Current and future immunotherapies for thyroid cancer.

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List of abbreviations:

AbTg	anti-thyroglobulin antibody
AbTPO	anti-thyroid peroxidase antibody
AITD	autoimmune thyroid disorders
ATC	anaplastic thyroid cancer
CCL	chemokine (C-C motif) ligand
CCR	chemokine (C-C motif) receptor
CT	calcitonin
CTL	cytotoxic T cells
CTLA-4	**Cytotoxic T-Lymphocyte Antigen4**
CXCL	chemokine (C-X-C motif) ligand
CXCR	chemokine (C-X-C motif) receptor
DAC	5-aza-2'-deoxycytidine
DC	dendritic cell
DTC	differentiated thyroid carcinoma
EMT	epithelial-to-mesenchymal transition
FTC	follicular thyroid cancer
hPPCT	human PPCT
IFN	interferon
LN	lymph node
MTC	medullary thyroid cancer
NK	natural killer
NMR	**nuclear magnetic resonance**
NSCLC	non-small-cell lung cancer
OS	overall survival
PD-1	programmed death-1
PDTC	**aggressive thyroid cancer**
PFS	progression-free survival
PPCT	preprocalcitonin
PTC	papillary thyroid cancer
RET	rearranged during transfection
rT3	**reverse triiodothyronine**
SCLC	small-cell lung cancer
T3	**triiodothyronine**
T4	**thyroxine**
TC	thyroid cancer
Tg	thyroglobulin
TILN	tumor-involved lymph nodes
TKI	tyrosine kinase inhibitor
TLR3	toll-like receptor 3
TNF	tumor necrosis factor
Treg	Regulatory CD4+ T cells
TSH	**thyroid stimulating hormone**
TSI	thyroid stimulating immunoglobulin
UILN	uninvolved

Abstract

Introduction: Cancer immunotherapies were approved in recent years, including the immune checkpoint inhibitors. Experience with ipilimumab (CTLA-4 antagonist), nivolumab and pembrolizumab (PD-1 antagonists), and atezolizumab (PD-L1 antagonist) has shown **that** the impact on overall survival in cancer patients is paramount. Immune checkpoint inhibitors **target** the immune system, and **they can be applied works across multiple cancers**; the response rate is ranging from 20 to 40%. **Many studies have shown that thyroid cancer (TC) cells produce cytokines, and chemokines, inducing several tumor-promoting effects. Targeting and/or lowering cytokines, and chemokines concentrations within the tumor microenvironment would produce a therapeutic benefit.** In TC, increased Treg and PD-1^{+} T cell frequencies are indicative of aggressive disease; and PD-L1 expression correlates with a greater risk of recurrence.

Area covered: Searching in literature, few pioneering studies have evaluated immunotherapy in thyroid cancer. More recently it has been described one case of anaplastic thyroid cancer treated with vemurafenib and nivolumab, with substantial regression and complete radiographic and clinical remission.

Expert Opinion/Commentary: The use of immune checkpoint inhibitors in aggressive TC has not yet been extensively investigated, and further studies in large number of TC patients, are urgently needed.

1. Immunotherapy.

Cancer immunotherapies were approved in recent years, including preventive and therapeutic cancer vaccines [1], the first "immune checkpoint inhibitors" [2-4], a bi-specific T-cell engager, and an oncolytic virus [5] (**Table 1**). Of these, immune checkpoint inhibitors that target the programmed death-1 (PD-1) pathway generated the most interest, with response rates across tumor types that average 20-30% [6]. A pressing challenge is transforming the majority of patients from immunotherapy non-responders to responders. This will likely require potent combination immunotherapies that effectively harness the cancer-immunity cycle [7].

Cancer therapies result in tumor cell death and release of tumor antigens, which are presented by dendritic cells in the tumor-draining lymph nodes (LN) to prime and activate tumor immunity. Tumor-specific T cells then gain access to the circulation and traffic to tumors, where they infiltrate the tumor mass. T cell-mediated lysis of cancer cells releases more tumor antigens, thus perpetuating the cycle. Multiple opportunities for therapeutic intervention that enhance tumor immunity are possible at each step of this cycle [7].

The immune system has an important role in the recognition and eradication of tumor cells ("immune surveillance"). The knowledge of the relationships between the immune system and cancers leaded to the identification of key molecules that influence them. Cancer cells try to escape recognition and destruction by the immune system through multiple mechanisms. For example, tumor cells can dysregulate immune cell activity (of T cells and natural killer cells) activating T cell inhibitory pathways (checkpoint), as Cytotoxic T-Lymphocyte Antigen4 (CTLA-4), Programmed Death-1 (PD-1), or suppression of NK cell activity, and others.

CTLA-4 is a protein receptor that downregulates immune responses, functioning as an immune checkpoint. CTLA-4 is constitutively expressed in regulatory T cells, and upregulated in conventional T cells after activation. Binding to CD80 or CD86 on the surface of antigen-

presenting cells, it acts as an "off" switch. Anti-CTLA4 agents act in the priming phase of immune response through the inhibition of the interaction between CTLA-4 on T cell and B7 on antigen-presenting cell. The inhibition of negative regulation through binding of CTLA-4 has been shown to promote stimulation of adaptive immunity and potentiation of T cell activation. CTLA-4-blocking antibodies have shown to be efficient in various murine malignancy models upon administration as monotherapy.

Ipilimumab, a fully human monoclonal antibody that blocks CTLA-4, was the first successfully developed drug belonging to a new class of therapeutics called "immune checkpoint inhibitors" [8].

Activated T-cells express also PD-1, another immune checkpoint target, that mediates immunosuppression. Its ligands PD-L1 (B7-H8) and PD-L2 (B7-DC) are expressed on many tumor and stroma cells and other cell types, as leucocytes. The immunosuppressive action of the PD-1 receptor is activated in the effector phase of the interaction between T lymphocytes and tumor cells. A new class of drugs that block PD-1, the PD-1 inhibitors, activate the immune system to attack different types of cancer with varying success [9].

Experience with ipilimumab (a CTLA-4 antagonist), nivolumab and pembrolizumab (PD-1 antagonists) and atezolizumab (a PD-L1 antagonist) in treating cancer has defined several key principles of cancer immunotherapy with immune checkpoint inhibitors. Immune checkpoint inhibitors target the immune system, engaging T cells with inherent capacity for adaptability and memory, underlining the durable responses and long-term survival observed with these agents, and they can be applied works across multiple cancers. Moreover, the side effects of immune checkpoint inhibitors are distinct from those of chemotherapy and targeted agents. Finally, the efficacy of immunotherapy can be improved by combining immune checkpoint inhibitors with other treatment strategies [10].

1.1. Cancer immunotherapy with immune checkpoint inhibitors has unique patterns of clinical benefit

The impact on overall survival (OS) of immune checkpoint inhibitors therapy is paramount. Ipilimumab prolongs OS with no impact on overall response rate (10%) or progression-free survival (PFS) in melanoma [2,11]. **However the rate of PFS at week 12 was slightly higher in the ipilimumab-alone treated group [2]. There was a 24% reduction in the risk of progression in the ipilimumab–dacarbazine group as compared with the dacarbazin [11].**

A recent phase III study making a comparison between nivolumab and everolimus in metastatic kidney cancer showed no difference in median PFS, but the median OS for patients treated with nivolumab was longer (25 versus 19.6 months) [10]. **However the median PFS was slightly longer (4.6 months) in the nivolumab group than in the everolimus group (4.4 months) [12].**

This clinical benefit pattern may be related to the immune response evolution over time, or to pseudo-progression, defined as an increase in size of the tumor mass on imaging from immune infiltration rather than tumor growth. A **non-conventional** response pattern led to the development of immune-related response criteria, which preserve the potential of benefiting from immunotherapy despite apparent disease progression on imaging [13]. Thus, immunotherapy should be continued in the face of apparent disease progression by imaging if the patient is doing well, and/or until progressive disease is confirmed by a second scan 4 weeks later.

