Lenvatinib in the Therapy of Aggressive Thyroid Cancer:

State of the Art and New Perspectives with Patents Recently Applied

Ferrari SM[a], Ruffilli I[2a], Centanni M[b], Virili C[2b], Materazzi G[c],

Alexopoulou M[3a], Miccoli M[4a], Antonelli A*[5a], Fallahi P[6a].

[a] Department of Clinical and Experimental Medicine, University of Pisa, Via Savi, 10, I-56126, Pisa, Italy; **+39-050-992318;**

[b] Department of Medico-Surgical Sciences and Biotechnologies, "Sapienza" University of Rome, Latina, Italy; **+39-06-49972604**;

[c] Department of Surgical, Medical, Molecular Pathology and Critical Area, University of Pisa, Pisa, Italy; **+39-050-997706.**

Ferrari SM	sm.ferrari@int.med.unipi.it
Ruffilli I	ilaria.ruffilli@gmail.com
Centanni M	marco.centanni@uniroma1.it
Virili C	camillavirili@libero.it
Materazzi G	gabriele.materazzi@unipi.it
Alexopoulou M	dr.m.alexopoulou@gmail.com
Miccoli M	mario.miccoli@med.unipi.it
Antonelli A	alessandro.antonelli@med.unipi.it
Fallahi P	poupak@int.med.unipi.it

*Corresponding author's and person to whom reprints requests should be addressed:
Alessandro Antonelli, MD, Prof
Director: Immuno-Endocrine Section of Internal Medicine
Professor of Medicine
Head, Laboratory of Primary Human Cells
Department of Clinical and Experimental Medicine
University of Pisa, School of Medicine,
Honorary Editor, "Drugs" (IF=5.000)
Via Savi, 10, I-56126, Pisa, Italy
Phone: +39-050-992318
Fax: +39-050-993472
e-mail: alessandro.antonelli@med.unipi.it

Abstract

Background and Objective: Lenvatinib is an oral, multitargeted tyrosine kinase inhibitor (TKI) of vascular endothelial growth factor receptors (VEGFR1-VEGFR3), fibroblast growth factor receptors (FGFR1-FGFR4), platelet-derived growth factor receptor (PDGFR)α, rearranged during transfection (RET), and v-kit (KIT) signaling networks implicated in tumor angiogenesis.
Method: Here we review the scientific literature about lenvatinib in the treatment of thyroid cancer.

Results: *In vitro* studies have shown antineoplastic activity of lenvatinib in differentiated thyroid cancer (DTC), mainly because of its antiangiogenetic effects, but a slight effect on thyroid cancer cell proliferation has been shown.

In vivo phase II, and phase III studies in patients with aggressive DTC not responsive to radioiodine, have shown that lenvatinib administration was associated with an amelioration in progression-free survival (PFS) with respect to placebo (median PFS 18.2 vs. 3.6 months). However overall survival was not significantly changed. Lenvatinib is also effective in patients resistant to sorafenib as salvage therapy.

Adverse effects of any grade occur in more than 40% of lenvatinib-treated patients, mainly hypertension, diarrhea, asthenia or fatigue, nausea, decreased appetite, and decreased weight. Discontinuations of the therapy because of adverse effects occur in about 14% of patients. Moreover, deaths considered to be drug-related can occur.

Conclusion: On the base of the above mentioned considerations, it is necessary to prove the effectiveness of lenvatinib in the context of associated moderate to severe toxicities requiring frequent dose reduction and delays, and for this reason many interesting patents have been recently applied.

Running Title: Lenvatinib in aggressive thyroid cancer

Keywords: anaplastic thyroid cancer; dedifferentiated thyroid cancer; follicular thyroid cancer; lenvatinib; papillary thyroid cancer; tyrosine kinase inhibitors.

1. Introduction

The most frequent endocrine malignancy is thyroid cancer (TC), with more than 60,000 cases each year in the US alone. Differentiated thyroid cancer (DTC) accounts for more than 90 % of all thyroid malignancies and its incidence has been rising continuously [1-4].

Several risk factors are associated with DTC, such as the exposure to ionizing radiations in childhood or adolescence, that can cause especially papillary thyroid cancer (PTC) [5] as well as secondary radiations, or nuclear explosions or nuclear accidents [6, 7]. Exposure to low doses of radiations may cause the onset of thyroid nodules and cancer, too [8, 9]. Iodine deficiency is another risk factor, associated with a higher frequency of follicular thyroid cancer (FTC), while an increased frequency of PTC has been shown in iodine deficient areas, after the introduction of iodine prophylaxis [10, 11]. Hashimoto's thyroiditis is associated with PTC and thyroid lymphoma [12-15]. PTCs and FTCs are treated with total thyroidectomy and aggressive PTCs and FTCs with successive radioactive iodine (RAI) remnant ablation with 131I, too [7, 16].

Basal and rTSH-stimulated thyroglobulin (Tg) determination, and neck ultrasonography are the key elements in the follow up of patients with PTC and FTC, previously been submitted to surgery [7, 17, 18]. For more than 95 % of DTC patients, surgical treatment, RAI ablation, and thyroid-stimulating hormone (TSH) suppressive therapy reach an overall survival (OS) rate of about 98 % in 5 years. However, locoregional recurrence occurs in up to 10-20 % and distant metastases in approximately 5-10 % at 10 years. Two-thirds of these patients will become RAI-refractory and for this reason they will never be cured with RAI therapy, with a 3-year OS rate lower than 50 %.

Until recently, chemotherapy was the only treatment in patients with advanced DTC, but its humble effectiveness and significant toxicity needed for compelling advances in the therapy of advanced DTC. Over the last decade, substantial progress has been made in the management of RAI-refractory DTC. Thanks to the new knowledges in TC biology, novel targeted therapies for this disease have been developed, including the tyrosine kinase inhibitors (TKIs). In DTC, sorafenib was first approved as salvage treatment, and then in 2015, also lenvatinib was approved by the US Food and Drug Administration (FDA) for the treatment of RAI-refractory TC.

Here we review the scientific literature about lenvatinib in the treatment of TC.

2. Molecular pathways involved in the development of TC

Different molecular pathways are involved in the pathogenesis of TC (**Figure 1**).

About 40-50 % of gene alterations in PTCs are *BRAF* mutations, 30-40 % rearranged during transfection/PTC (*RET/PTC*), and 10 % *RAS* mutations, without overlaps. An increased prevalence of *BRAF* mutations (up to 70 %) has been reported in dedifferentiated papillary thyroid cancer (DePTCs)

[19, 20]. Approximately 35 % of gene alterations in FTCs are PAX8-/peroxisome proliferator-activated receptor (PPAR)γ rearrangement (*PAX8-/PPARγ*), 40-50 % *RAS* mutations [21, 22].

