Molina JR, Kasperbauer JL, et al. Enhanced survival in
21 locoregionally confined anaplastic thyroid carcinoma: a single-institution
22 experience using aggressive multimodal therapy. *Thyroid*. 2011;21:25–30.

23 8. Onoda N, Kashiwagi S, Noda S, et al. High efficacy of chemoradiation therapy
24 sensitized by weekly docetaxel for anaplastic thyroid cancer. *Anticancer Res*.
25 2013;33:3445–3448.

- 1 9. Onoda N, Sugino K, Higashiyama T, et al. The Safety and Efficacy of Weekly
2 Paclitaxel Administration for Anaplastic Thyroid Cancer Patients: A
3 Nationwide Prospective Study. *Thyroid*. 2016;26:1293–1299.
- 4 10. Smallridge RC, Ain KB, Asa SL, et al. American Thyroid Association
5 guidelines for management of patients with anaplastic thyroid cancer. *Thyroid*.
6 2012;22:1104–1139.
- 7 11. Antonelli A, Fallahi P, Ferrari SM, et al. New targeted therapies for thyroid
8 cancer. *Curr Genomics*. 2011;12:626–631.
- 9 12. Antonelli A, Fallahi P, Ulisse S, et al. Tyrosine kinase inhibitors for the
10 therapy of anaplastic thyroid cancer. *Int J Endocr Oncol*. 2015;2:135–142.
- 11 13. Smallridge RC, Marlow LA, Copland JA. Anaplastic thyroid cancer:
12 molecular pathogenesis and emerging therapies. *Endocr Relat Cancer*.
13 2009;16:17–44.
- 14 14. Xing M. BRAF mutation in thyroid cancer. *Endocr Relat Cancer*.
15 2005;12:245–262.
- 16 15. Perri F, Pezzullo L, Chiofalo MG, et al. Targeted therapy: a new hope for
17 thyroid carcinomas. *Crit Rev Oncol Hematol*. 2015;94:55–63.
- 18 16. Zoghlami A, Roussel F, Sabourin JC, et al. BRAF mutation in papillary
19 thyroid carcinoma: predictive value for long-term prognosis and radioiodine
20 sensitivity. *Eur Ann Otorhinolaryngol Head Neck Dis*. 2014;131:7–13.
- 21 17. Elisei R, Ugolini C, Viola D, et al. BRAF (V600E) mutation and outcome of
22 patients with papillary thyroid carcinoma: a 15-year median follow-up study. *J*
23 *Clin Endocrinol Metab*. 2008;93:3943–3949.

- 1 18. Smith N, Nucera C. Personalized therapy in patients with anaplastic thyroid
2 cancer: targeting genetic and epigenetic alterations. *J Clin Endocrinol Metab.*
3 2015;100:35–42.
- 4 19. Xing M. Molecular pathogenesis and mechanisms of thyroid cancer. *Nat Rev*
5 *Cancer.* 2013;13(3):184–199.
- 6 20. Smallridge RC, Marlow LA, Copland JA. Anaplastic thyroid cancer:
7 molecular pathogenesis and emerging therapies. *Endocr Relat Cancer.* 2009;
8 16(1):17–44.
- 9 21. Liu Z, Hou P, Ji M, et al. Highly prevalent genetic alterations in receptor
10 tyrosine kinases and phosphatidylinositol 3-kinase/akt and mitogen-activated
11 protein kinase pathways in anaplastic and follicular thyroid cancers. *J Clin*
12 *Endocrinol Metab.* 2008;93:3106–3116.
- 13 22. Fagin JA, Matsuo K, Karmakar A, et al. High prevalence of mutations of the
14 p53 gene in poorly differentiated human thyroid carcinomas. *J Clin Invest.*
15 1993;91:179–184.
- 16 23. Quiros RM, Ding HG, Gattuso P, et al. Evidence that one subset of anaplastic
17 thyroid carcinomas are derived from papillary carcinomas due to BRAF and
18 p53 mutations. *Cancer.* 2005;103:2261–2268.
- 19 24. Nikiforova MN, Nikiforov YE. Molecular genetics of thyroid cancer:
20 implications for diagnosis, treatment and prognosis. *Expert Rev Mol Diagn.*
21 2008;8:83–95.
- 22 25. Ruggeri RM, Campenni A, Baldari S, et al. What is New on Thyroid Cancer
23 Biomarkers. *Biomark Insights.* 2008;3:237–252.

