Thyroid disorders induced by checkpoint inhibitors.

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Short title: Thyroid disorders and checkpoint inhibitors.

Abstract

Immune checkpoint inhibitors are drugs that inhibit the "checkpoint molecules". Different types of cancer immune checkpoint inhibitors have been approved recently: CTLA-4 monoclonal antibodies (as ipilimumab); anti-PD-1 monoclonal antibodies (as pembrolizumab and nivolumab); and anti-PD-L1 monoclonal antibodies (as atezolizumab, avelumab, and durmalumab). The increased immune response induced by these agents leads to immune-related adverse events (irAEs), that can vary from mild to fatal, according to the organ system and severity. Immune-related endocrine toxicities are thyroid dysfunctions, hypophysitis, adrenal insufficiency, and type 1 diabetes mellitus, and are usually irreversible in 50%. In particular, hypophysitis is the most frequent anti-CTLA-4-antibodies-related irAE, while thyroid abnormalities (as hypothyroidism, thyrotoxicosis, painless thyroiditis, or even "thyroid storm") are more frequently associated with anti-PD-1-antibodies. The combination of anti-CTLA-4-antibodies, with anti-PD-1-antibodies, is associated with about 30% of irAEs. Clinical signs and symptoms vary according to the influenced target organ. Endocrinopathies can often be managed by the treating oncologist. However in more severe cases (i.e. in the presence of insulin-dependent diabetes, adrenal insufficiency, or disorders of gonadal hormones, or severe hyperthyroidism, or hypothyroidism, or long-lasting management of hypophysitis) an endocrinological evaluation, and a prompt therapy, are needed.

Keywords: immune checkpoint inhibitors; CTLA-4; PD-1; PD-L1; thyroid disorders; hypophysitis.

1 Introduction

In cancer immunotherapy the immune system is manipulated to treat cancer. Different types of cancer immunotherapies have been approved recently, as precautionary and therapeutic cancer vaccines [1], immune checkpoint inhibitors (ICI) [2, 3], a bi-specific T-cell engager, and an oncolytic virus [4].

Other studies are evaluating the involvement of cytokines and chemokines, in human cancer, and their possible modulation [5-7].

The immune system recognizes and destroys foreign or tumor cells, through the antigens recognition by T-cells that involves the interaction of the major histocompatibility complex molecules, with the antigen presenting cells (APC).

Cancer therapies lead cancer cells to die and release tumor antigens, presented by dendritic cells in the tumor-draining lymph nodes in order to activate tumor immunity. In this way, tumor-specific T cells enter the circulation towards tumors, infiltrating the tumor mass. Cancer cells lysis, mediated by T-cells, leads to a higher release of tumor antigens, perpetuating the cycle [8].

Immune checkpoints regulate the immune system, and are determinant for self-tolerance, that prevents the immune system from attacking cells randomly. “Stimulatory checkpoint molecules” belong to the tumor necrosis factor (TNF) receptor superfamily (CD27, CD40, OX40, GITR and CD137), while others are members of the B7-CD28 superfamily (CD28 and ICOS); also different “inhibitory checkpoint molecules” exist, and among them CTLA-4 (Cytotoxic T-Lymphocyte-Associated protein 4, known as CD152, whose expression on Treg cells serves to control T cell proliferation) is the target of Bristol-Myers Squibb's melanoma drug ipilimumab (Yervoy), approved by Food and Drug Administration (FDA) in March 2011 [9], and PD-1 (Programmed Death 1 receptor; with the two ligands PD-L1 and PD-L2) is the target of Merck & Co.'s melanoma drug pembrolizumab (Keytruda), approved by FDA in September 2014 [10].

Immune checkpoint inhibitors are drugs that inhibit the “inhibitory checkpoint molecules”.

CTLA-4 is constitutively expressed by regulatory T cells and upregulated after T cells activation, acting as an "off" switch. CTLA-4 binds the B7 ligand on APC. Binding CTLA-4, ICI prevent it from binding with B7, and allow B7 to bind with CD28, in this way inducing the immune system to attack tumor cells [11].