A meta-analysis of 5000 patients with advanced melanoma reported that ipilimumab rendered melanoma a chronic disease in 20% of patients [14]. Early data with single-agent nivolumab [4,15,16], pembrolizumab [17], and combination of ipilimumab/nivolumab [18,**19**] suggest that PD-1 antagonists could result in even more long-term responders. Relative to CTLA-4 blockade, PD-1/PD-L1 blockade appears to double the number of long-term responders. PD-1 blockade also appears to give higher

objective response rate (ORR)s and longer PFS than CTLA-4 blockade [20,21]. The effectiveness of the combination of CTLA-4 and PD-1/PD-L1 blockade is even higher, and it is likely that OS will continue to improve with effective combination immunotherapies.

PD-L1 overexpression predicts activity as well as better survival for patients treated with immune checkpoint inhibitors [22].

1.2. Immunotherapy with immune checkpoint inhibitors targets a broad range of tumor types

Because cancer immunotherapy **targets** the immune system, it can be effective independently from **tumor stage** or driver mutations, as ipilimumab has long-term benefit for cutaneous, ocular and mucosal melanomas, which have distinct biology [23-28]. There was no difference between BRAF- and NRAS-mutated melanoma in duration of response or OS with ipilimumab [29]. Moreover, the efficacy of PD-1 blockade extends to multiple tumor types, including kidney cancer, small-cell lung cancer (SCLC), non-small-cell lung cancer (NSCLC), Hodgkin's lymphoma, head and neck cancer, esophageal and gastric cancers, hepatocellular cancer, bladder cancer, breast cancer and others [6,30]. **However PD-1 blockade often is more effective in certain subtypes of cancer. For example clinical activity appears more likely in triple negative PD-L1+ breast cancer [31].** Thus, it is possible to achieve long-term survival in some patients across a range of distinct tumor types, where the different response rates across tumors reflect different immunogenicity.

1.3. Spectrum of toxicities

Nearly, every organ can be affected with immune-related adverse effects. Published clinical trial data suggest that overall 7–19% of patients receiving anti-PD1/PD-L1 antibodies experienced grade 3–5 adverse events [12,20,32-36]. The discontinuation rate of immune checkpoint inhibitors due to adverse events ranged from 3% to 8% for anti-PD-1/PD-L1 antibodies [12,17,20,21,32-34], and up to 15% for

ipilimumab [20,21]. The discontinuation rate due to adverse effects with the combination treatment of nivolumab, plus ipilimumab, is significantly higher, at a rate of 36% [20]. Time to onset of immune-related adverse events, however, is variable [37]. The addition of immune checkpoint inhibitors with chemotherapy does not accentuate the cytotoxic toxicities of chemotherapy, but does add the additional risk of immune-related toxicities [38].

1.4. Immune-related endocrine toxicities

Immune-related endocrine toxicities include hypophysitis, thyroid dysfunction, adrenal insufficiency and type 1 diabetes mellitus. Immune-related endocrine toxicities are one of the few toxicities which are commonly irreversible, with studies showing recovery of the pituitary–thyroid axis in up to 50% of patients [39] and in 50–60% for the pituitary–gonadal axis [39-41]. Very few cases of corticotroph recovery have been reported [40].

About the onset time of immune-related adverse events, hypophysitis was reported in patients in the initial pembrolizumab clinical trial database with a time to onset as far as 1.7 months, similarly to the onset of hypophysitis described in ipilimumab clinical trials. In a phase III nivolumab trial conducted in advanced melanoma patients, thyroid dysfunctions occurred with a time to onset ranging from 24 days to 11.7 months from the beginning of the therapy [42].

1.5. Thyroid disorders induced by immunotherapy

Thyroid abnormalities are the second most common endocrine-related immune adverse effect secondary to anti-CTLA-4-antibodies [39] and can occur in 1–6% of patients treated with immune checkpoint inhibitors [40,41]. The incidence is closer to 10% in patients treated with single agent anti-PD-1/PD-L1 [20,21]. It may present as thyrotoxicosis, hypothyroidism, painless thyroiditis, or more severe disorders such as “thyroid storm” [40]. Most cases of anti-CTLA-4 thyroiditis are subclinical

and occur after 2–4 infusions [39]. **Also other less frequent endocrine disorders have been reported such as euthyroid Graves' ophthalmopathy [43,44].**

1.6. Mechanisms leading to thyroid disorders

Thyroid disorders induced by immunotherapy are due to an autoimmune reaction against thyroid leading to a destructive thyroiditis with release of thyroid antigen and then antibody production. The beginning hyperthyroid phase is characterized by high thyroglobulin (Tg) levels in most of the patients (without anti-Tg as well as elevated anti-Tg and thyroid stimulating immunoglobulin (TSI), in 40 and 50% of the subjects, respectively) with evidence of thyroiditis in patients with available imaging. In the major part of these patients, normalization of Tg is in favour of a destructive process leading to permanent hypothyroidism [41,45-50].

In patients with high TSI levels, it is not probable that this can lead to thyrotoxicosis as available imaging in patients were less indicative of Graves' (an ultrasonographic hypoechoic pattern of the thyroid).

Cancer patients treated with anti-CTLA-4 or anti-PD-1, with previous diagnosed autoimmune thyroid disorders (AITD), or circulating antithyroglobulin antibodies (AbTg), antithyroperoxidase antibodies (AbTPO), more frequently develop a thyroid dysfunction or a destructive thyroiditis [45-50] (in about 80% of cases). **However thyroid dysfunctions may occur also in patients without circulating thyroid autoantibody [51].**

Interestingly, an elevated antitumor response was present in some series of patients with circulating anti-thyroid autoantibodies, suggesting an association between inflammatory and tumor response [45]. Moreover, the appearance of AbTg was associated with prolonged survival [52].

1.7. Management of thyroid disorders

Thyroid function tests are to be checked at baseline and then every 2 months while on treatment with immune checkpoint inhibitors [53]. Immune checkpoint therapy can be continued for grade 1–2 hyperthyroidism, and treatment is initiated for symptoms of hyperthyroidism [54,55]. In case of symptomatic hyperthyroidism (grade 3), immune checkpoint inhibitor therapy should be withheld and corticosteroids commenced (oral prednisolone 1–2 mg/kg/day) [54]. If necessary antithyroid medications (methimazole, propilthyouracil, or carbimazole) may be indicated. In case of severe hyperthyroidism (grade 4), immune check-point inhibitor therapy should be permanently stopped, and methylprednisone (1–2 mg/kg/day; IV) should be administered for 3 days, followed by oral prednisolone (1–2 mg/kg/day), which is tapered over at least 1 month [54].

To distinguish the pathogenesis of hyperthyroidism, thyroid ultrasound and the study of thyroid vascularization could be useful, in fact hyper-vascularity is associated with Graves' disease, while hypo-vascularity is associated with destructive thyroiditis.

Primary hypothyroidism is diagnosed if thyroid stimulating hormone (TSH) levels are increased in presence of a low free thyroxine (T4) level, whereas hypophysitis is characterized by a low TSH and low free T4. In case of primary hypothyroidism immune checkpoint inhibitor therapy can be continued with appropriate levothyroxine replacement [42]. Hypophysitis can present as fatigue, headaches, and visual field defects. Diagnosis is established according to levels of pituitary hormones (ACTH, TSH, FSH, LH, GH, IGF-1 and prolactin) and nuclear magnetic resonance (NMR) imaging showing an enlarged pituitary, with/without necrosis. For grade ≥2 toxicity it is adviced to withhold immune checkpoint inhibitor therapy and start high-dose corticosteroids (methylprednisolone 125 mg daily intravenously for 3 days with a switch to oral prednisone 1-2 mg/kg daily upon improvement of symptoms). The corticosteroid therapy (which is quite frequent in neoplastic subjects) may have a direct effect on the pituitary by reducing TSH levels. Furthermore corticosteroid therapy can be associated with an "euthyroid sick

syndrome" (also known as nonthyroidal illness syndrome) characterized by low serum triiodothyronine (T3) and elevated reverse T3 (rT3). This might affect differential diagnosis when evaluating thyroid function tests [42].