- 1 26. Penna GC, Vaisman F, Vaisman M, et al. Molecular Markers Involved in
2 Tumorigenesis of Thyroid Carcinoma: Focus on Aggressive Histotypes.
3 Cytogenet Genome Res. 2016;150:194–207.
- 4 27. Su X, Jiang X, Wang W, et al. Association of telomerase reverse transcriptase
5 promoter mutations with clinicopathological features and prognosis of thyroid
6 cancer: a meta-analysis. *Onco Targets Ther.* 2016;9:6965–6976.
- 7 28. Ferrara N. Vascular endothelial growth factor: basic science and clinical
8 progress. *Endocr Rev.* 2004;25:581–611.
- 9 29. Sato K. Vascular endothelial growth factors and thyroid disorders. *Endocr J.*
10 2001;48:635–646.
- 11 30. Bunone G, Vigneri P, Mariani L, et al. Expression of angiogenesis stimulators
12 and inhibitors in human thyroid tumors and correlation with clinical
13 pathological features. *Am J Pathol.* 1999;155:1967–1976.
- 14 31. Lennard CM, Patel A, Wilson J, et al. Intensity of vascular endothelial growth
15 factor expression is associated with increased risk of recurrence and decreased
16 disease-free survival in papillary thyroid cancer. *Surgery.* 2001;129:552–558.
- 17 32. Gulubova M, Ivanova K, Ananiev J, et al. VEGF expression, microvessel
18 density and dendritic cell decrease in thyroid cancer. *Biotechnol Biotechnol*
19 *Equip.* 2014;28:508–517.
- 20 33. Herbst RS. Review of epidermal growth factor receptor biology. *Int J Radiat*
21 *Oncol Biol Phys.* 2004;59(Suppl. 2):21–26.
- 22 34. Knauf JA. Does the epidermal growth factor receptor play a role in the
23 progression of thyroid cancer? *Thyroid.* 2011;21:1171–1174.
- 24 35. Yeh MW, Rougier JP, Park JW, et al. Differentiated thyroid cancer cell
25 invasion is regulated through epidermal growth factor receptor-dependent

- 1 activation of matrix metalloproteinase (MMP)-2/gelatinase A. *Endocr Relat*
2 *Cancer*. 2006;13:1173–1183.
- 3 36. Bond JA, Wyllie FS, Rowson J, et al. In vitro reconstruction of tumour
4 initiation in a human epithelium. *Oncogene*. 1994;9:281–290.
- 5 37. Hou P, Liu D, Shan Y, et al. Genetic alterations and their relationship in the
6 phosphatidylinositol 3-kinase/Akt pathway in thyroid cancer. *Clin Cancer Res*.
7 2007;13:1161–1170.
- 8 38. Mahlknecht U, Hoelzer D. Histone acetylation modifiers in the pathogenesis
9 of malignant disease. *Mol Med*. 2000;6:623–644.
- 10 39. Marks P, Rifkind RA, Richon VM, et al. Histone deacetylases and cancer:
11 causes and therapies. *Nat Rev Cancer*. 2001;1:194–202.
- 12 40. **Zhang L, Zhang Y, Mehta A, et al. Dual inhibition of HDAC and EGFR**
13 **signaling with CUDC-101 induces potent suppression of tumor growth**
14 **and metastasis in anaplastic thyroid cancer. *Oncotarget*. 2015;6:9073–**
15 **9085.**
- 16 41. Xiong Y, Zhang L, Kebebew E. MiR-20a is upregulated in anaplastic thyroid
17 cancer and targets LIMK1. *PLoS One*. 2014;9:e96103.
- 18 42. Keir ME, Butte MJ, Freeman GJ, et al. PD-1 and its ligands in tolerance and
19 immunity. *Annu Rev Immunol*. 2008;26:677–704.
- 20 43. Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and activity of anti-PD-L1
21 antibody in patients with advanced cancer. *N Engl J Med*. 2012;366:2455–65.
- 22 44. Ahn S, Kim TH, Kim SW, et al. Comprehensive screening for PD-L1
23 expression in thyroid cancer. *Endocr Relat Cancer*. 2017;24:97–106.