PD-1 is present on T, B, and NK cells, and binds to PD-L1, expressed by tumor cells, preventing apoptosis of the cell expressing PD-L1 by the immune system. ICI, that bind either PD-L1 or PD-1, prevent this process [11].

FDA approved anti-CTLA-4 monoclonal antibodies (as ipilimumab), anti-PD-1 monoclonal antibodies (as pembrolizumab and nivolumab), and anti-PD-L1 monoclonal antibodies (as atezolizumab, avelumab, and durmalumab) [11].

Different characteristics of cancer immunotherapy with ICI have been reported [8, 12]: ICI involve T cells with innate capacity for adaptability and memory, underlying the long-lasting responses and survival reported with these therapies; ICI treat the immune system, despite either tumor histology or the absence/presence of driver mutations; the side effects associated with ICI are different from those of chemotherapy and targeted therapies; the effectiveness of immunotherapy can be ameliorated by the combination among ICI and other treatment strategies [8, 12].

2 Cancer immunotherapy with ICI targets different tumor types

Cancer immunotherapy has the immune system as target, for this reason its effectiveness is independent from tumor type, or the absence/presence of driver mutations.

For example ipilimumab has a long-lasting effect in mucosal, ocular, and cutaneous melanomas, that have different biology [13-18], and no differences were present between NRAS- and BRAF-mutated melanoma regarding the duration of response or overall survival (OS) [19]. Subgroup analyses conducted in the phase 3 trial MDX010-20 indicate ipilimumab has a survival long-lasting effect across patient populations, also in those subjects with a known poor prognosis (for example with poor performance status or raised lactate dehydrogenase) [12, 20]. Ipilimumab has also shown to have activity in old patients and those with stable asymptomatic brain metastases, supporting the hypothesis of a potential benefit with immunotherapy in wide patient populations [21, 22].

Also the effectiveness of PD-1 blockade has been reported in multiple tumor types, as Hodgkin's lymphoma, kidney cancer, esophageal and gastric cancers, small-cell lung cancer (SCLC), hepatocellular cancer, non-small-cell lung cancer (NSCLC), head and neck cancer, breast cancer, bladder cancer, and others [12]. In particular, PD-1 blockade is more efficacious in some cancer subtypes (i.e. the clinical activity is more promising in triple negative PD-L1+ breast cancer [23]). Therefore, long-term survival can be obtained in some patients across a range of distinct tumor types, in whom the different response rates denote different tumor immunogenicity.

The activity of the anti-PD1 antibody nivolumab has been investigated in different tumor types. In phase 1 trials [24, 25], median OS was 9.6 months, 16.8 months and >22 months, in patients with NSCLC, melanoma and renal cell carcinoma, respectively.

Other ICI, as other anti-PD-1/PD-L1 agents, are also under evaluation in different solid tumors and haematological malignancies [12].

3 ICI toxicities

Checkpoint inhibitors have a different safety profile from chemotherapy or targeted therapy. The treatment with the anti-PD-1 antibodies nivolumab and pembrolizumab and the anti-CTLA-4 antibody ipilimumab has improved survival in metastatic lung and renal cancer and in melanoma [26]. The raised immune response induced by these therapies has led to a specific group of side effects known as immune-related adverse events (irAEs), resulting from immune activation after the CTLA-4 and/or PD-1 inhibition [26], and can vary from mild to fatal, according to the organ system and severity [27].

As ICI induce immune cells to attack normal tissues, every organ can be damaged, as skin, lungs, heart, muscles, bowels, liver, endocrine tissues, eyes, kidneys, and central nervous system [11, **28**, **29**]. According to clinical trial results about 7–19% of patients treated with anti-PD-1/PD-L1 antibodies developed grade 3–5 adverse events. The discontinuation rate of ICI caused by adverse events ranged 3-8% for anti-PD-1/PD-L1 antibodies, and up to 15% for ipilimumab. The discontinuation rate due to adverse effects observed combining nivolumab and ipilimumab, is significantly higher (36% of rate) [8]. Of note, delayed toxicities can be observed months or even years after the last received dose of immune checkpoint inhibitor [11].