Regular monitoring of thyroid function tests during the treatment period should allow for early detection of hypothyroidism, prior to the patient becoming symptomatic. If the patient is symptomatic, the clinical situation should be evaluated on an individual basis to evaluate whether the immune checkpoint inhibitor should be withheld [40,54,56].

2. Aggressive thyroid cancer.

More than 90% of thyroid cancer (TC) are differentiated thyroid carcinomas arising from follicular cells (DTC) [papillary thyroid cancer (PTC) 90%, follicular thyroid cancer (FTC) 10%], 5% are medullary thyroid cancer (MTC), and <5% are anaplastic thyroid cancer (ATC), or other rare histological types.

Thyroid cancer derived from follicular epithelium.

PTC and FTC patients are usually treated with total thyroidectomy, and radioiodine is performed in patients with an elevated risk, and evaluated in DTC patients with an intermediate risk. Throughout tumor progression, DTC cells can lose the iodide uptake ability, and become resistant to radioiodine; this worsens the prognosis, and DTC becomes "aggressive dedifferentiated TC" (**PDTC**).

Thyroid surgery has minimal effect in ATC, and the other therapies are not beneficial [57,58]. Further attempts in the development of new drugs are going on owing to the absence of specific and effective drugs for the treatment of **PDTC**.

Thyroid cancer derived from C-cells.

Surgery is the only curative treatment of MTC; however, no definitive treatment is possible for recurrent or metastatic MTC.

Molecular pathways alterations in thyroid cancer and targeted therapies.

In the last years, different genetic alterations in the molecular pathways, determinant for the development and progression of TC, have been identified (BRAF mutations, **TERT promoter mutations,** RAS mutations, RET/PTC gene rearrangements, RET mutations). Tyrosine kinase inhibitors (TKIs) are small organic compounds able to inhibit tyrosine kinases auto-phosphorylation and activation. Most of them are multi-kinase inhibitors acting on the above reported molecular pathways, and consequently in growth, angiogenesis, and local and distant spread of TC and for this reason are now considered as new therapies of aggressive TC, as they can induce clinical responses and stabilization of the disease [57,58]. Median PFS is improved by these drugs, but no significant OS increase has been shown until now; side effects are common, and they may be severe.

New attempts are needed to find novel, more effective and safe therapies in patients with **PDTC**. Moreover, immunotherapy in **PDTC** has not yet been extensively evaluated.

3. Immune response in TC.

Many studies have evaluated the immune response, and the involvement of cytokines and chemokines, or immune competent cells in human TC.

3.1 Evaluation of the immune response in human TC: cytokines and chemokines

In the last few years, experimental evidence has accumulated showing that chemokine (C-X-C motif) receptor (CXCR)3, and its ligands [the interferon (IFN)-γ inducible chemokine (C-X-C motif) ligand (CXCL)9, CXCL10, CXCL11] play an important role in the immune pathogenesis of autoimmune disorders involving thyroid and endocrine glands [59,60]. In fact, after IFN-γ stimulation, thyroid epithelial cells secrete CXCL10, which induces the chemotaxis of type-1 T-helper lymphocytes, that further secrete IFN-γ, thus initiating and perpetuating the autoimmune process. In PTC, RET/PTC rearrangements, and BRAF or RAS activating mutations lead to the up-regulation of CXCL10,

stimulating proliferation and invasion [61,62]. Furthermore, elevated basal levels of Toll-like receptor 3 (TLR3) and non-canonical WNT5a RNA have been reported in PTC cell lines. TLR3 are functional in PTC cells, leading to increase significantly CXCL10 levels, the TLR3 signaling end product [63]. In primary cultures of PTC cells, IFN-γ induced a CXCL10 release more than ten times higher than in normal follicular thyroid cells. Furthermore a deregulation of CXCL10 secretion was observed, in fact PPAR-gamma agonists inhibited CXCL10 secretion in normal thyroid follicular cells, and stimulated it in PTCs [64].

Also ATC cells produce CXCL10, basally and under the influence of cytokines. However, the pattern of modulation by IFN-γ, tumor necrosis factor (TNF)-α or thiazolidinediones strongly varies, suggesting that the intracellular pathways involved in the chemokine modulation in ATC have different types of deregulation [65]. ATC cell lines are sensitive to lysis by ex vivo expanded natural killer (NK) cells, and the lysis was abrogated upon blockade of NKG2D **[66]**. Moreover, ATC cell lines produced high levels of CXCL10 and stimulated migration of expanded NK cells, and ATC tumors were enriched for NK cells expressing the cognate chemokine receptor CXCR3 [66].

In different human carcinoma types, mast cell infiltrate increases in the tumoral tissue and mast cell density correlates with a bad prognosis. A recent study suggested that mast cells are recruited into thyroid cancer, promoting proliferation, survival and invasive ability of cancer cells, in this way contributing to thyroid cancer growth and invasiveness, principally mediated by histamine, CXCL1 and CXCL10 (mast-cell-derived mediators) [67].

A study assessed the expression of the chemokine receptors chemokine (C-C motif) receptor (CCR)3, CCR7, and CXCR4 in tumor and nonmalignant thyroid tissues from patients with PTC. Expression of these receptors in PTC was correlated with the clinical pathological condition of PTC. The results suggested that CCR3, CCR7, and CXCR4 are increasingly expressed in tumor cells from more aggressive PTC, being CXCR4 expression a potential marker for enhanced tumor aggressiveness [68].

The importance of mast cells derived mediators (as the chemokines CXCL1, CXCL10, and CXCL8) in the context of TC has been demonstrated. CXCL1 and CXCL10 contribute to the stimulation of cell proliferation, while CXCL8 is involved in the acquisition of TC malignant traits inducing or enhancing the epithelial mesenchymal transition and stem-like features of TC cells [69].

CXCL8 displays several tumor-promoting effects. Targeting and/or lowering CXCL8 concentrations within the tumor microenvironment would produce a therapeutic benefit. It was demonstrated that IFN-γ inhibits CXCL8 secretion and in turn the migration of a **BRAF^{V600E}** mutated thyroid cell line [70].

Furthermore, primary ATC cells produce chemokine (C-C motif) ligand (CCL)2 basally and after cytokines stimulation, with an extremely variable pattern of modulation, suggesting different types of deregulation in the chemokine modulation. Serum CCL2 is increased in ATC patients. Further studies will be necessary to evaluate if CCL2 might be used as a marker in the follow-up of ATC patients [71].

The inhibition of chemokine signaling could be considered as a novel therapeutic approache for the treatment of refractory forms of TC.

3.2 Evaluation of the immune response in human TC: PD-1$^+$ T cell, PD-L1, Treg, cytotoxic T cells and NK cells

To characterize the immune response to metastatic PTC, CD4+ T cell polarization was assessed, such as the role of PD-1 and T cell exhaustion. T cell subset frequencies were compared in Uninvolved (UILN) and tumor-involved lymph nodes (TILN) and assessed for correlation with recurrent disease and extranodal invasion. Regulatory CD4+ T cells (Treg) were enriched in TILN compared with UILN and further elevated in TILN from patients with recurrent disease. PD-1$^+$ T cells were present at high frequency in TILN and markedly enriched in TILN that showed evidence of extranodal invasion. In TILN, Treg frequency correlated with PD-1$^+$ T cell frequencies. The Authors suggested that increased Treg and PD-1$^+$ T cell frequencies in LN may be indicative of aggressive recurrent PTC [72].

In another study $BRAF^{V600E}$ mutated patients samples showed higher PD-L1 mRNA levels with respect to $BRAF^{WT}$ TC patients [73].