Mutations of the *RET* proto-oncogene are determinant in the familial forms and also in the sporadic forms of medullary thyroid carcinoma (MTC).

Thanks to the knowledge of the molecular pathways at the basis of TC progression, it has been possible to develop new drugs able to block oncogenic kinases (V600EBRAF, RET/PTC) or signaling kinases [as vascular endothelial growth factor receptor (VEGFR) and platelet-derived growth factor receptors (PDGFRs)] associated with cellular growth and proliferation [20].

3. TKIs

TKIs, competing with the ATP-binding site of the catalytic domain of tyrosine kinases (TKs), are small compounds able to modulate TK-dependent oncogenic pathways [23], reducing autophosphorylation and TK activation, and in this way inhibiting the following activation of intracellular signaling pathways.

TC initiation and progression involves multiple genetic and epigenetic alterations, of which *RET/PTC* gene rearrangements, *RET* or *BRAF* or *RAS* mutations, and *VEGFR-2* mutations leading to the activation of the MAPK, the mTOR and PI3K-AKT signaling pathways are crucial [20]. TKIs can interact with one or several TKs, as a single TKI can target multiple TKs [24].

As TKIs may induce clinical responses and stabilization of disease, they are currently used for the treatment of aggressive TC [DTC, MTC and anaplastic thyroid cancer (ATC)]. Vandetanib and cabozantinib have been approved for the treatment of MTC, and sorafenib and lenvatinib for DTC refractory to RAI [25-28]. These drugs protract median progression-free survival (PFS); however major side effects are frequent. New attempts are going on to find new more efficacious and safe compounds, and to personalize the therapy in each TC patient.

4. Lenvatinib

Lenvatinib is an oral, multitargeted TKI of VEGFR 1 through 3 (VEGFR1-VEGFR3), fibroblast growth factor receptors 1 through 4 (FGFR1-FGFR4), PDGFRα, RET, and v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (KIT) signaling networks involved in tumor angiogenesis [29]. It is used in form of the mesylate salt (CAS number 857890-39-2), for the treatment of dedifferentiated thyroid cancer (DeTC) that does not respond to RAI, and it is either locally recurrent or metastatic.

4.1 Pharmacokinetics (PK)

Lenvatinib is rapidly absorbed in the gut, the peak blood plasma concentrations is reached after 1-4 hours (in fasting condition). Bioavailability is about 85 %. Lenvatinib circulates nearly completely (98–99 %) bound to proteins (mainly albumin) [30].

It is metabolized by the CYP3A4 liver enzyme to desmethyl-lenvatinib (M2). Lenvatinib and M2 are oxidized by aldehyde oxidase (AO) to other substances called M2' and M3' [31]; these are the main metabolites in the feces. It is also metabolized to the N-oxide M3 by a CYP enzyme. Also non-enzymatic metabolization occurs [30, 32].

The terminal half-life is 28 hours; about 2/3 are excreted via the feces, and 1/4 via the urine [30].

5. *In vitro* studies

A first study evaluated lenvatinib in *RET* gene fusion-driven preclinical models. Lenvatinib decreased auto-phosphorylation of *KIF5B-RET*, *CCDC6-RET*, and *NcoA4-RET*, inhibited the growth of *CCDC6-RET* human thyroid and lung cancer cell lines, and suppressed tumorigenicity of *RET* gene fusion-transformed NIH3T3 cells [33]. A second study evaluated the antitumor activity of lenvatinib against human TC xenograft models in nude mice. Orally administered lenvatinib showed antitumor activity in xenograft models of 5 DTC, 5 ATC, and 1 MTC xenograft models. Lenvatinib also showed antiangiogenetic activity in 5 DTC and 5 ATC xenografts, while the antiproliferative activity was shown *in vitro* only in 2 of 11 TC cell lines (i.e., RO82-W-1 and TT cells). Lenvatinib was also able to inhibit the RET phosphorylation in TT cells with the activated mutation C634W [34]. Most of the cancers have intrinsic or evasive resistance to VEGF inhibitors (by multiple mechanisms). Serum angiopoietin-2 (Ang2), and Tie2 [its receptor, that identifies a highly pro-angiogenic subset of macrophages, called "Tie2-expressing macrophages" (TEM)], have been shown to be potential

biomarkers of VEGF inhibitor response in different cancers. Ang2-Tie2 have critical roles in pericyte-mediated vessel stabilization. A study evaluated a novel strategy to bypass the resistance by a combination of lenvatinib (VEGFR, FGFR, and RET inhibitor) and golvatinib (E7050, c-Met, Tie2, and EphB4 inhibitor). Golvatinib, in combination with lenvatinib, inhibited the stabilization of pericytes and TEM differentiation, as shown by *in vitro* studies. In TC models, golvatinib plus lenvatinib inhibited pericyte development and TEM infiltration, resulting in perfusion disorder and apoptosis. These preclinical studies suggested the combination of multi-targeting TKIs can sensitize cancer to VEGF inhibitors [35].

6. *In vivo* studies

6.1. Phase I studies, and pharmacokinetic

In order to evaluate the biological and clinical activity of lenvatinib, and its safety, 77 patients were subdivided in 3 cohorts and treated per os twice daily in 28-day cycles: 18 with intermittent twice-daily dosing of 0.1-3.2 mg, 33 with twice-daily dosing of 3.2-12 mg, and 26 with twice-daily dosing of 10 mg (expanded melanoma cohort). The maximum tolerated dose was 10 mg per os twice daily. Hypertension (43 %), proteinuria (39 %), fatigue (42 %), and nausea (25 %) were drug-related toxicities. Partial response (PR) was obtained in 15.6 % of patients (melanoma, 5/29 patients; thyroid, 3/6; pancreatic, 1/2; lung, 1; renal, 1; endometrial, 1/4; and ovarian, 1/5), and stable disease (SD) in 24.7 % [objective response rate (ORR) was 40.3 %]. In multivariate analyses, longer PFS was linked to increased baseline systolic blood pressure and decreased angiopoietin-1 ratio (after 2 hours) in patients with melanoma.
This study concluded that the toxicity profile, PKs, and antitumor activity of lenvatinib were promising [36].
Another phase I study enrolled Japanese patients with solid tumors in 2 cohorts, treating them with 20 or 24 mg of lenvatinib orally on a once-daily continuous schedule (on a 28-day cycle, according to a conventional 3+3 dose escalation scheme).
Nine patients received lenvatinib (20 mg in 3 subjects and 24 mg in 6 subjects). The most common adverse events (AEs) were thrombocytopenia, increased circulating TSH, and hypertension (89 %), and then leukopenia, headache, and proteinuria (78 %). PR was shown in 1 patient, and SD was maintained for more than 6 months by 3 patients. This study showed that Japanese patients with solid tumors tolerated the once-daily continuous dose of 24 mg of lenvatinib and it had promising antitumor activity [37].
A population PK analysis used pooled data from 15 clinical studies, including 8 phase I studies in healthy subjects, 4 phase I studies in patients with solid tumours, 2 phase II studies in patients with TC and 1 phase III study in patients with RAI-refractory DTC (RR-DTC). Data were collected from 779 subjects treated with 3.2-32 mg oral lenvatinib in tablets or capsules, mainly once daily. The population mean value for lenvatinib apparent clearance (CL/F) was 6.56 l h(-1), and was independent of dose and time. The relative bioavailability of lenvatinib in capsule form was 90 % vs. tablets [percent coefficient of variation (% CV) 30.2]. Dose, pH-elevating agents, alanine aminotransferase, aspartate aminotransferase or bilirubin levels, or renal function, age, sex, race do not affect lenvatinib PK. The significant effects of several covariates on lenvatinib PK variability were small, and not clinically relevant [32].