- 1 45. Flaherty KT, Infante JR, Daud A, et al. Combined BRAF and MEK inhibition
2 in melanoma with BRAF V600 mutations. *N Engl J Med.* 2012;367:1694–
3 1703.
- 4 46. Kurata K, Onoda N, Noda S, et al. Growth arrest by activated BRAF and MEK
5 inhibition in human anaplastic thyroid cancer cells. *Int J Oncol.*
6 2016;49:2303–2308.
- 7 47. Maverakis E, Cornelius LA, Bowen GM, et al. Metastatic melanoma - a
8 review of current and future treatment options. *Acta Derm Venereol.*
9 2015;95:516–524.
- 10 48. Lim AM, Taylor GR, Fellowes A, et al. BRAF Inhibition in BRAFV600E-
11 Positive Anaplastic Thyroid Carcinoma. *J Natl Compr Canc Netw.*
12 2016;14:249–254.
- 13 49. Cabanillas ME, Busaidy NL, Khan SA, et al. Molecular diagnostics and
14 anaplastic thyroid carcinoma: the time has come to harvest the high hanging
15 fruit. *Int J Endocr Oncol* 2016. 3:221–233.
- 16 50. Subbiah V, Bang YJ, Lassen UN, et al. ROAR: a phase 2, open-label study in
17 patients (pts) with BRAF V600E–mutated rare cancers to investigate the
18 efficacy and safety of dabrafenib (D) and trametinib (T) combination therapy.
19 2016 ASCO Annual Meeting. *J Clin Oncol* 34, 2016 (suppl; abstr TPS2604).
- 20 51. Kim KB, Cabanillas ME, Lazar AJ, et al. Clinical responses to vemurafenib in
21 patients with metastatic papillary thyroid cancer harboring BRAF(V600E)
22 mutation. *Thyroid.* 2013;23:1277–1283.
- 23 52. Nehs MA, Nucera C, Nagarkatti SS, et al. Late intervention with anti-
24 BRAF(V600E) therapy induces tumor regression in an orthotopic mouse
25 model of human anaplastic thyroid cancer. *Endocrinology.* 2012;153:985–994.

- 1 53. Rosove MH, Peddi PF, Glaspy JA. BRAF V600E inhibition in anaplastic
2 thyroid cancer. *N Engl J Med.* 2013;368:684–685.
- 3 54. Prager GW, Koperek O, Mayerhoefer ME, et al. Sustained Response to
4 Vemurafenib in a BRAFV600E-Mutated Anaplastic Thyroid Carcinoma
5 Patient. *Thyroid.* 2016;26:1515–1516.
- 6 55. Marten KA, Gudena VK. Use of vemurafenib in anaplastic thyroid carcinoma:
7 a case report. *Cancer Biol Ther.* 2015;16:1430–1433.
- 8 56. Hyman DM, Puzanov I, Subbiah V, et al. Vemurafenib in Multiple
9 Nonmelanoma Cancers with BRAF V600 Mutations. *N Engl J Med.*
10 2015;373:726–736.
- 11 57. Kanthou C, Tozer GM. Microtubule depolymerizing vascular disrupting
12 agents: novel therapeutic agents for oncology and other pathologies. *Int J Exp*
13 *Pathol.* 2009;90:284–294.
- 14 58. Dowlati A, Robertson K, Cooney M, et al. A phase I pharmacokinetic and
15 translational study of the novel vascular targeting agent combretastatin a-4
16 phosphate on a single-dose intravenous schedule in patients with advanced
17 cancer. *Cancer Res.* 2002;62:3408–3416.
- 18 59. Sosa JA, Balkissoon J, Lu SP, et al. Thyroidectomy followed by fosbretabulin
19 (CA4P) combination regimen appears to suggest improvement in patient
20 survival in anaplastic thyroid cancer. *Surgery.* 2012;152:1078–1087.
- 21 60. Sosa JA, Elisei R, Jarzab B, et al. Randomized safety and efficacy study of
22 fosbretabulin with paclitaxel/carboplatin against anaplastic thyroid carcinoma.
23 *Thyroid.* 2014;24:232–240.
- 24 61. Kloos RT, Ringel MD, Knopp MV, et al. Phase II trial of sorafenib in
25 metastatic thyroid cancer. *J Clin Oncol.* 2009;27:1675–1684.