The most frequent ipilimumab-induced side effects involve gastrointestinal tract (colitis and diarrhoea), skin (pruritus and cutaneous rash), liver (autoimmune hepatitis) and endocrine system (hypophysitis and thyroid dysfunction). Immune-related arthritis, neuropathy, myositis, and uveitis happen occasionally [1].

A systematic review and meta-analysis [**30**] was conducted on 81 articles, with a total of 1265 patients from 22 clinical trials to evaluate the incidence and nature of irAEs in oncologic patients administered with anti-CTLA-4 antibodies (ipilimumab and tremelimumab). The reported irAEs were skin lesions (rash, pruritus, and vitiligo); colitis; hepatitis,

hypophysitis, thyroiditis (less frequently); sarcoidosis, uveitis, Guillain-Barré syndrome, immune-mediated cytopenia and polymyalgia rheumatic/Horton (rarely). The overall incidence of all-grade irAEs was 72% (95% CI, 65–79%). The overall incidence of high-grade irAEs was 24% (95% CI, 18–30%). The risk of irAEs depended on the dosage, with incidence of all-grade irAEs being evaluated to 61% (95% CI, 56–66%) for ipilimumab 3 mg/kg and 79% (95% CI, 69–89%) for ipilimumab 10 mg/kg. About 0.86% of patients died owing to irAEs [**30**].

The safety profile of PD-1 blockade is similar and also includes pneumonitis of grade 1-2 [1]. Combination therapy did not cause new toxicities or deaths [1].

4 Endocrine irAEs

Immune-related endocrine toxicities are thyroid dysfunction, hypophysitis, adrenal insufficiency and type 1 diabetes mellitus (T1DM). In comparison to other irAEs, endocrinopathies are often irreversible manifestations [**31**], and papers show recovery of the pituitary–thyroid axis in up to 50% of patients [**32**] and in 50–60% for the pituitary–gonadal axis [**32-34**], while few cases of corticotroph recovery have been described [**33**].

Endocrine glands release hormones affecting cell function in a different location. The pituitary (or hypophysis), adrenal glands and thyroid are the endocrine organs that are usually affected by ICI. As the activity of the endocrine system is mediated by feedback loops among organ systems, the inflammation of a single gland causes the dysfunction of the affected organ and of downstream target organs [**35**]. Even if different systems can be affected simultaneously, a patient can have only thyroid or adrenal symptoms. Moreover, immune checkpoint blockade, and overall blockade of the PD-1/PD-L1 pathway alone or combined with anti-CTLA-4, has also been associated with the onset of autoimmune T1DM [**35**].

About the onset time of irAEs, it is usually from 7 weeks with ipilimumab and 10 weeks with nivolumab [**36**].

Endocrine irAEs have been reported in 0–29% of patients [**37**]. Ipilimumab-induced hypophysitis is the most frequent irAE grade 3/4; hypothyroidism and hyperthyroidism are next in frequency. Little is known for tremelimumab, that is associated with fewer reported endocrinopathies (0–8.3%) [**37**]. The incidence of endocrinopathies after the therapy with PD-1/PD-L1 inhibitors is different from that with anti-CTLA-4 antibodies, as they have different mechanism of action. The induction of CTLA-4 in T cells occurs in the early stages of their response to antigens, while the PD-1/PD-L1 pathway regulates inflammatory reaction both in peripheral tissues and neoplastic microenvironment, and it is activated downstream of the immune response [**37**].