6.2. Phase II studies

A phase II trial enrolled 58 patients with advanced RR-DTC with a disease progression during the earlier 12 months, and administered them with lenvatinib (24 mg once daily) in 28-day cycles (till reaching disease progression, toxicity, withdrawal, or death); circulating levels of 51 cytokines and angiogenic factors were measured. The primary endpoint was the ORR; secondary endpoints included PFS and safety.
After 14 months of follow-up, patients had an ORR of 50 %, and only PRs were reported. Median time to response (TTR) was 3.6 months, median response duration 12.7 months, and median PFS 12.6 months. The ORR for the 17 patients who received previously VEGF therapy was 59 %. Lower baseline levels of angiopoietin-2 were associated with the tumor response and longer PFS. Grade 3 and 4 treatment-emergent AEs [weight loss (12 %), hypertension (10 %), proteinuria (10 %), and diarrhea (10 %)] occurred in 72 % of patients.
The comforting response rates, median TTR, and PFS in patients treated with lenvatinib led to the need of further investigations in a phase III trial [38].

A phase II trial was conducted in 59 patients with unresectable progressive MTC, who had been treated in the 12 previous months with lenvatinib (24 mg daily, on a 28-day cycle), until disease progression, toxicity, withdrawal, or death.

Lenvatinib ORR was 36 %; all PRs. ORR was similar between patients with (35 %) or without (36 %) previous anti-VEGFR treatment. Disease control rate (DCR) was 80 %; 44 % had SD. Among responders, median TTR was 3.5 months. Median PFS was 9.0 months. AEs were diarrhea (14 %), hypertension (7 %), decreased appetite (7 %), fatigue, dysphagia and higher alanine aminotransferase levels (5 % each). The status of *RET* was not correlated with the obtained results. Low baseline levels of angiopoietin-2, HGF, and IL-8 were associated with tumor reduction and longer PFS.

This study suggested that lenvatinib is effective in patients with documented progressive MTC [39].

6.3. Phase III studies

In a phase III, randomized, double-blind, multicenter study involving patients with progressive TC (refractory to iodine-131), 261 patients were administered with lenvatinib (24 mg daily in a 28-day cycle) and 131 received placebo. DTC patients in the placebo group could receive open-label lenvatinib, at the time of disease progression. The primary endpoint was PFS, and secondary endpoints were response rate, OS, and safety.

The median PFS was significantly ($P < 0.001$) longer (18.3 months) in the lenvatinib group, than in the placebo group (and 3.6 months). The response rate was 64.8 %. In the lenvatinib group 4 complete responses and 165 PRs were observed, the response rate was 64.8 %, vs. 1.5 % in the placebo group ($P < 0.001$); OS was not reached in both groups. AEs of any grade (treatment related) occurred in > 40 % of patients in the lenvatinib group, and were: hypertension (67.8 % of patients), diarrhea (59.4 %), asthenia or fatigue (59.0 %), nausea (41.0 %), and decreased appetite (50.2 %) and decreased weight (46.4 %). Thirty-seven patients in the lenvatinib group (14.2 %) and 3 patients in the placebo group (2.3 %) discontinued the study drug owing to AEs. Moreover, during the treatment 6/20 deaths in the lenvatinib group, were considered to be drug-related.

This study demonstrated that the therapy with lenvatinib in patients with iodine-131-refractory TC led to significant improvements in PFS and response rate. However, patients who received lenvatinib had more AEs, even severe [28].

Because of the results of this phase III study, lenvatinib received an FDA and European Medicines Agency (EMA) approval in February and March 2015 for the treatment of patients with locally recurrent or metastatic, progressive, RAI-refractory DTC [40].

A subanalysis evaluated the efficacy and safety of lenvatinib in Japanese patients (30 treated with lenvatinib and 10 with placebo) who participated in Study of (E7080) Lenvatinib in Differentiated Cancer of the Thyroid (SELECT) and the results were assessed according to the SELECT population (lenvatinib, n = 261; placebo, n = 131).

In Japanese patients, median PFS in those receiving lenvatinib was 16.5 months, and in the ones receiving placebo was 3.7 months, but the difference was not significant ($P = 0.067$). Overall response rates for lenvatinib were 63.3 % and for placebo 0 %. OS was not significantly different. The lenvatinib safety profile in Japanese patients was not significantly different from the one of the overall SELECT population, apart from higher incidences of hypertension (any grade: Japanese, 87 %; overall, 68 %; grade ≥ 3: Japanese, 80 %; overall, 42 %). Japanese patients had more dose reductions (Japanese, 90 %; overall, 67.8 %), but fewer discontinuations due to AEs (Japanese, 3.3 %; overall, 14.2 %). This study suggested that in Japanese patients with RAI-refractory DTC, lenvatinib had similar clinical results as the overall SELECT population [41].

A study evaluated tumor size changes linked to the therapy with lenvatinib, in the SELECT phase III, randomized, double-blind, multicenter study. Patients showing complete response or PR were considered responders to lenvatinib (n = 169), 76/92 nonresponders had at least one postbaseline tumor evaluation and were included in this analysis. Patients treated with lenvatinib (responders, -51.9 %; nonresponders, -20.2 %) had a median maximum percentage change in tumor size of -42.9 %. The reduction of tumor size was higher at the first evaluation (median, -24.7 % at 8 wk after randomization), then the rate of change diminished but was continuous (-1.3% per mo). In a multivariate model, percentage change in tumor size at the first evaluation was a slightly significant positive predictor for PFS.