A study evaluated PD-L1 expression in PTC and its variants, to determine its prognostic value to predict clinical outcome in these patients. PD-L1 immunostaining was principally localized in cytoplasm and occasionally in plasma tumor cell membranes. Patients with increased PD-L1 membrane or cytoplasmic positivity had significantly shorter median disease free survival (36 months, and 49 months, respectively) with respect to those with PD-L1 negative tumors (both 186 months). Considering $PD\text{-}L1^{+}$ and $PD\text{-}L1^{-}$ patients and PTC staging, an increased cytoplasmic positivity was present in all four stages of PTC showing a greater risk of recurrence and a poor prognosis. These data suggest PD-L1 positive expression in PTC correlates with a greater risk of recurrence and shortened disease free survival leading to hypothesize that it could be considered as a prognostic marker for PTC [74].

However, it may be possible that PD-L1 expression in TC simply correlates with a yet undefined and more aggressive subtype of TC.

Other studies support the presence of an antitumor immune response in advanced TCs linked to cytotoxic T cells (CTL) and NK cells. This antitumor response is likely blunted by the presence of immunosuppressive pathways within the microenvironment, facilitated by tumor-associated macrophages or increased expression of negative regulators of CTL function [75].

Recently it has been shown that PD-L1 was positively expressed in 53.2% of PTCs [76], while a very low expression of PD-L1 in MTC was observed [77].

4. Immunotherapy in TC.

4.1 Experimental models of immunotherapy in TC

Along the lines of other studies that performed a preclinical evaluation of immunotherapy in other kinds of cancer (for example colon cancer, melanoma, etc.) [78-80], the effect of immune checkpoint inhibitors has been evaluated in syngenic immune-competent models of TC in mice [78-80].

In an immuno competent orthotopic mouse model of ATC, combined PLX4720 and dasatinib treatment exerted a significant antineoplastic response, and immune cell infiltration was increased by PLX4720 treatment [81].

In another paper, $BRAF^{V600E}$ cells showed significantly higher baseline expression of PD-L1 at mRNA and protein levels *versus* $BRAF^{WT}$ cells. Treating all cell lines with MEK inhibitor, PD-L1 expression decreased. BRAF inhibitor treatment decreased PD-L1 expression in $BRAF^{V600E}$ cells, but increased it in $BRAF^{WT}$ cells. Immunocompetent mice (B6129SF1/J) implanted with syngenic 3747 $BRAF^{V600E/WT}$ $P53^{-/-}$ murine tumor cells were randomized to control, PLX4720, anti PD-L1 antibody and their combination. The combination dramatically reduced tumor volume, compared to PLX4720 or PD-L1 antibody alone. Immunohistochemistry analysis revealed intense CD8+ CTL infiltration and cytotoxicity and favorable CD8+: Treg ratio compared to each individual treatment. These results suggested anti PD-L1 treatment potentiates the effect of BRAF inhibitor on tumor regression and intensifies anti tumor immune response in an immune-competent model of ATC [73].

NY-ESO-1 is one of the most immunogenic members of the cancer/testis antigen family, and demethylating and deacetylating agents can increase its levels. This cytoplasmic antigen is considered a potent target for cancer immunotherapy. A study evaluated the baseline expression of NY-ESO-1 messenger RNA and protein before and after exposure to 5-aza-2'-deoxycytidine (DAC) (72 hours) in different TC cell lines. None of the TC cell lines showed baseline expression of NY-ESO-1. However, the NY-ESO-1 gene expression increased in BCPAP, TPC-1, and 8505c cell lines after DAC treatment. Furthermore, in vivo DAC treatment increased NY-ESO-1 expression in an orthotopic mouse model

with BCPAP cells, suggesting that many differentiated TC cells can be pressed to express immune antigens, that successively can be utilized in TCR-based immunotherapeutic interventions [82].

A recent study evaluated the effects of NK cell-based immunotherapy for pulmonary metastasis of ATC. Human NK cells (NK-92MI) were retrovirally transduced to express the effluc gene. Human ATC cells (CAL-62) were transduced with the effluc and Rluc genes. To evaluate the targetability of NK cells to ATC tumors, NK-92MI cells expressing the effluc gene (NK/F) were administered through the tail vein of nude mice with a pulmonary metastasis or tumor xenograft. An ATC pulmonary metastasis mouse model was generated, and NK cells significantly inhibited the growth of the metastasis. These results suggest that NK cells are able to target ATC tumors and that NK cell-based immunotherapy may be effective for pulmonary metastases of ATC [83].

No effective therapies are known for patients suffering from progressive MTC, a calcitonin (CT)-secreting C cell tumor. As CT, which arises from the precursor protein preprocalcitonin (PPCT), is expressed by almost all MTCs, these molecules may represent target antigens for immunotherapy against MTC. A study investigated whether DNA immunization induced cellular and humoral immune responses against human PPCT (hPPCT) in mice. The results showed that cellular and humoral immune responses against hPPCT can be generated by DNA immunization and increased by coinjection of the GM-CSF gene, suggesting the use of DNA immunization as a potential novel immunotherapeutic therapy for MTC patients [84].

4.2 Immunotherapy of TC in humans

In a pioneering study human Tg (altered with the diazonium derivatives of arsanilic and sulfanilic acids, and with Freund adjuvant) was used to induce autoimmune thyroiditis in humans with untreatable metastatic TC. There was minimal toxicity during the therapy, and it was possible to create

autoimmune thyroiditis in 3/8 patients. Antibodies to Tg were induced in 5/8 patients (62.5%). However, the clinical response to the immunotherapy was minimal [85].

Recently some studies investigated the role of dendritic cells (DCs) within the immune system and how to generate and activate these cells to induce cytotoxic immunity in tumors. DC vaccinations in patients with endocrine malignancies (mainly in metastasized MTC) resulted in tumor-specific immunity and partial clinical responses in some cases. For these reasons, new DC vaccination protocols for the treatment of patients with endocrine tumors have now been conducted [86].

Furthermore it was shown that oncolytic virus dl922-947 reduces IL-8/CXCL8 and MCP-1/CCL2 expression and impairs angiogenesis and macrophage infiltration in ATC [87].

More recently it has been described a 52-year-old male who was diagnosed with ATC and initially treated with a thyroidectomy and LN dissection, followed by chemotherapy. Next generation sequencing was then performed to guide therapy and the tumor was found to have BRAF and PD-L1 positivity, and it was subsequently treated with vemurafenib and nivolumab. This led to substantial regression of ATC, and in complete radiographic and clinical remission 20 months after the beginning of the treatment with nivolumab [88].

Conclusion.

In aggressive TC, TKI therapy prolongs median PFS, but until now no significant increase has been observed on OS; side effects are common, and severe [57,89-91]. New efforts are needed to obtain new effective and safe therapies for **PDTC** patients.

Many studies have shown that TC cells produce cytokines, and chemokines, that induce several tumor-promoting effects. Targeting and/or lowering cytokines, and chemokines concentrations within the tumor microenvironment would produce a therapeutic benefit.

Increased Treg and PD-1^+ T cell frequencies are indicative of aggressive, recurrent PTC. PD-L1 positive expression in PTC correlates with a greater risk of recurrence and shortened disease free survival. Moreover, other studies support the presence of an anti-tumor immune response in advanced thyroid cancers linked to CTL and NK cells, that is blunted by the presence of immunosuppressive pathways within the microenvironment, facilitated by tumor-associated macrophages or increased expression of negative regulators of CTL function [72-75].

Few pioneering studies have evaluated immunotherapy in TC [85,86]. More recently it has been described one case of ATC treated with vemurafenib and nivolumab, with substantial regression and in complete radiographic and clinical remission 20 months after the beginning of the treatment with nivolumab [88].

The use of immune checkpoint inhibitors in aggressive TC has not yet been extensively investigated, and further studies in large number of TC patients, are urgently needed.

Expert commentary.