The incidence of thyroid disorders as hypothyroidism, thyrotoxicosis, painless thyroiditis, or even ‘thyroid storm’ [**33**] is approximately 10% in patients administered with single agent anti-PD-1/PD-L1 [8, **38, 39**], but also euthyroid Graves’ ophthalmopathy and other less common endocrine disorders have been described [**40, 41**].

4.1 Hypophysitis

Hypophysitis related to anti-CTLA-4 therapy varies between 0.4-17% [**32**], and this variation could be due to differences in hormonal monitoring, and drug dose.

Combination of nivolumab with ipilimumab resulted in hypophysitis in about 8% of cases [26].

Anti-CTLA-4-related hypophysitis seems to be more common in men than in women [**34**], as for example one paper reported a prevalence of anti-CTLA-4-related hypophysitis of 3.6% in women and 15.6% in men [P=0.02; OR 4.73 (95% CI 1.27–30.79)] [**42**], but these data are in contrast with idiopathic autoimmune hypophysitis, that has a men to women ratio of

1:3 [**34**]. This phenomenon is supposed to be caused by the increased melanoma prevalence in men in these trials [**43**].
Regarding age, a retrospective review showed a significantly older population (mean age of 68.2±2.4 years with respect to 59.9±1.0; P=0.005) with ipilimumab-related hypophysitis [**42**]. The review was conducted in 154 adult patients with metastatic melanoma and treated with ipilimumab between March 2008 and December 2013, and evaluated the prevalence of hypophysitis, the clinical course and treatment outcomes in hypophysitis, and identified the risk factors for the development of hypophysitis to define optimal strategies for its management [**42**]. The Authors concluded that male gender and older age are risk factors for hypophysitis [**42**].
Fatigue and/or muscle weakness and headache are present in 89% of patients diagnosed with hypophysitis, even if these symptoms are nonspecific [**43**]. Anorexia, nausea, weight loss, alterations in mental status, visual changes, temperature intolerance and arthralgias are described with a frequence of 10.5–21.1% [**34**]. Low levels of sodium (range 113-134 mEq/l) have been shown, too, in patients with anti-CTLA-4-related hypophysitis [**34**]. The morbidity linked to anti-CTLA-4-related hypophysitis seems to be associated with secondary adrenal insufficiency [27], that could be life threatening if not treated.
Adrenocorticotropic hormone and/or thyroid stimulating hormone (TSH) deficiency are the most frequent pituitary hormone abnormalities in anti-CTLA-4-related hypophysitis patients, also low insulin-like growth factor 1 levels are present [**34**].

4.2 Hyperglycaemia

PD-1 pathway blockade can cause diabetes mellitus [**44-47**]. Anti-PD-1 therapy led to 8 cases of T1DM with another case in a patient administered with anti-PD-L1 therapy. Seven/9 T1DM patients had diabetic ketoacidosis and 2 patients had severe hyperglycemia [**44-47**]. GAD65 antibodies (T1DM marker) and diabetic ketoacidosis were present in 5 patients treated with nivolumab [**44-47**]. Three/5 patients had T1DM-specific autoantibodies (GAD65), and 40% the upregulation of CD8+ T-cell response to a T1DM antigen [**45**].

4.3 Adrenalitis

Even if rarely, adrenalitis and following primary adrenal insufficiency caused by the therapy with ipilimumab have been shown [**48, 49**]. A 56-year-old woman with metastatic melanoma showed fatigue and headache after 4 doses of ipilimumab. Hypophysitis and secondary adrenal insufficiency were diagnosed for the presence of low morning cortisol and corticotropin levels and of pituitary enlargement. She started hydrocortisone. Bilateral enlargement of adrenal glands was shown by surveillance-computed tomography (CT) scan of the abdomen, while before the treatment with ipilimumab, her adrenal glands were normal in size. She did not respond to cosyntropin stimulation demonstrating primary adrenal insufficiency. Her adrenal glands normalized in size after 6 weeks, and this change in size of the adrenal glands indicates an ipilimumab-related autoimmune adrenalitis [**48**].
In a 79-year-old imaging data of newly symmetrically and smoothly enlarged, hypermetabolic adrenal glands have been shown after ipilimumab therapy, indicating drug-induced adrenalitis and not metastatic disease [**49**].