These findings suggest that tumor size changes associated with lenvatinib are characterized by two phases: a first rapid decline, and then a slower, continuous shrinkage [42].

7. Anecdotal studies

The TKI sorafenib is a common first-line therapy for advanced DTC, even if responses do not last longer. A retrospective study determined the effectiveness of salvage therapy after first-line sorafenib failure, in patients with metastatic DTC (group 2). Patients who received first-line sorafenib only (group 1) were evaluated for comparison of OS. PFS, best response, and median OS were measured. Salvage therapy consisted of cabozantinib (n = 4), sunitinib (n = 4), vemurafenib (n = 3), lenvatinib (n = 3), and pazopanib (n = 3).

Sixty-four patients with DTC were included: 35 in group 1 and 25 in group 2, and the groups were well-balanced. The 64 patients treated with first-line sorafenib had median OS of 37 months, and patients administered with salvage therapy had a significantly longer median than the ones with sorafenib alone (58 vs. 28 months, P = 0.013). Best responses with first-line sorafenib were PR in 2/15 (13 %), SD in 10/15 (67 %), and progressive disease in 3/15 (20 %) patients. With salvage therapy, PRs were seen in 7/17 (41 %) and SD in 10/17 (59 %) patients. Median PFS was 7.4 months with first-line sorafenib and 11.4 months with salvage therapy.

This study concluded that targeted drugs, and lenvatinib, are efficacious salvage treatments after sorafenib failure, in spite of similar mechanisms of action, and should be offered to patients with DTC [43].

A case report described the rapid control of T3 thyrotoxicosis in patients with metastatic FTC treated with lenvatinib [44].

8. Limits and drug resistance

Despite TKIs have a less toxicity respect to cytotoxic chemotherapy, they cause significant side effects (like fatigue, hypertension, cutaneous rash, mucositis, hand-and-foot syndrome, nausea, diarrhea, vomiting, and also thyroid dysfunction [45]), that can cause the discontinuation of the therapy with TKIs.

Patients with DTC following a TKI-therapy in the clinical trials gave contrasting outcomes, probably caused by drug resistance arising from the activation of other mitogenic signals [46].

TKIs act as an antiangiogenetic drugs, blocking tumor growth without removing tumor cells. Therefore, the combination of TKIs has been recently suggested [46], even if possible interaction between those are yet to be clarified [47].

Testing the sensitivity of primary TC cells from each subject to different TKIs could ameliorate the efficacy of the treatments [48, 49].

By human tumor cells, disease orientated *in vitro* drug screening has some predictive value for the activity of clinical responses [50, 51], and may be useful to prevent the administration of inefficacious chemotherapeutics to patients [52]. Chemosensitivity tests *in vitro* gave a prediction of *in vivo* efficacy in 60 % of cases [53], while there is an association of about 90 % between a negative chemosensitivity test *in vitro* and an ineffectiveness of the chemotherapy *in vivo* [51], permitting to avoid the administration of inactive drugs to patients.

Primary TC cell cultures have been usually obtained from surgical biopsies performed for therapeutic or diagnostic procedures. Recently we demonstrated the possibility to overcome the problem of surgical procedures using fine-needle aspiration (FNA) cytology obtaining primary cell cultures from FNA samples of ATC (FNA-ANA); this paves the way to the use of FNA-ANA to test the sensitivity to different drugs in each patient. Thanks to this, unnecessary surgical procedures and the administration of inefficacious drugs could be avoided [48, 53-63].

9. Recent patents on lenvatinib in aggressive thyroid cancer

Many patents have been applied in the last years, and we are reporting here some of them.

Cell-targeted serine protease constructs (truncated serine protease polypeptides and fusion proteins) are provided, that can be used in methods for targeted cell killing, as for the treatment of proliferative diseases, like cancer. A truncated serine protease polypeptide refers to an engineered serine protease that is truncated such that the leader sequence, positioned N-terminally, has been removed or replaced with a heterologous sequence. Recombinant serine proteases, such as Granzyme B polypeptides, are provided with improved stability and cell toxicity. In order to increase its effectiveness, a serine protease therapeutic of the embodiments can be administered also in conjunction with a chemotherapeutic agent, as lenvatinib [64]. Another invention provides an anti-human Notch4 antibody or a Notch4 binding fragment with neutralizing activity against human Notch4. The Inventors obtained a mouse anti-human Notch4 antibody with a high neutralizing activity and binding affinity towards human Notch4 and determined the complementarity determining region (CDR) sequence of the mouse

anti-human Notch4 antibody. This led to the production of a humanized antibody with the variable region of heavy and light chains and the CDR sequence of the mouse anti-human Notch4 antibody [65]. The antitumor effect of the combined use of Antibody B [constituted by the heavy chain variable region of HK3 (human kappa light chain subgroup 3) and the light chain variable region of L3] and lenvatinib mesylate in a FTC238 human thyroid cancer cell line xenograft model has been shown. The change in relative tumor volume (RTV) (N=5, mean±standard error) of the control (non-treated) group, Antibody B administration group (twice-a-week tail vein administration), lenvatinib mesylate administration group (once-a-day oral administration), and Antibody B (twice-a-week tail vein administration)+lenvatinib mesylate (once-a-day oral administration) combination group were reported [65].

A method for treating or ameliorating a solid tumor (as for example thyroid cancer) present in humans comprises administering intratumorally to the subject a unit dose of *C. novyi* colony forming units (CFUs) suspended in a pharmaceutically acceptable carrier or solution. The method includes a possible co-treatment protocol by the administration to the patient of a therapy selected from the group consisting of chemotherapy, radiation therapy, immunotherapy, and for example the *C. novyi* NT spores, simultaneously or at different times, as decided by the physician [66].

Methods for treating solid tumors (as thyroid cancer) and hematological cancers by synergistic combinations of BET (bromodomain and extra-terminal) bromodomain inhibitor thieno-triazolo-1,4-diazepine (JQ1) and/or its analogs with certain kinase inhibitors, anti-apoptotic agents and other specific anti-neoplastic agents have been shown. The synergistic effect of combining therapeutic agents leads to the use of lower dosages of one or more of the therapeutic agent(s) and/or less frequent administration to the patient, reducing the toxicity associated with the administration of the agent(s), and improving the efficacy [67].