- 1 62. Gupta-Abramson V, Troxel AB, Nellore A, et al. Phase II trial of sorafenib in
2 advanced thyroid cancer. *J Clin Oncol.* 2008;26:4714–4719.
- 3 63. Hoftijzer H, Heemstra KA, Morreau H, et al. Beneficial effects of sorafenib on
4 tumor progression, but not on radioiodine uptake, in patients with
5 differentiated thyroid carcinoma. *Eur J Endocrinol.* 2009;161:923–931.
- 6 64. ** Brose MS, Nutting CM, Jarzab B, et al. Sorafenib in radioactive iodine-
7 refractory, locally advanced or metastatic differentiated thyroid cancer: a
8 randomised, double-blind, phase 3 trial. *Lancet.* 2014;384:319–328. The first
9 phase III trial of sorafenib conducted in patients with radioactive iodine-
10 refractory, locally advanced or metastatic differentiated thyroid cancer.
- 11 65. Savvides P, Nagaiah G, Lavertu P, et al. Phase II trial of sorafenib in patients
12 with advanced anaplastic carcinoma of the thyroid. *Thyroid.* 2013;23:600–
13 604.
- 14 66. Chen G, Nicula D, Renko K, et al. Synergistic anti-proliferative effect of
15 metformin and sorafenib on growth of anaplastic thyroid cancer cells and their
16 stem cells. *Oncol Rep.* 2015;33:1994–2000.
- 17 67. Wells SA Jr, Gosnell JE, Gagel RF, et al. Vandetanib for the treatment of
18 patients with locally advanced or metastatic hereditary medullary thyroid
19 cancer. *J Clin Oncol.* 2010;28:767–772.
- 20 68. Thornton K, Kim G, Maher VE, et al. Vandetanib for the treatment of
21 symptomatic or progressive medullary thyroid cancer in patients with
22 unresectable locally advanced or metastatic disease: U.S. Food and Drug
23 Administration drug approval summary. *Clin Cancer Res.* 2012;18:3722–
24 3730.

- 1 69. Robinson BG, Paz-Ares L, Krebs A, et al. Vandetanib (100 mg) in patients
2 with locally advanced or metastatic hereditary medullary thyroid cancer. *J Clin*
3 *Endocrinol Metab.* 2010;95:2664–2671
- 4 70. ** Wells SA Jr, Robinson BG, Gagel RF, et al. Vandetanib in patients with
5 locally advanced or metastatic medullary thyroid cancer: a randomized,
6 double-blind phase III trial. *J Clin Oncol.* 2012;30:134–141. The first phase III
7 trial of vandetanib conducted in patients with medullary thyroid cancer.
- 8 71. Wunderlich A, Khoruzhyk M, Roth S, et al. Pretherapeutic drug evaluation by
9 tumor xenografting in anaplastic thyroid cancer. *J Surg Res.* 2013;185:676–
10 683.
- 11 72. Leboulleux S, Bastholt L, Krause T, et al. Vandetanib in locally advanced or
12 metastatic differentiated thyroid cancer: a randomised, double-blind, phase 2
13 trial. *Lancet Oncol.* 2012;13:897–905.
- 14 73. Kim DW, Jo YS, Jung HS, et al. An orally administered multitarget tyrosine
15 kinase inhibitor, SU11248, is a novel potent inhibitor of thyroid oncogenic
16 RET/papillary thyroid cancer kinases. *J Clin Endocrinol Metab.*
17 2006;91:4070–4076.
- 18 74. Cohen EEW, Needles BM, Cullen KJ, et al. Phase 2 study of sunitinib in
19 refractory thyroid cancer. *Journal of Clinical Oncology.* 2008;26:abstract
20 6025.
- 21 75. Goulart B, Carr L, Martins RG, et al. Phase II study of sunitinib in iodine
22 refractory, well-differentiated thyroid cancer (WDTC) and metastatic
23 medullary thyroid carcinoma (MTC). *Journal of Clinical Oncology.*
24 2008;26:abstract 6062.