The use of immune checkpoint inhibitors in aggressive TC has not yet been investigated and further studies are urgently needed. **Since it has been shown that PD-L1 was positively expressed in 53.2% of PTCs [76], and since PD-L1 positive patients had a significantly higher response rate than PD-L1 negative [92],** it is reasonable to expect an overall 20-40% response rate in TC patients by immune checkpoint inhibitors, such as in other types of cancer.

A pressing challenge is transforming the majority of cancer patients from immunotherapy non-responders to responders. TC has some peculiarities that will be used to increase the responder rate. In fact, about 20% of TC patients are also affected by AITD. Patients treated with anti-CTLA-4 or anti-PD-1, with previous diagnosed AITD, or circulating AbTg, or AbTPO, more frequently develop a thyroid dysfunction or a thyroiditis [45-50], strongly suggesting that immunotherapy stimulates the

immune process associated with AITD in these patients. In the major part of these cancer patients, thyroid dysfunctions by checkpoint inhibitors lead to a destructive thyroiditis leading to permanent hypothyroidism. The induction of a destructive thyroiditis could be theoretically beneficial in patients with TC and residual thyroid tissue, because it could lead to a partial destruction of the TC tissue itself; this point needs to be investigated.

However, the prevalence of the appearance of a thyroid disorder in patients treated with immunotherapy is low (5-10%). In consideration that with immune checkpoint inhibitors therapy the appearance of a destructive thyroiditis is observed in up to 80% of cancer patients, with AITD [45-50], it could be hypothesized a higher response rate in TC patients with AITD, and a more potent antineoplastic effect in these patients, opening the way to a selective use of immune checkpoint inhibitors in TC patients with AITD.

It is also necessary to consider that a destructive thyroiditis leading to destruction of the tumoral TC tissue will not result in a thyroid dysfunction, since TC patients have been previously submitted to total thyroidectomy, and euthyroidism is maintained with L-T4 therapy.

The stronger antineoplastic immune response observed in cancer patients treated with immune checkpoint inhibitors in presence of AITD [45], and the observation that the appearance of AbTg was associated with prolonged survival [52], suggest that also cancer patients (not TC) with AITD are more likely to be treated with immune checkpoint inhibitors.

Furthermore in TC patients without AITD, it could be speculated that the induction of an autoimmune process against a specific antigen present in the tumor itself could evoke a stronger immune response specifically directed against the antigen itself. With this view, it has to be noticed that thyroid has antigens uniquely expressed by the thyroid tissue itself. For example, Tg is an antigen expressed specifically and uniquely by the thyroid tissue. In fact, Tg is very useful as tumoral marker in patients with PTC, FTC, or **PDTC**, after thyroidectomy and radioiodine ablation, since the presence of

circulating Tg is a specific indicator of the presence of residual thyroid tissue. This generates the opportunity to evaluate in aggressive PTC, or FTC, or **PDTC** if immunotherapy could be specifically directed and enhanced against the thyroid tissue by pre-induction of an autoimmune reaction against a specific antigen of the tissue itself, that in this case is Tg. Considering that the responder rate with immune checkpoint inhibitors is ranging from 20 to 40% (in other cancers) [7,21,57,89-91,93], and since the appearance of a destructive thyroiditis is observed in up to 80% of cancer patients with circulating AbTg (after immune checkpoint inhibitors therapy) [45-50], it is possible to hypothesize that a specific immunization with Tg, in **PDTC** patients (initially without AITD), will lead to the appearance of AbTg in about 60% of them [85], increasing the number of responders to immune checkpoint inhibitors. Furthermore, an elevated antineoplastic response was observed in cancer patients treated with immune checkpoint inhibitors in presence of AITD [45], and the appearance of AbTg was associated with prolonged survival [52]. So it is possible to hypothesize that the combination of the induction of an autoimmune reaction against Tg, and immune checkpoint inhibitors, will induce in **PDTC** a more potent antineoplastic effect (than immune checkpoint inhibitors alone), and a prolonged survival.

Since the presence of AbTg was associated with a more potent antineoplastic effect of immune checkpoint inhibitors, and prolonged survival [45,52], it will be possible to hypothesize that the combination of the induction of an autoimmune reaction against Tg, and immune checkpoint inhibitors therapy, will be used also in other cancers to induce the appearance of AbTg, increasing the responder rate, and the antineoplastic response.

Theoretically, in case that a cancer antigen specific immunization might induce a stronger and specifically directed immune response (against the cancer that contains the above mentioned antigen), the induction of an autoimmune reaction could be used also in other cancers that contain specific

antigens, or that overexpress an antigen (for example, CT, or PPCT [84] in MTC, or CEA in colon cancer, etc.), in association with immune checkpoint inhibitors therapy.

Five-year view.

Immune checkpoint inhibitors will be used in different types of aggressive TC in humans (**PDTC**, MTC, ATC); it is reasonable to expect an overall 20-40% response rate in TC-P by immune checkpoint inhibitors. Probably, the effectiveness of the combination of CTLA-4 and PD-1/PD-L1 blockade on OS will be proved to be higher, than single therapy (such as in other cancers).

A pressing challenge is transforming the majority of cancer patients from immunotherapy non-responders to responders. Since about 20% of TC-P are also affected by AITD, and since the appearance of a destructive thyroiditis is observed in up to 80% of cancer patients with AITD (leading to destruction of the TC tissue) [45-50], it will be investigated if AITD lead to a higher response rate by immune checkpoint inhibitors in TC patients with AITD, and to a more potent antineoplastic effect; opening the way to a selective use of immune checkpoint inhibitors in TC patients with AITD.

The stronger antineoplastic immune response observed in cancer patients treated with immune checkpoint inhibitors in presence of AITD [45], and the observation that the appearance of AbTg was associated with prolonged survival [52], could be investigated to evaluate if also other cancer patients (not TC) with AITD are more likely to be treated with immune checkpoint inhibitors.

Furthermore in TC patients without AITD, the combination of the induction of an autoimmune reaction against Tg, and immune checkpoint inhibitors therapy, could be evaluated to induce a higher response rate, and a more potent antineoplastic effect (than immune checkpoint inhibitors alone).

Since the presence of AbTg was associated with a more potent antineoplastic effect of immune checkpoint inhibitors, and prolonged survival [45,52], the combination of the induction of an autoimmune reaction against Tg, and immune checkpoint inhibitors, could be investigated also in other types of cancer, to induce the appearance of AbTg, increasing the responder rate, and the antineoplastic response.

The induction of an autoimmune reaction will be investigated also in other cancers that contain specific antigens, or that overexpress an antigen (for example, CT, or PPCT in MTC, or CEA in colon cancer, etc.), in association with immune checkpoint inhibitors therapy.

Key issues.

- Cancer immunotherapies were approved in recent years, including the immune checkpoint inhibitors: ipilimumab (a CTLA-4 antagonist), nivolumab and pembrolizumab (PD-1 antagonists), and atezolizumab (a PD-L1 antagonist).
- Immune checkpoint inhibitors engage T lymphocytes (Treg and PD-1^+ T-cells) with inherent capacity for adaptability and memory. This mechanism of action underlies the durable responses and long-term survival observed with these agents.
- Immune checkpoint inhibitors **target** the immune system, and can work regardless of either cancer histology, or the presence of driver mutations.
- The impact on "overall survival" of immune checkpoint inhibitors is paramount. Ipilimumab rendered melanoma a chronic disease in 20% of patients. Immune checkpoint inhibitors that target the PD-1 pathway show response rates, across tumor types, that average 20-30%. Combining CTLA-4 and PD-1/PD-L1 blockade is even more effective, with response rate ranging 20-40%.
- A pressing challenge is transforming the majority of cancer patients from immunotherapy non-responders to responders, with potent combination of immunotherapies.
- Many studies have shown that TC cells produce cytokines, and chemokines, that induce several tumor-promoting effects. Targeting and/or lowering cytokines, and chemokines concentrations within the tumor microenvironment would produce a therapeutic benefit.
- Increased Treg and PD-1^+ T cell frequencies in TC are indicative of aggressive, recurrent TC. PD-L1 positive expression in PTC correlates with a greater risk of recurrence and shortened disease free survival. Moreover, other studies support the presence of an antitumor immune

response in advanced TC linked to CTL and NK cells, that is blunted by the presence of immunosuppressive pathways within the microenvironment.