Another invention provides methods of treating or delaying progression of cancer using an anti-angiogenesis agent (as a VEGF antagonist, like an anti-VEGF antibody) in combination with a PD-1 axis binding antagonist (as anti-PD-L1 antibody) and an anti-OX40 antibody (like an antibody binding human OX40). A nonpeptide small molecule VEGF antagonist can include for example lenvatinib [68]. Moreover, a method for treating solid tumors (as thyroid cancer, even advanced or metastatic) in a patient includes (1) determining the presence or absence of a *H-RAS* mutation in the patient, and then (2) administering a therapeutically effective amount of a farnesyltransferase inhibitor (FTI) to the patient if a *H-RAS* mutation is detected. Some drugs can be used in combination with the FTI treatment, as lenvatinib [69].

A method of preventing, treating or lessening the severity of a proliferative disease (as thyroid cancer) in a patient administered with a therapeutically effective amount of a bicyclic pyrazolone compound, used to inhibit or modulate the activity of receptor TKs, particularly Axl, c-Met, Mer, and Ron. Such compound comprises also a therapeutic agent selected from the group consisting of chemotherapeutic agents and anti-proliferative agents, like lenvatinib [70].

Another invention is about methods for the treatment of cancer combining radiation, cerium oxide nanoparticles (CONPs; nanometer-sized crystals of cerium oxide of about 1-20 nm in longest dimension) and at least one chemotherapeutic agent, to enhance radiation-induced and chemotherapy-induced cancer cell death and to reduce the toxicity associated with radiation therapy and chemotherapy. The cancer treated by the method of the present invention is a solid tumor, as thyroid cancer [71].

A method for treating cancer (for example thyroid cancer) in a patient administering a combination therapy comprising an antagonist of a Programmed Death 1 protein (PD-1) and a multiple receptor TK (multi-RTK) inhibitor has been shown. The multi-RTK inhibitor my be represented by lenvatinib [72].

Conclusion

Lenvatinib is an oral, multitargeted TKI of VEGFR1-VEGFR3, FGFR1-FGFR4, PDGFRα, RET, and v-kit (KIT) signaling networks implicated in tumor angiogenesis.

In vitro studies have shown antineoplastic activity of lenvatinib in DTC, mainly because of its antiangiogenetic effects, but a slight effect on TC cells proliferation has been shown.

In vivo phase II, and phase III studies in patients with aggressive DTC not responsive to RAI have shown that lenvatinib administration was linked to a significant PFS amelioration with respect to placebo (median PFS 18.2 vs. 3.6 months). However OS was not significantly changed. Lenvatinib is also effective in patients resistant to sorafenib as salvage therapy.

AEs of any grade occur in more than 40 % of lenvatinib-treated patients, mainly hypertension, diarrhea, asthenia or fatigue, nausea, decreased appetite, and decreased weight. Discontinuations of the therapy because of AEs occur in about 14 % of patients. Moreover, deaths considered to be drug-related can occur.

Many interesting patents have been recently applied.

On the base of the above mentioned considerations, it is necessary to prove the benefit of lenvatinib in the context of associated moderate to severe toxicities requiring frequently dose reduction and delays [29, 73].

Current & Future Developments

In vitro studies have shown the antineoplastic activity of lenvatinib in DTC, especially thanks to its antiangiogenetic effects.

In vivo phase II, and phase III studies in patients with aggressive DTC not responsive to radioiodine, have shown that lenvatinib administration was associated with an amelioration in PFS vs. placebo (median PFS 18.2 vs. 3.6 months), even if overall survival was not significantly changed.

Adverse effects of any grade occur in more than 40 % of patients administered with lenvatinib, and discontinuations of the therapy because of adverse effects occur in about 14 % of patients. Furthermore, deaths considered to be drug-related can occur.

Further studies are ongoing and many interesting patents have been recently applied.

Future investigations are necessary to prove the effectiveness of lenvatinib in the context of associated moderate to severe toxicities requiring frequent dose reduction and delays.

Ethics Approval and Consent to Participate
Not applicable.

Human and Animal Rights
No Animals/Humans were used for studies that are base of this research.

Consent for Publication
Not applicable.

CONFLICT OF INTEREST
The authors declare no conflict of interest, financial or otherwise.

References:

[1]   American Thyroid Association (ATA) Guidelines Taskforce on Thyroid Nodules and Differentiated Thyroid Cancer, Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, *et al.* Revised American Thyroid Association management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid 2009; 19(11): 1167-214.

[2]   American Cancer Society. Thyroid cancer: what are the key statistics about thyroid cancer? Revised January 12, 2015. www.cancer.org/cancer/thyroidcancer/detailedguide/thyroid-cancer-key-statistics. Accessed February, 2018.

[3]   Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. CA Cancer J Clin 2007; 57(1): 43-66.

[4]   American Cancer Society. Cancer Facts & Figures 2016. Atlanta: American Cancer Society; 2016.

[5]   Schlumberger M, Chevillard S, Ory K, Dupuy C, Le Guen B, de Vathaire F. [Thyroid cancer following exposure to ionizing radiation]. Cancer Radiother 2011; 15(5): 394-9.

[6]   Fushiki S. Radiation hazards in children - lessons from Chernobyl, Three Mile Island and Fukushima. Brain Dev 2013; 35(3): 220-7.