- 1 76. ** Carr LL, Mankoff DA, Goulart BH, et al. Phase II study of daily sunitinib
2 in FDG-PET-positive, iodine-refractory differentiated thyroid cancer and
3 metastatic medullary carcinoma of the thyroid with functional imaging
4 correlation. *Clin Cancer Res.* 2010;16:5260–5268. A phase II trial of sunitinib
5 conducted in patients with thyroid cancer.
- 6 77. Grande E, Capdevila J, Díez JJ, et al. A significant response to sunitinib in a
7 patient with anaplastic thyroid carcinoma. *J Res Med Sci.* 2013;18:623–625.
- 8 78. Ravaud A, de la Fouchardière C, Caron P, et al. A multicenter phase II study
9 of sunitinib in patients with locally advanced or metastatic differentiated,
10 anaplastic or medullary thyroid carcinomas: mature data from the THYSU
11 study. *Eur J Cancer.* 2017;76:110–117.
- 12 79. Cohen EE, Rosen LS, Vokes EE, et al. Axitinib is an active treatment for all
13 histologic subtypes of advanced thyroid cancer: results from a phase II study. *J*
14 *Clin Oncol.* 2008;26:4708–4713.
- 15 80. Cohen EE, Tortorici M, Kim S, et al. A Phase II trial of axitinib in patients
16 with various histologic subtypes of advanced thyroid cancer: long-term
17 outcomes and pharmacokinetic/pharmacodynamic analyses. *Cancer*
18 *Chemother Pharmacol.* 2014;74:1261–1270.
- 19 81. Sherman SI, Jarzab B, Cabanillas ME, et al. A phase II trial of the
20 multitargeted kinase inhibitor E7080 in advanced radioiodine (RAI)-refractory
21 differentiated thyroid cancer (DTC). *Journal of Clinical Oncology.*
22 2011;29:abstract 5503.
- 23 82. Tohyama O, Matsui J, Kodama K, et al. Antitumor activity of lenvatinib
24 (e7080): an angiogenesis inhibitor that targets multiple receptor tyrosine

- 1 kinases in preclinical human thyroid cancer models. *J Thyroid Res.*
2 2014;2014:638747.
- 3 83. Takahashi S, Kiyota N, Yamazaki T, et al. Phase II study of lenvatinib in
4 patients with differentiated, medullary, and anaplastic thyroid cancer: Final
5 analysis results. 2016 ASCO Annual Meeting. *J Clin Oncol* 34, 2016 (suppl;
6 abstr 6088).
- 7 84. Antonelli A, Bocci G, La Motta C, et al. CLM94, a novel cyclic amide with
8 anti-VEGFR-2 and antiangiogenic properties, is active against primary
9 anaplastic thyroid cancer in vitro and in vivo. *J Clin Endocrinol Metab.*
10 2012;97:E528–E536.
- 11 85. Ferrari SM, Fallahi P, La Motta C, et al. Antineoplastic activity of the
12 multitarget tyrosine kinase inhibitors CLM3 and CLM94 in medullary thyroid
13 cancer in vitro. *Surgery.* 2014;156:1167–1176.
- 14 86. Antonelli A, Bocci G, Fallahi P, et al. CLM3, a multitarget tyrosine kinase
15 inhibitor with antiangiogenic properties, is active against primary anaplastic
16 thyroid cancer in vitro and in vivo. *J Clin Endocrinol Metab.* 2014;99:E572–
17 E581.
- 18 87. Fallahi P, Ferrari SM, La Motta C, et al. CLM29 and CLM24,
19 pyrazolopyrimidine derivatives, have antitumoral activity in vitro in anaplastic
20 thyroid cancer, with or without BRAF mutation. *Endocrine.* 2016;53:136–144.
- 21 88. Croyle M, Akeno N, Knauf JA, et al. RET/PTC-induced cell growth is
22 mediated in part by epidermal growth factor receptor (EGFR) activation:
23 evidence for molecular and functional interactions between RET and EGFR.
24 *Cancer Res.* 2008;68:4183–4191.

- 1 89. Lopez JP, Wang-Rodriguez J, Chang CY, et al. Gefitinib (Iressa) potentiates
2 the effect of ionizing radiation in thyroid cancer cell lines. *Laryngoscope*.
3 2008;118:1372–1376.
- 4 90. Nobuhara Y, Onoda N, Yamashita Y, et al. Efficacy of epidermal growth
5 factor receptor-targeted molecular therapy in anaplastic thyroid cancer cell
6 lines. *Br J Cancer*. 2005;92:1110–1116.
- 7 91. ** Pennell NA, Daniels GH, Haddad RI, et al. A phase II study of gefitinib in
8 patients with advanced thyroid cancer. *Thyroid*. 2008;18:317–323. The phase
9 II trial of gefitinib conducted in patients with anaplastic thyroid cancer.
- 10 92. Fury MG, Solit DB, Su YB, et al. A phase I trial of intermittent high-dose
11 gefitinib and fixed-dose docetaxel in patients with advanced solid tumors.
12 *Cancer Chemother Pharmacol*. 2007;59:467–475.
- 13 93. Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and activity of anti-PD-L1
14 antibody in patients with advanced cancer. *N Engl J Med*. 2012;366:2455-
15 2465.
- 16 94. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune
17 correlates of anti-PD-1 antibody in cancer. *N Engl J Med*. 2012;366:2443-
18 2454.
- 19 95. Wu H, Sun Y, Ye H, et al. Anaplastic thyroid cancer: outcome and the
20 mutation/expression profiles of potential targets. *Pathol Oncol Res*.
21 2015;21:695-701.
- 22 96. Brauner E, Gunda V, Vanden Borre P, et al. Combining BRAF inhibitor and
23 anti PD-L1 antibody dramatically improves tumor regression and anti tumor
24 immunity in an immunocompetent murine model of anaplastic thyroid cancer.
25 *Oncotarget*. 2016;7:17194-17211.