- Few pioneering studies have evaluated immunotherapy in TC in humans.
- More recently it has been described one case of ATC treated with vemurafenib and nivolumab, with substantial regression and complete radiographic and clinical remission.
- The use of immune checkpoint inhibitors in aggressive TC has not yet been extensively investigated, and further studies in large number of TC patients, are urgently needed.

References.

1. Emens LA, Ascierto PA, Darcy PK, et al. Cancer immunotherapy: Opportunities and challenges in the rapidly evolving clinical landscape. Eur J Cancer 2017;81:116-29

   ****An important review about cancer immunotherapy, as a new established way to treat cancer**

2. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med 2010;363:711-23

   **** Ipilimumab, with/without a gp100 peptide vaccine, with respect to gp100 alone, improved overall survival in patients with previously treated metastatic melanoma**

3. Robert C, Ribas A, Wolchok JD, et al. Anti-programmed death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a phase 1 trial. Lancet 2014;384:1109-17

4. Topalian SL, Sznol M, McDermott DF, et al. Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. J Clin Oncol 2014;32:1020-30

   **** Overall survival in patients with advanced treatment-refractory melanoma treated with nivolumab compares favorably with that in literature studies of similar patient populations**

5. Andtbacka RH, Kaufman HL, Collichio F, et al. Talimogene Laherparepvec Improves Durable Response Rate in Patients With Advanced Melanoma. J Clin Oncol 2015;33:2780-8

6. Lipson EJ, Forde PM, Hammers HJ, et al. Antagonists of PD-1 and PD-L1 in Cancer Treatment. Semin Oncol 2015;42:587-600

7. Chen DS, Mellman I. Oncology meet immunology: the cancer-immunity cycle. Immunity 2013; 39:1-10

8. **Grosso JF, Jure-Kunkel MN. CTLA-4 blockade in tumor models: an overview of preclinical and translational research. Cancer Immun 2013;13:5**
9. **Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer 2012; 12:252-64**
10. Ascierto PA, Marincola FM. What have we learned from cancer immunotherapy in the last 3 years? J Transl Med 2014;12:141
11. Robert C, Thomas L, Bondarenko I, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med 2011;364:2517-26
12. Motzer RJ, Escudier B, McDermott DF, et al. Nivolumab versus Everolimus in Advanced Renal-Cell Carcinoma. N Engl J Med 2015;373:1803-13
13. Wolchok JD, Hoos A, O'Day S, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res 2009;15:7412-20
14. Schadendorf D, Hodi FS, Robert C, et al. Pooled Analysis of Long-Term Survival Data From Phase II and Phase III Trials of Ipilimumab in Unresectable or Metastatic Melanoma. J Clin Oncol 2015;33:1889-94
15. McDermott DF, Drake CG, Sznol M, et al. Survival, Durable Response, and Long-Term Safety in Patients With Previously Treated Advanced Renal Cell Carcinoma Receiving Nivolumab. J Clin Oncol 2015;33:2013-20
16. Gettinger SN, Horn L, Gandhi L, et al. Overall Survival and Long-Term Safety of Nivolumab (Anti-Programmed Death 1 Antibody, BMS-936558, ONO-4538) in Patients With Previously Treated Advanced Non-Small-Cell Lung Cancer. J Clin Oncol 2015;33:2004-12
17. Herbst RS, Baas P, Kim DW, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet 2016;387:50

18. Hodi FS, Chesney J, Pavlick AC, et al. Combined nivolumab and ipilimumab versus ipilimumab alone in patients with advanced melanoma: 2- year overall survival outcomes in a multicentre, randomised, controlled, phase 2 trial. Lancet Oncol 2016;17:1558-68
19. **Wolchok JD, Chiarion-Sileni V, Gonzalez R, et al. Overall Survival with Combined Nivolumab and Ipilimumab in Advanced Melanoma. N Engl J Med 2017; 377: 1345-56**
20. Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma. N Engl J Med 2015;373:23-34
21. Robert C, Schachter J, Long GV, et al. Pembrolizumab versus Ipilimumab in Advanced Melanoma. N Engl J Med 2015;372:2521-32
22. **Aguiar PN Jr, De Mello RA, Hall P, Tadokoro H, Lima Lopes G. PD-L1 expression as a predictive biomarker in advanced non-small-cell lung cancer: updated survival data. Immunotherapy 2017; 9:499-506**
23. Maio M, Danielli R, Chiarion-Sileni V, et al. Efficacy and Safety of ipilimumab in patients with pre-treated, uveal melanoma. Ann Oncol 2013;24:2911-15
24. Luke JJ, Callahan MK, Postow MA, et al. Clinical activity of ipilimumab for metastatic uveal melanoma: a retrospective review of the Dana-Farber Cancer Institute, Massachusetts General Hospital, Memorial Sloan-Kettering Cancer Center, and University Hospital of Lausanne experience. Cancer 2013;119:3687-95
25. Kelderman S, van der Kooij MK, van den Eertwegh AJ, et al. Ipilimumab in pretreated metastatic uveal melanoma patients. Results of the Dutch Working group on Immunotherapy of Oncology (WIN-O). Acta Oncol 2013;52:1786-88
26. Zimmer L, Vaubel J, Morh P, et al. Phase II DeCOG-study of ipilimumab in pretreated and treatment-naïve patients with metastatic uveal melanoma. PLoS One 2015;10:e0118564

27. Del Vecchio M, Di Guardo L, Ascierto PA, et al. Efficacy and safety of ipilimumab 3mg/kg in patients with pretreated, metastatic, mucosal melanoma. Eur J Cancer 2014;50:121-7
28. Postow MA, Luke JJ, Bluth MJ, et al. Ipilimumab for patients with advanced mucosal melanoma. Oncologist 2013;18:726-32
29. Ascierto PA, Simeone E, Sileni VC, et al. Clinical experience with ipilimumab 3 mg/kg: real-work efficacy and safety data from an expanded access programme cohort. J Transl Med 2014;12:116
30. Ascierto PA, Addeo R, Carteni G, et al. The role of immunotherapy in solid tumors: report of the Campania Society of Oncology Immunotherapy (SCITO) meeting, Naples 2014. J Transl Med 2014;12:291
31. Emens LA. Breast Cancer Immunotherapy: Facts and Hopes. Clin Cancer Res. 2017 Aug 11. pii: clincanres.3001.2017. doi: 10.1158/1078-0432.CCR-16-3001
32. Robert C, Long GV, Brady B, et al. Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med 2015;372:320-30
33. Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. N Engl J Med 2015;373:1627-39
34. Brahmer J, Reckamp KL, Baas P, et al. Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer. N Engl J Med. 2015;373:123-35
35. Ribas A, Puzanov I, Dummer R, et al. Pembrolizumab versus investigator-choice chemotherapy for ipilimumabrefractory melanoma (KEYNOTE-002): a randomised, controlled, phase 2 trial. Lancet Oncol 2015;16:908-18
36. Roberts K, Culleton V, Lwin Z, O'Byrne K, Hughes BG. Immune checkpoint inhibitors: Navigating a new paradigm of treatment toxicities. Asia Pac J Clin Oncol 2017;13:277-88

37. Weber JS, Kähler KC, Hauschild A. Management of immune-related adverse events and kinetics of response with ipilimumab. J Clin Oncol 2012;30:2691-7

38. Langer CJ, Gadgeel SM, Borghaei H, et al. Carboplatin and pemetrexed with or without pembrolizumab for advanced, non-squamous non-small-cell lung cancer: a randomised, phase 2 cohort of the open-label KEYNOTE-021 study. Lancet Oncol 2016;17:1497-508

39. Corsello SM, Barnabei A, Marchetti P, et al. Endocrine side effects induced by immune checkpoint inhibitors. J Clin Endocrinol Metab 2013;98:1361-75