[7]   Miccoli P, Antonelli A, Spinelli C, Ferdeghini M, Fallahi P, Baschieri L. Completion total thyroidectomy in children with thyroid cancer secondary to the Chernobyl accident. Arch Surg 1998; 133(1): 89-93.
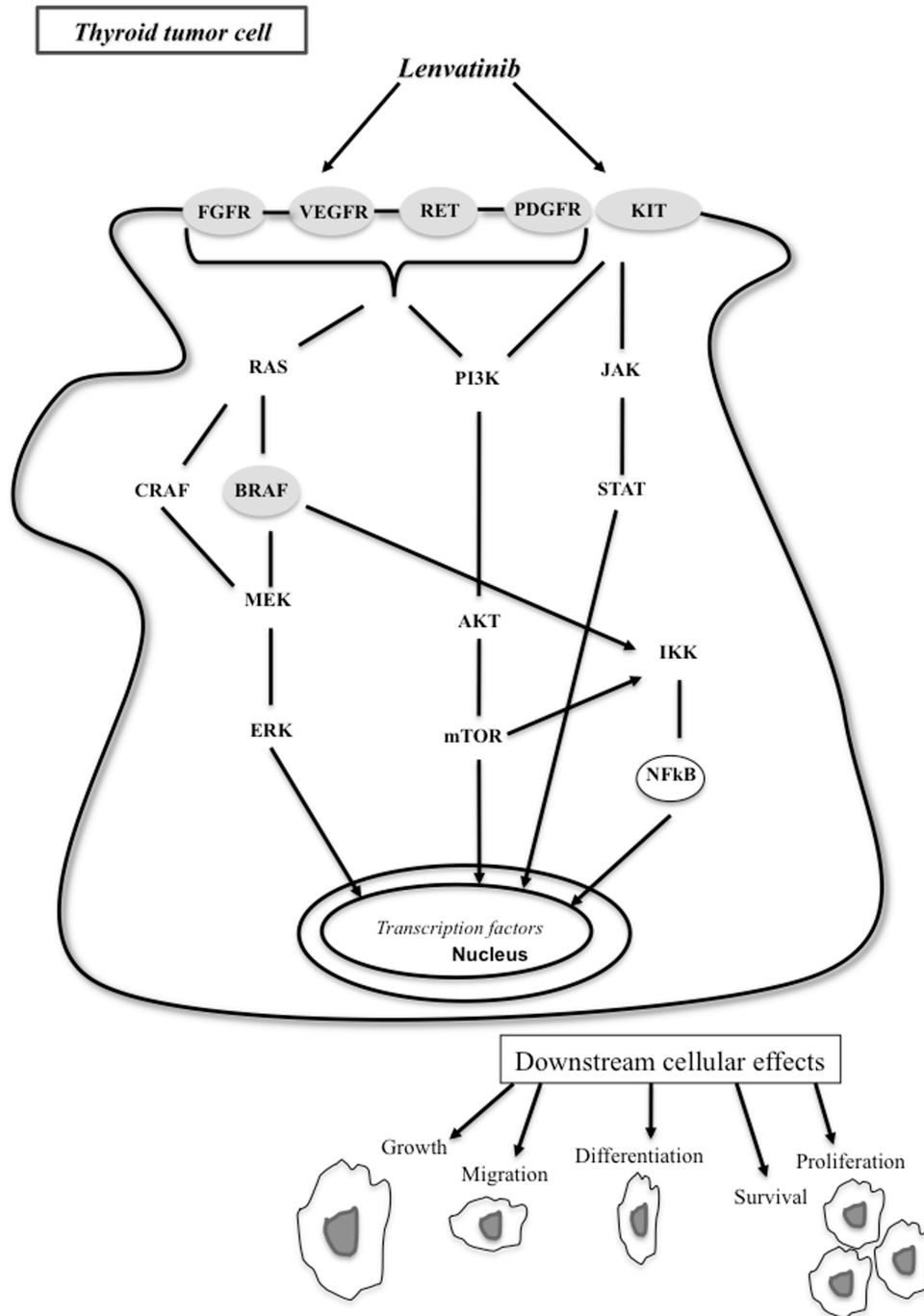
[8]   Suzuki K, Yamashita S. Low-dose radiation exposure and carcinogenesis. Jpn J Clin Oncol 2012; 42(7): 563-8.

[9]   Antonelli A, Silvano G, Gambuzza C, Bianchi F, Tana L, Baschieri L. Is occupationally induced exposure to radiation a risk factor of thyroid nodule formation? Arch Environ Health 1996; 51(3): 177-80.

[10]   Knobel M, Medeiros-Neto G. Relevance of iodine intake as a reputed predisposing factor for thyroid cancer. Arq Bras Endocrinol Metabol 2007; 51(5): 701-12.

[11]   Donati L, Antonelli A, Bertoni F, Moscogiuri D, Andreani M, Venturi S, et al. Clinical picture of endemic cretinism in central Apennines (Montefeltro). Thyroid 1992; 2(4): 283-90.

[12]   Stein SA, Wartofsky L. Primary thyroid lymphoma: a clinical review. J Clin Endocrinol Metab 2013; 98(8): 3131-8.

[13]   Jankovic B, Le KT, Hershman JM. Clinical Review: Hashimoto's thyroiditis and papillary thyroid carcinoma: is there a correlation? J Clin Endocrinol Metab 2013; 98(2): 474-82.

[14]   Antonelli A, Ferri C, Fallahi P, Nesti C, Zignego AL, Maccheroni M. Thyroid cancer in HCV-related mixed cryoglobulinemia patients. Clin Exp Rheumatol 2002; 20(5): 693-6.

[15]   Antonelli A, Ferri C, Fallahi P, Pampana A, Ferrari SM, Barani L, et al. Thyroid cancer in HCV-related chronic hepatitis patients: a case-control study. Thyroid 2007; 17(5): 447-51.

[16]   Haugen BR. Radioiodine remnant ablation: current indications and dosing regimens. Endocr Pract 2012; 18(4): 604-10.

[17]   Antonelli A, Miccoli P, Ferdeghini M, Di Coscio G, Alberti B, Iacconi P, et al. Role of neck ultrasonography in the follow-up of patients operated on for thyroid cancer. Thyroid 1995; 5(1): 25-8.

[18]   Antonelli A, Miccoli P, Fallahi P, Grosso M, Nesti C, Spinelli C, et al. Role of neck ultrasonography in the follow-up of children operated on for thyroid papillary cancer. Thyroid 2003; 13(5): 479-84.

[19]   Krause DS, Van Etten RA. Tyrosin kinases as targets for cancer therapy. N Engl J Med 2005; 353(2): 172-87.

[20]   Nikiforov YE, Nikiforova MN. Molecular genetics and diagnosis of thyroid cancer. Nat Rev Endocrinol 2011; 7(10): 569-80.

[21]   Lemoine NR, Mayall ES, Wyllie FS, Williams ED, Goyns M, Stringer B, et al. High frequency of ras oncogene, activation in all stages of human thyroid tumorigenesis. Oncogene 1989; 4(2): 159-64.

[22]   Antonelli A, Ferrari SM, Fallahi P, Frascerra S, Piaggi S, Gelmini S, et al. Dysregulation of secretion of CXC alpha-chemokine CXCL10 in papillary thyroid cancer: modulation by peroxisome proliferator-activated receptor-gamma agonists. Endocr Relat Cancer 2009; 16(4): 1299-311.

[23]   Lorusso PM, Eder JP. Therapeutic potential of novel selective-spectrum kinase inhibitors in oncology. Expert Opin Investig Drugs 2008; 17(7): 1013-28.

[24]   Antonelli A, Ferri C, Ferrari SM, Sebastiani M, Colaci M, Ruffilli I, et al. New targeted molecular therapies for dedifferentiated thyroid cancer. J Oncol 2010; 2010: 921682.

[25]   Brose MS, Nutting CM, Jarzab B, Elisei R, Siena S, Bastholt L, et al. Sorafenib in radioactive iodine-refractory, locally advanced or metastatic differentiated thyroid cancer: a randomised, double-blind, phase 3 trial. Lancet 2014; 384(9940): 319-28.

[26]   Wells SA Jr, Robinson BG, Gagel RF, Dralle H, Fagin JA, Santoro M, et al. Vandetanib in patients with locally advanced or metastatic medullary thyroid cancer: a randomized, double-blind phase III trial. J Clin Oncol 2012; 30(2): 134-41.