4.4 Thyroid disorders

Thyroid abnormalities (as hypothyroidism, thyrotoxicosis, painless thyroiditis, or even “thyroid storm” [**33**]) are the second most common irAEs associated with anti-CTLA-4-antibodies [**32**] and are present in 1–6% of patients administered with ICI [**33, 34**].

In primary hypothyroidism, high TSH with low-to-normal free thyroxine (FT4) or triiodothyronine (T3) are present, while in hypothyroidism caused by pituitary dysfunction low to mid-normal levels of TSH with a low FT4 have been shown [**34**]. However, the measurement of T3 in patients with any acute illnesses could be not precise, and the best evaluation of primary thyroid dysfunction is TSH measurement [**50**].

In clinical trials conducted in patients administered with ipilimumab, the incidence of secondary hypothyroidism was 7.6% (4.3–11.0%), and primary hypothyroidism was shown in 5.6% of patients (5.2–5.9%) [**34, 42, 51-61**], even if data about the diagnostic test values, aetiology or management of hypothyroidism are not reported in most of these studies.

A retrospective analysis of endocrine irAEs was conducted in 156 patients with melanoma treated with ipilimumab in clinical trials [**43**]. Pituitary-, thyroid-, and adrenal-related hormone tests, radiographic data and clinical history of patients were evaluated in order to identify cases of thyroiditis, adrenal dysfunction, hypophysitis, and hypothyroidism. The total incidence of hypophysitis was 8% and of hypothyroidism/thyroiditis 6%, while primary adrenal dysfunction was not frequent. The combined treatment with ipilimumab and nivolumab showed an incidence of 22% for either thyroiditis or hypothyroidism and 9% for hypophysitis [**43**].

Euthyroid Graves' ophthalmopathy and other less frequent endocrine disorders were also observed [**40, 41**]. A study reported 3 patients with metastatic melanoma administered with ipilimumab alone or combined with bevacizumab, who developed thyroiditis, and euthyroid Graves' ophthalmopathy [**40**].

Thyroid disorders are the most frequent endocrine irAEs caused by tremelimumab (0%–5.2%), following a pattern similar to ipilimumab [**62**]. Graves' hyperthyroidism was induced by tremelimumab in a 55-year-old man with metastatic melanoma after 8 years of treatment. The patient had no personal or family history of thyroid or autoimmune diseases. He was treated with carbimazole as block and replace therapy, with no complications, and the treatment with tremelimumab was temporarily discontinued and started again when he was euthyroid. Graves' hyperthyroidism was not reported again since the discontinuation of block and replace therapy [**63**].

About 39.0-54.2% of patients treated with PD-1 antibodies show an immune related adverse event, too [**34**]. The most common endocrine adverse event with PD-1 antibody therapy was hypothyroidism (almost 5.9% of incidence), while hyperthyroidism in 1.0–4.7% of patients [**34**].

Ten patients showed painless thyroiditis syndrome after the therapy with anti-PD-1 monoclonal antibodies for metastatic malignancies [**64**]. Six patients had a transient thyrotoxicosis (with absence of thyrotropin binding inhibitory immunoglobulins), while 4 patients showed the presence of positive anti-thyroid antibodies. All thyrotoxic patients were administered with temporary beta-blockers and had spontaneous resolution of thyrotoxicosis and subsequently developed hypothyroidism. Four patients showed hypothyroidism without a preceding thyrotoxicosis, after 6-8 weeks from the beginning of the treatment. All the patients had positive anti-thyroid antibodies and were treated with a thyroid hormone replacement therapy for at least 6 months [**64**].

Another report showed a serological worsening of autoimmune thyroid disease in 2 patients treated with nivolumab, 1 with Hashimoto's thyroiditis, and 1 with a probable subclinical Hashimoto's thyroiditis [**65**].