40. Joshi MN, Whitelaw BC, Palomar MTP, et al. Immune checkpoint inhibitor related hypophysitis and endocrine dysfunction: clinical review. Clin Endocrinol 2016;85:331-9

41. Byun DJ, Wolchok JD, Rosenberg LM, et al. Cancer immunotherapy - immune checkpoint blockade and associated endocrinopathies. Nat Rev Endocrinol 2017;13:195-207

**** This review describes the current data regarding immune-related endocrinopathies, including hypophysitis, thyroid dysfunction and diabetes mellitus**

42. Villadolid J, Amin A. Immune checkpoint inhibitors in clinical practice: update on management of immune-related toxicities. Transl Lung Cancer Res 2015; 4: 560-75

43. Min L, Vaidya A, Becker C. Thyroid autoimmunity and ophthalmopathy related to melanoma biological therapy. Eur J Endocrinol 2011; 164: 303-7

44. Borodic G, Hinkle DM, Cia Y. Drug-induced graves disease from CTLA-4 receptor suppression. Ophthal Plast Reconstr Surg 2011; 27: e87-8

45. Alhusseini M, Samantray J. Hypothyroidism in Cancer Patients on Immune Checkpoint Inhibitors with anti-PD1 Agents: Insights on Underlying Mechanisms. Exp Clin Endocrinol Diabetes 2017;125:267-9

46. Brilli L, Danielli R, Ciuoli C, et al. Prevalence of hypophysitis in a cohort of patients with metastatic melanoma and prostate cancer treated with ipilimumab. Endocrine 2017: published online 12 April 2017, doi:10.1007/s12020-017-1289-2

47. Orlov S, Salari F, Kashat L, Walfish PG. Induction of painless thyroiditis in patients receiving programmed death 1 receptor immunotherapy for metastatic malignancies. J Clin Endocrinol Metab 2015;100:1738-41

48. De Velasco G, Je Y, Bossé D, et al. Comprehensive Meta-analysis of Key Immune-Related Adverse Events from CTLA-4 and PD-1/PD-L1 Inhibitors in Cancer Patients. Cancer Immunol Res 2017;5:312-8

49. Shang YH, Zhang Y, Li JH, Li P, Zhang X. Risk of endocrine adverse events in cancer patients treated with PD-1 inhibitors: a systematic review and meta-analysis. Immunotherapy 2017;9:261-72

50. Osorio JC, Ni A, Chaft JE, et al. Antibody-mediated thyroid dysfunction during T-cell checkpoint blockade in patients with non-small-cell lung cancer. Ann Oncol 2017;28:583-9

51. Torino F, Barnabei A, Paragliola R, et al. Thyroid dysfunction as an unintended side effect of anticancer drugs. Thyroid 2013; 23: 1345-66

52. De Remigis A, de Gruijl TD, Uram JN, et al. Development of thyroglobulin antibodies after GVAX immunotherapy is associated with prolonged survival. Int J Cancer 2015;13:127-37

*** An important study about GVAX (vaccine made of a tumor cell type transfected with GM-CSF) immunotherapy, that induces the appearance of TgAbs recognizing a unique antigenic repertoire and associating with prolonged survival**

53. Champiat S, Lambotte O, Barreau E et al. Management of immune checkpoint blockade dysimmune toxicities: a collaborative position paper. Ann Oncol 2016;27:559-74

54. Merck. Pembrolizumab US full prescribing information. 2014. Available at: https://www.merck.com/product/usa/pi_circulars/k/keytruda/keytruda_pi.pdf. [Accessed April 2017]

55. Naidoo J, Page DB, Li BT et al. Toxicities of the anti-PD-1 and anti-PD-L1 immune checkpoint antibodies. Ann Oncol 2015;26:2375-91

56. Genentech. Atezolizumab US full prescribing information. 2016. Available at: http://www.gene.com/download/pdf/tecentriq_prescribing.pdf. [Accessed April 2017]

57. Ferrari SM, Fallahi P, Politti U, et al. Molecular Targeted Therapies of Aggressive Thyroid Cancer. Front Endocrinol (Lausanne) 2015;6:176

58. Fallahi P, Mazzi V, Vita R, et al. New therapies for dedifferentiated papillary thyroid cancer. Int J Mol Sci 2015;16:6153-82

*** An important review evaluating the state of art of targeted therapies in the approach of dedifferentiated papillary thyroid cancer**

59. Antonelli A, Ferrari SM, Giuggioli D, et al. Chemokine (C-X-C motif) ligand (CXCL)10 in autoimmune diseases. Autoimmun Rev 2014;13:272-80

60. Antonelli A, Ferrari SM, Frascerra S, et al. CXCL9 and CXCL11 chemokines modulation by peroxisome proliferator-activated receptor-alpha agonists secretion in Graves' and normal thyrocytes. J Clin Endocrinol Metab 2010;95:E413-20

61. Melillo RM, Castellone MD, Guarino V, et al. The RET/PTC–RAS–BRAF linear signaling cascade mediates the motile and mitogenic phenotype of thyroid cancer cells. Journal of Clinical Investigation 2005;115:1068-81

62. Puxeddu E, Knauf JA, Sartor MA, et al. RET/PTC-induced gene expression in thyroid PCCL3 cells reveals early activation of genes involved in regulation of the immune response. Endocrine-Related Cancer 2005;12:319-34

63. McCall KD, Harii N, Lewis CJ, et al. High basal levels of functional Toll-like receptor 3 (TLR3) and noncano- nical Wnt5a are expressed in papillary thyroid cancer and are coordinately decreased by phenylmethimazole together with cell proliferation and migration. Endocrinology 2007;148:4226-37

64. Antonelli A, Ferrari SM, Fallahi P, 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;16:1299-311

65. Antonelli A, Ferrari SM, Fallahi P, et al. Variable modulation by cytokines and thiazolidinediones of the prototype Th1 chemokine CXCL10 in anaplastic thyroid cancer. Cytokine 2012;59:218-22

66. Wennerberg E, Pfefferle A, Ekblad L, et al. Human anaplastic thyroid carcinoma cells are sensitive to NK cell-mediated lysis via ULBP2/5/6 and chemoattract NK cells. Clin Cancer Res 2014;20:5733-44

67. Melillo RM, Guarino V, Avilla E, et al. Mast cells have a protumorigenic role in human thyroid cancer. Oncogene 2010;29:6203-15

68. González HE, Leiva A, Tobar H, et al. Altered chemokine receptor expression in papillary thyroid cancer. Thyroid 2009;19:957-65

69. Visciano C, Prevete N, Liotti F, Marone G. Tumor-Associated Mast Cells in Thyroid Cancer. Int J Endocrinol 2015:705169

70. Rotondi M, Coperchini F, Awwad O, et al. Effect of Interferon-γ on the Basal and the TNFα-Stimulated Secretion of CXCL8 in Thyroid Cancer Cell Lines Bearing Either the RET/PTC Rearrangement Or the BRAF V600e Mutation. Mediators Inflamm 2016:8512417

71. Ferrari SM, Elia G, Piaggi S, et al. CCL2 is Modulated by Cytokines and PPAR-g in Anaplastic Thyroid Cancer. Anticancer Agents Med Chem 2017: published online 19 July 2017, doi: 10.2174/1871520617666170719152349

72. French JD, Kotnis GR, Said S, et al. Programmed death-1+ T cells and regulatory T cells are enriched in tumor-involved lymph nodes and associated with aggressive features in papillary thyroid cancer. J Clin Endocrinol Metab 2012;97:E934-43

****An important study about immune reponse in thyroid cancer, concluding that increased Treg and PD-1(+) T cell frequencies in LN may be indicative of aggressive recurrent PTC**

73. Brauner E, Gunda V, Vanden Borre P, et al. Combining BRAF inhibitor and anti PD-L1 antibody dramatically improves tumor regression and anti tumor immunity in an immunocompetent murine model of anaplastic thyroid cancer. Oncotarget 2016;7:17194-211