[27]   Cabanillas ME, Brose MS, Ramies DA, Lee Y, Miles D, Sherman SI. Antitumor activity of cabozantinib (XL184) in a cohort of patients (pts) with differentiated thyroid cancer (DTC). American Society of Clinical Oncology - 48th annual meeting. Chicago, USA (2012).

[28]   Schlumberger M, Tahara M, Wirth LJ, Robinson B, Brose MS, Elisei R, et al. Lenvatinib versus placebo in radioiodine-refractory thyroid cancer. N Engl J Med 2015; 372(7): 621-30.

[29]   Costa R, Carneiro BA, Chandra S, Pai SG, Chae YK, Kaplan JB, et al. Spotlight on lenvatinib in the treatment of thyroid cancer: patient selection and perspectives. Drug Des Devel Ther 2016; 10: 873-84.

[30]   Haberfeld H. Austria-Codex (in German). Vienna: Österreichischer Apothekerverlag, ed. (2015).

[31]   Inoue K, Mizuo H, Kawaguchi S, Fukuda K, Kusano K, Yoshimura T. Oxidative metabolic pathway of lenvatinib mediated by aldehyde oxidase. Drug Metab Dispos 2014; 42: 1326–33.

[32]   Gupta A, Jarzab B, Capdevila J, Shumaker R, Hussein Z. Population pharmacokinetic analysis of lenvatinib in healthy subjects and patients with cancer. Br J Clin Pharmacol 2016; 81(6): 1124-33.

[33]   Okamoto K, Kodama K, Takase K, Sugi NH, Yamamoto Y, Iwata M, *et al.* Antitumor activities of the targeted multi-tyrosine kinase inhibitor lenvatinib (E7080) against RET gene fusion-driven tumor models. Cancer Lett 2013; 340(1): 97-103.
[34]   Tohyama O, Matsui J, Kodama K, Hata-Sugi N, Kimura T, Okamoto K, *et al.* Antitumor activity of lenvatinib (e7080): an angiogenesis inhibitor that targets multiple receptor tyrosine kinases in preclinical human thyroid cancer models. J Thyroid Res 2014; 2014: 638747.
[35]   Nakazawa Y, Kawano S, Matsui J, Funahashi Y, Tohyama O, Muto H, *et al.* Multitargeting strategy using lenvatinib and golvatinib: maximizing anti-angiogenesis activity in a preclinical cancer model. Cancer Sci 2015; 106(2): 201-7.
[36]   Hong DS, Kurzrock R, Wheler JJ, Naing A, Falchook GS, Fu S, *et al.* Phase I Dose-Escalation Study of the Multikinase Inhibitor Lenvatinib in Patients with Advanced Solid Tumors and in an Expanded Cohort of Patients with Melanoma. Clin Cancer Res 2015; 21(21): 4801-10.
[37]   Nakamichi S, Nokihara H, Yamamoto N, Yamada Y, Honda K, Tamura Y, *et al.* A phase 1 study of lenvatinib, multiple receptor tyrosine kinase inhibitor, in Japanese patients with advanced solid tumors. Cancer Chemother Pharmacol 2015; 76(6): 1153-61.
[38]   Cabanillas ME, Schlumberger M, Jarzab B, Martins RG, Pacini F, Robinson B, *et al.* A phase 2 trial of lenvatinib (E7080) in advanced, progressive, radioiodine-refractory, differentiated thyroid cancer: A clinical outcomes and biomarker assessment. Cancer 2015; 121(16): 2749-56.
[39]   Schlumberger M, Jarzab B, Cabanillas ME, Robinson B, Pacini F, Ball DW, *et al.* A Phase II Trial of the Multitargeted Tyrosine Kinase Inhibitor Lenvatinib (E7080) in Advanced Medullary Thyroid Cancer. Clin Cancer Res 2016; 22(1): 44-53.
[40]   Nair A, Lemery SJ, Yang J, Marathe A, Zhao L, Zhao H, *et al.* FDA Approval Summary: Lenvatinib for Progressive, Radio-iodine-Refractory Differentiated Thyroid Cancer. Clin Cancer Res 2015; 21(23): 5205-8.
[41]   Kiyota N, Schlumberger M, Muro K, Ando Y, Takahashi S, Kawai Y, *et al.* Subgroup analysis of Japanese patients in a phase 3 study of lenvatinib in radioiodine-refractory differentiated thyroid cancer. Cancer Sci 2015; 106(12): 1714-21.
[42]   Robinson B, Schlumberger M, Wirth LJ, Dutcus CE, Song J, Taylor MH, *et al.* Characterization of Tumor Size Changes Over Time From the Phase 3 Study of Lenvatinib in Thyroid Cancer. J Clin Endocrinol Metab 2016; 101(11): 4103-09.
[43]   Dadu R, Devine C, Hernandez M, Waguespack SG, Busaidy NL, Hu MI, *et al.* Role of salvage targeted therapy in differentiated thyroid cancer patients who failed first-line sorafenib. J Clin Endocrinol Metab 2014; 99(6): 2086-94.
[44]   Danilovic DL, de Camargo RY, Castro G Jr, Papadia C, Marui S, Hoff AO. Rapid Control of T3 Thyrotoxicosis in Patients with Metastatic Follicular Thyroid Cancer Treated with Lenvatinib. Thyroid 2015; 25(11): 1262-4.
[45]   Fallahi P, Ferrari SM, Vita R, Di Domenicantonio A, Corrado A, Benvenga S, *et al.* Thyroid dysfunctions induced by tyrosine kinase inhibitors. Expert Opin Drug Saf 2014; 13(6): 723-33.
[46]   Gild ML, Bullock M, Robinson BG, Clifton-Bligh R. Multikinase inhibitors: a new option for the treatment of thyroid cancer. Nat Rev Endocrinol 2011; 7(10): 617-24.
[47]   Liu R, Liu D, Xing M. The Akt inhibitor MK2206 synergizes, but perifosine antagonizes, the BRAF(V600E) inhibitor PLX4032 and the MEK1/2 inhibitor AZD6244 in the inhibition of thyroid cancer cells. J Clin Endocrinol Metab 2012; 97(2): E173-82.
[48]   Antonelli A, Ferrari SM, Fallahi P, Berti P, Materazzi G, Minuto M, *et al.* Thiazolidinediones and antiblastics in primary human anaplastic thyroid cancer cells. Clin Endocrinol (Oxf) 2009; 70(6): 946-53.
[49]   Antonelli A, Bocci G, La Motta C, Ferrari SM, Fallahi P, Ruffilli I, *et al.* CLM94, a novel cyclic amide with anti-VEGFR-2 and antiangiogenic properties, is active against primary anaplastic thyroid cancer in vitro and in vivo. J Clin Endocrinol Metab 2012; 97(4): E528-36.
[50]   Newell DR. Flasks, fibres and flanks--pre-clinical tumour models for predicting clinical antitumour activity. Br J Cancer 2001; 84(10): 1289-90.
[51]   Schroyens W, Tueni E, Dodion P, Bodecker R, Stoessel F, Klastersky J. Validation of clinical predictive value of in vitro colorimetric chemosensitivity assay in head and neck cancer. Eur J Cancer 1990; 26(7): 834-8.
[52]   Antonelli A. "Molecular profiling and ways towards personalized medicine in advanced differentiated thyroid cancer". Curr Genomics 2014; 15(3): 161.
[53]   Antonelli A, Ferrari SM, Fallahi P, Berti P, Materazzi G, Marchetti I, *et al.* Evaluation of the sensitivity to chemotherapeutics or thiazolidinediones of primary anaplastic thyroid cancer cells obtained by fine-needle aspiration. Eur J Endocrinol 2008; 159(3): 283-91.