- 1 97. Ohta K, Endo T, Haraguchi K, et al. Ligands for peroxisome proliferator-
2 activated receptor gamma inhibit growth and induce apoptosis of human
3 papillary thyroid carcinoma cells. *J Clin Endocrinol Metab.*
4 2001;86:2170–2177.
- 5 98. Grommes C, Landreth GE, Heneka MT. Antineoplastic effects of peroxisome
6 proliferator-activated receptor gamma agonists. *Lancet Oncol.*
7 2004;5:419–429.
- 8 99. Klopper JP, Hays WR, Sharma V, et al. Retinoid X receptor-gamma and
9 peroxisome proliferator-activated receptor-gamma expression predicts thyroid
10 carcinoma cell response to retinoid and thiazolidinedione treatment. *Mol*
11 *Cancer Ther.* 2004;3:1011–1020.
- 12 100. Park JW, Zarnegar R, Kanauchi H, et al. Troglitazone, the peroxisome
13 proliferator-activated receptor-gamma agonist, induces antiproliferation and
14 redifferentiation in human thyroid cancer cell lines. *Thyroid.*
15 2005;15:222–231.
- 16 101. Fröhlich E, Machicao F, Wahl R. Action of thiazolidinediones on
17 differentiation, proliferation and apoptosis of normal and transformed
18 thyrocytes in culture. *Endocr Relat Cancer.* 2005;12:291–303.
- 19 102. Hayashi N, Nakamori S, Hiraoka N, et al. Antitumor effects of peroxisome
20 proliferator activate receptor gamma ligands on anaplastic thyroid carcinoma.
21 *Int J Oncol.* 2004;24:89–95.
- 22 103. Aiello A, Pandini G, Frasca F, et al. Peroxisomal proliferator-activated
23 receptor-gamma agonists induce partial reversion of epithelial-mesenchymal
24 transition in anaplastic thyroid cancer cells. *Endocrinology.*
25 2006;147:4463–4475.

- 1 104. Marlow LA, Reynolds LA, Cleland AS, et al. Reactivation of suppressed
2 RhoB is a critical step for the inhibition of anaplastic thyroid cancer growth.
3 *Cancer Res.* 2009;69:1536–1544.
- 4 105. Antonelli A, Ferrari SM, Fallahi P, et al. Thiazolidinediones and antiproliferatives in
5 primary human anaplastic thyroid cancer cells. *Clin Endocrinol (Oxf).*
6 2009;70:946–953.
- 7 106. Antonelli A, Fallahi P, Ferrari SM, et al. Dedifferentiated thyroid cancer: a
8 therapeutic challenge. *Biomed Pharmacother.* 2008;62:559–563.
- 9 107. Smallridge RC, Copland JA, Brose MS, et al. Efatutazone, an oral PPAR- γ
10 agonist, in combination with paclitaxel in anaplastic thyroid cancer: results of
11 a multicenter phase 1 trial. *J Clin Endocrinol Metab.* 2013;98:2392–2400.
- 12 108. Nagayama Y, Shimamura M, Mitsutake N. Cancer Stem Cells in the Thyroid.
13 *Front Endocrinol (Lausanne).* 2016;7:20.
- 14 109. Malaguarnera R, Frasca F, Garozzo A, et al. Insulin receptor isoforms and
15 insulin-like growth factor receptor in human follicular cell precursors from
16 papillary thyroid cancer and normal thyroid. *J Clin Endocrinol Metab.*
17 2011;96:766–774.
- 18 110. Heiden KB, Williamson AJ, Doscas ME, et al. The sonic hedgehog signaling
19 pathway maintains the cancer stem cell self-renewal of anaplastic thyroid
20 cancer by inducing snail expression. *J Clin Endocrinol Metab.*
21 2014;99:E2178–E2187.
- 22 111. Tseng LM, Huang PI, Chen YR, et al. Targeting signal transducer and
23 activator of transcription 3 pathway by cucurbitacin I diminishes self-renewing
24 and radiochemoresistant abilities in thyroid cancer-derived CD133+ cells. *J*
25 *Pharmacol Exp Ther.* 2012;341:410–423.