74. Chowdhury S, Veyhl J, Jessa F, et al. Programmed death-ligand 1 overexpression is a prognostic marker for aggressive papillary thyroid cancer and its variants. Oncotarget 2016;7:32318-28

75. French JD, Bible K, Spitzweg C, et al. Leveraging the immune system to treat advanced thyroid cancers. Lancet Diabetes Endocrinol 2017;5:469-81

76. Bai Y, Niu D, Huang X, et al. PD-L1 and PD-1 expression are correlated with distinctive clinicopathological features in papillary thyroid carcinoma. Diagn Pathol 2017; 12: 72

77. Bongiovanni M, Rebecchini C, Saglietti C, et al. Very low expression of PD-L1 in medullary thyroid carcinoma. Endocr Relat Cancer 2017; 24: L35-L38

78. Selby MJ, Engelhardt JJ, Johnston RJ, et al. Preclinical Development of Ipilimumab and Nivolumab Combination Immunotherapy: Mouse Tumor Models, In Vitro Functional Studies, and Cynomolgus Macaque Toxicology. PLoS One 2016;11:e0161779

79. Mellman I, Hubbard-Lucey VM, Tontonoz MJ, et al. De-Risking Immunotherapy: Report of a Consensus Workshop of the Cancer Immunotherapy Consortium of the Cancer Research Institute. Cancer Immunol Res 2016;4:279-88

80. Shin DS, Ribas A. The evolution of checkpoint blockade as a cancer therapy: what's here, what's next? Curr Opin Immunol 2015;33:23-35

81. Vanden Borre P, Gunda V, McFadden DG, et al. Combined BRAF(V600E)- and SRC-inhibition induces apoptosis, evokes an immune response and reduces tumor growth in an immunocompetent orthotopic mouse model of anaplastic thyroid cancer. Oncotarget 2014;5:3996-4010

82. Gunda V, Frederick DT, Bernasconi MJ, et al. A potential role for immunotherapy in thyroid cancer by enhancing NY-ESO-1 cancer antigen expression. Thyroid 2014;24:1241-50

83. Zhu L, Li XJ, Kalimuthu S, et al. Natural Killer Cell (NK-92MI)-Based Therapy for Pulmonary Metastasis of Anaplastic Thyroid Cancer in a Nude Mouse Model. Front Immunol 2017;8:816

84. Haupt K, Siegel F, Lu M, et al. Induction of a cellular and humoral immune response against preprocalcitonin by genetic i: a potential new treatment for medullary thyroid carcinoma. Endocrinology 2001;142:1017-23

85. Gerfo PL, Feind C, Weber C, et al. Immunotherapy of thyroid cancer by induction of autoimmune thyroiditis. Surgery 1983;94:959-65

86. Schott M. Immunesurveillance by dendritic cells: potential implication for immunotherapy of endocrine cancers. Endocr Relat Cancer 2006;13:779-95

87. Passaro C, Borriello F, Vastolo V, et al. The oncolytic virus dl922-947 reduces IL-8/CXCL8 and MCP-1/CCL2 expression and impairs angiogenesis and macrophage infiltration in anaplastic thyroid carcinoma. Oncotarget 2016; 7: 1500-15

88. Kollipara R, Schneider B, Radovich M, et al. Exceptional Response with Immunotherapy in a Patient with Anaplastic Thyroid Cancer. Oncologist 2017; 22:1149-51

****An important study about immune checkpoint inhibitors therapy in a 62-year-old male who was diagnosed with ATC. The patient continues to be in complete radiographic and clinical remission 20 months after the beginning of the treatment with nivolumab**

89. Fallahi P, Di Bari F, Ferrari SM, et al. Selective use of vandetanib in the treatment of thyroid cancer. Drug Des Devel Ther 2015; 9:3459-70

90. Ferrari SM, Politti U, Spisni R, et al. Sorafenib in the treatment of thyroid cancer. Expert Rev Anticancer Ther 2015;15:863-74

91. Fallahi P, Ferrari SM, Di Bari F, et al. Cabozantinib in Thyroid Cancer. Recent Pat Anticancer Drug Discov 2015;10:259-69

92. Zhang T, Xie J, Arai S, et al. The efficacy and safety of anti-PD-1/PD-L1 antibodies for treatment of advanced or refractory cancers: a meta-analysis. Oncotarget 2016; 7: 73068-79

93. Haugen BR, Alexander EK, Bible KC, et al. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid 2016;26:1-133

Table 1. Main experimental or approved immunotherapies in human cancer.

Main drugs	Cancer types	Basic mechanism	Main reported effects	Major disadvantages	Approved Immunotherapies	Reference
Immune checkpoint blockade						
anti-CTLA-4 (ipilimumab)	melanoma (ocular and mucosal melanomas)	- Activates pre-existing anticancer T cell responses, by blocking CTLA-4 function, and possibly triggering new ones	-Prolongs OS with no impact on overall response rate or PFS	-A clinical benefit has been reached by only few patients -About 35 % of patients showed severe immune-related adverse events	-For unresectable or metastatic melanoma	2, 11, 23-28
anti-PD-1 and anti-PD-L1 (nivolumab)	NSCLC, SCLC, kidney cancer, Hodgkin's lymphoma, head and neck cancer, esophageal and gastric cancers, hepatocellular cancer, bladder cancer, breast cancer	-It prevents the binding between PD-1 and its ligands PD-L1 and PD-L2	-It is possible to achieve long-term survival in some patients across a range of distinct tumor types, where the different response rates across tumors reflect different immunogenicity	-A clinical benefit has been reached by only few patients	-For the treatment of advanced melanoma, advanced NSCLC, advanced renal cell carcinoma, classical Hodgkin lymphoma, advanced squamous cell carcinoma of the head and neck, urothelial carcinoma, MSI-H or dMMR metastatic colorectal cancer, and hepatocellular carcinoma	6, 10, 12, 30
Inhibition of chemokine signaling	Many types of cancers, such as thyroid cancer; metastatic renal cell Carcinoma; metastatic melanoma		-May offer novel therapeutic approaches for the treatment of refractory forms of cancer			70, 71
IL-2		-Host's		-A low rate of	- Proleukin®	

IFN-α		immune system stimulation -Host's immune system stimulation		response and a significative risk of systemic Inflammation -A low rate of response and toxicity at high-dose	(aldesleukin) is approved for the treatment of metastatic renal cell carcinoma and metastatic melanoma - INTRON ® A (Interferon alfa-2b is approved for the treatment of hairy cell leukemia, malignant melanoma, AIDS-related Kaposi's sarcoma, follicular non-Hodgkin's lymphoma, and condyloma acuminata	
<u>Cell-based therapies</u> DCs vaccinations	endocrine malignancies (mainly in metastasized MTC)	- Immunization by antigen-loaded DCs	-Resulted in tumor-specific immunity	-Partial clinical responses in some cases	-Sipuleucel-T is the first DCs-based cancer vaccine for men with asymptomatic or minimally symptomatic metastatic castration-resistant prostate cancer	86
oncolytic virus dl922-947	ATC and melanoma	- Acts killing tumor cells and also inducing a long-lasting CD8 T cell-mediated anti-tumor response	-Reduces IL-8/CXCL8 and MCP-1/CCL2 expression and impairs angiogenesis and macrophage infiltration	-No OS improvement and no effect has been seen in the case of melanoma spread to other internal organs	-Talimogene laherparepvec (T-VEC) has been approved by the FDA for the treatment of surgically unresectable skin and lymph node lesions in patients with advanced melanoma	87

ATC, anaplastic thyroid cancer; DCs, dendritic cells; DTC, differentiated thyroid cancer; FDA, Food and Drugd Administration; MTC, medullary thyroid cancer; NSCLC, non-small-cell lung cancer; OS, overall survival; PFS, progression-free survival; SCLC, small-cell lung cancer.