[54]   Antonelli A, Ferrari SM, Fallahi P, Berti P, Materazzi G, Barani L, *et al.* Primary cell cultures from anaplastic thyroid cancer obtained by fine-needle aspiration used for chemosensitivity tests. Clin Endocrinol (Oxf) 2008; 69(1): 148-52.

[55]   Ferrari SM, Fallahi P, La Motta C, Bocci G, Corrado A, Materazzi G, *et al.* Antineoplastic activity of the multitarget tyrosine kinase inhibitors CLM3 and CLM94 in medullary thyroid cancer in vitro. Surgery 2014; 156(5): 1167-76.

[56]   Antonelli A, Bocci G, La Motta C, Ferrari SM, Fallahi P, Corrado A, *et al.* CLM29, a multi-target pyrazolopyrimidine derivative, has anti-neoplastic activity in medullary thyroid cancer in vitro and in vivo. Mol Cell Endocrinol 2014; 393(1-2): 56-64.

[57]   Antonelli A, Bocci G, Fallahi P, La Motta C, Ferrari SM, Mancusi C, *et al.* CLM3, a multitarget tyrosine kinase inhibitor with antiangiogenic properties, is active against primary anaplastic thyroid cancer in vitro and in vivo. J Clin Endocrinol Metab 2014; 99(4): E572-81.

[58]   Antonelli A, Bocci G, La Motta C, Ferrari SM, Fallahi P, Fioravanti A, *et al.* Novel pyrazolopyrimidine derivatives as tyrosine kinase inhibitors with antitumoral activity in vitro and in vivo in papillary dedifferentiated thyroid cancer. J Clin Endocrinol Metab 2011; 96(2): E288-96.

[59]   Aiello A, Pandini G, Frasca F, Conte E, Murabito A, Sacco A, *et al.* Peroxisomal proliferator-activated receptor-gamma agonists induce partial reversion of epithelial-mesenchymal transition in anaplastic thyroid cancer cells. Endocrinology 2006; 147(9): 4463-75.

[60]   Marlow LA, Reynolds LA, Cleland AS, Cooper SJ, Gumz ML, Kurakata S, *et al.* Reactivation of suppressed RhoB is a critical step for the inhibition of anaplastic thyroid cancer growth. Cancer Res 2009; 69(4): 1536-44.

[61]   Bravo SB, García-Rendueles ME, Seoane R, Dosil V, Cameselle-Teijeiro J, López-Lázaro L, *et al.* Plitidepsin has a cytostatic effect in human undifferentiated (anaplastic) thyroid carcinoma. Clin Cancer Res 2005; 11(21): 7664-73.

[62]   Karwowski JK, Nowels KW, McDougall IR, Weigel RJ. Needle track seeding of papillary thyroid carcinoma from fine needle aspiration biopsy. A case report. Acta Cytol 2002; 46(3): 591-5.

[63]   Uchida N, Suda T, Inoue T, Fujiwara Y, Ishiguro K. Needle track dissemination of follicular thyroid carcinoma following fine-needle aspiration biopsy: report of a case. Surg Today 2007; 37(1); 34-7.

[64] Rosenblum, M.G., Mohamedali, K.A., Cheung, L.H. Serine protease molecules and therapies. WO2014055836 A2 (2014).

[65] Sakamoto, Y., Adachi, Y., Matsui, J., Kato, Y., Ozawa, Y., Abe, T., Ito, K., Nakazawa, Y., Tachino, S., Suzuki, K., Agarwala, K., Hoshino, K., Katayama, M. Anti human Notch4 antibody. CA 2979759 A1 (2016).

[66] Saha, S., Zhou, S., Vogelstein, B., Kinzler, K.W. C. novyi for the treatment of solid tumors in humans. US20160051597 A1 (2016).

[67] Bradner, J.E., McKeown, M.R., Shipp, M.A., Chapuy, B., Wong, K., Chen, Z. Combination therapy for cancer using bromodomain and extra-terminal (bet) protein inhibitors. US20160279141 A1 (2016).

[68] Rhee, I.P., Kim, J., Huseni, M., Stefanich, E., Sukumaran, S., Li, C. Methods of treating cancer using anti-ox40 antibodies and pd-1 axis binding antagonists. US20170000885 (2017).

[69] Gualberto, A., Scholz, C.R. Methods of treating cancer patients with farnesyltransferase inhibitors. US20170051356 A1 (2017).

[70] Xi, N., Wu, Y. Bicyclic pyrazolone compounds and methods of use. US20150299219 A1 (2015).

[71] Baker, C. Treatment of cancer with a combination of radiation, cerium oxide nanoparticles, and a chemotherapeutic agent. WO2016011328 A1 (2016).

[72] Denker, A.E., Kato, Y., Tabata, K., Hori, Y. Combination of a PD-1 antagonist and a VEGFR/FGFR/RET tyrosine kinase inhibitor for treating cancer. WO2016140717 A1 (2016).

[73]   Capdevila J, Galofré JC, Grande E, Zafón Llopis C, Ramón Y Cajal Asensio T, Navarro González E, *et al.* Consensus on the management of advanced radioactive iodine-refractory differentiated thyroid cancer on behalf of the Spanish Society of Endocrinology Thyroid Cancer Working Group (GTSEEN) and Spanish Rare Cancer Working Group (GETHI). Clin Transl Oncol 2017; 19(3): 279-287

Figure Legend

Fig. 1. Molecular targets in the signaling pathways involved in aggressive thyroid cancer.

Figure 1