- 1 112. Hombach-Klonisch S, Natarajan S, Thanasupawat T, et al. Mechanisms of
2 therapeutic resistance in cancer (stem) cells with emphasis on thyroid cancer
3 cells. *Front Endocrinol (Lausanne)*. 2014;5:37.
- 4 113. Vicari L, Colarossi C, Giuffrida D, et al. Cancer stem cells as a potential
5 therapeutic target in thyroid carcinoma. *Oncol Lett*. 2016;12:2254-2260.
- 6 114. Engelman JA, Jänne PA. Mechanisms of acquired resistance to epidermal
7 growth factor receptor tyrosine kinase inhibitors in non-small cell lung cancer.
8 *Clin Cancer Res*. 2008;14:2895–2899.
- 9 115. ** McDermott U, Downing JR, Stratton MR. Genomics and the continuum of
10 cancer care. *N Engl J Med*. 2011;364:340–350. A relevant paper focusing on
11 the resistance to targeted therapies.
- 12 116. Kantarjian H, Giles F, Wunderle L, et al. Nilotinib in imatinib-resistant CML
13 and Philadelphia chromosome-positive ALL. *N Engl J Med*. 2006;354:2542–
14 2551.
- 15 117. Ibrahim N, Yu Y, Walsh WR, et al. Molecular targeted therapies for cancer:
16 sorafenib mono-therapy and its combination with other therapies (review).
17 *Oncol Rep*. 2012;27:1303–1311.
- 18 118. Koh YW, Shah MH, Agarwal K, et al. Sorafenib and Mek inhibition is
19 synergistic in medullary thyroid carcinoma in vitro. *Endocr Relat Cancer*.
20 2012;19:29–38.
- 21 119. Kandil E, Tsumagari K, Ma J, et al. Synergistic inhibition of thyroid cancer by
22 suppressing MAPK/PI3K/AKT pathways. *J Surg Res*. 2013;184:898–906.
- 23 120. Newell DR. Flasks, fibres and flanks--pre-clinical tumour models for
24 predicting clinical antitumour activity. *Br J Cancer*. 2001;84:1289–1290.

- 1 121. Schroyens W, Tueni E, Dodion P, et al. Validation of clinical predictive value
2 of in vitro colorimetric chemosensitivity assay in head and neck cancer. *Eur J*
3 *Cancer*. 1990;26:834–838.
- 4 122. Blumenthal RD, Goldenberg DM. Methods and goals for the use of in vitro
5 and in vivo chemosensitivity testing. *Mol Biotechnol*. 2007;35:185–197.
- 6 123. **Antonelli A, Ferrari SM, Fallahi P, et al. Evaluation of the sensitivity to
7 chemotherapeutics or thiazolidinediones of primary anaplastic thyroid cancer
8 cells obtained by fine-needle aspiration. *Eur J Endocrinol*. 2008;159:283–291.
9 The first study evaluating the sensitivity to tyrosine kinase inhibitors in
10 primary dedifferentiated thyroid cancer cells.
- 11 124. **Antonelli A, Ferrari SM, Fallahi P, et al. Primary cell cultures from
12 anaplastic thyroid cancer obtained by fine-needle aspiration used for
13 chemosensitivity tests. *Clin Endocrinol (Oxf)*. 2008;69:148–152. The first
14 study evaluating the sensitivity to drugs of primary anaplastic thyroid cancer
15 cells obtained by fine-needle aspiration.
- 16 125. Sasanakietkul T, Murtha TD, Javid M, et al. Epigenetic modifications in
17 poorly differentiated and anaplastic thyroid cancer. *Mol Cell Endocrinol*.
18 2017;S0303-7207:30291–30295. [Epub ahead of print].

19