Another case of nivolumab-induced painless thyroiditis was shown in a patient with no history of any thyroid disorders [**66**]. A 61-year-old man with no history of familial or personal thyroid disease with NSCLC was treated with radiotherapy and chemotherapy, and then, after the progression of cancer, with nivolumab [**67**]. After 3 infusions, the patient showed bilateral eyelid ptosis and bilateral conjunctival redness with chemosis. Ophthalmologic examination showed severe proptosis with

complete ophthalmoplegia, with normal thyroid function tests and without anti-thyroperoxidase or anti-TSH receptor antibodies. A strong bilateral proptosis with the expansion of the orbital adipose tissue, but no thickening of extraocular muscles, was shown by CT scan of the orbits. T2-weighted MRI showed inflammation of orbital adipose tissue. Nivolumab was withdrawn, and the patient treated with methylprednisolone (intravenously and once a week). After 3 cycles, a significant amelioration of left chemosis was observed. The patient died owing to massive hemoptysis, being euthyroid without thyroid autoimmunity 1 week prior to his death [**67**].

The case of a patient with metastatic squamous cell lung cancer who developed a symptomatic inflammatory myositis (confirmed with muscle biopsy and primary hypothyroidism), after nivolumab treatment, has been described. After initiation of corticosteroids and thyroid hormone replacement, there was an improvement of clinical and laboratory data [**68**].

The combined treatment with nivolumab and ipilimumab caused the highest incidences of hypothyroidism (17%) and hyperthyroidism (10%) of any grade [**38, 69**].

Fifty-one patients with advanced NSCLC were treated with pembrolizumab as part of KEYNOTE-001 (NCT01295827) [**70**]. Thyroid function and anti-thyroid antibodies levels were evaluated prospectively at each study visit, starting before the first treatment. At baseline 3/51 patients were hypothyroid and 48/51 were not. Ten/48 [21%, 95% confidence interval (CI) 10% to 35%] patients developed thyroid dysfunction and required thyroid replacement therapy. Eight/10 patients had anti-thyroid antibodies and developed thyroid dysfunction, in comparison to 3/38 who did not (80% vs 8%, P<0.0001). During the treatment with pembrolizumab, thyroid dysfunction occurred early (median, 42 days), and 6/10 patients experienced a transient hyperthyroidism (before hypothyroidism) that was not persistent. Hyperthyroidism and hypothyroidism were both asymptomatic. Interestingly, OS with pembrolizumab was significantly higher in subjects who developed thyroid dysfunction (hazard ratio, 0.29; 95% CI 0.09-0.94; P=0.04) [**70**].

Accordingly another paper showed the appearance of thyroglobulin antibodies (TgAbs) and was associated with prolonged survival [**71**]. The development of TgAbs was evaluated in patients treated with GVAX [a cancer vaccine constituted by whole tumor cells genetically modified to secrete the immune stimulatory cytokine, granulocyte-macrophage colony-stimulating factor (GM-CSF), and then irradiated to prevent further cell division] and/or ipilimumab and correlated TgAbs seroconversion with survival. TgAbs were evaluated in patients with pancreatic (n=53), prostate (n=35) or colon (n=8) cancer, before and after the treatment with GVAX alone (n=34), or combined with ipilimumab (n=42) or ipilimumab alone (n=20). TgAbs developed after GVAX, independently from the type of cancer (75% colon cancer, 76% pancreatic cancer and 81% in prostate) and co-administration of ipilimumab (75% in GVAX alone, and 78% if combined with ipilimumab). Of note, TgAbs seroconversion was associated with significantly prolonged survival (P=0.01 for pancreas and P=0.005 for prostate cancer) [**71**].

A prospective study [**72**] was conducted between April 2014 and October 2015, in 177 patients with metastatic melanoma administered with: 1. ipilimumab (n=15); 2. anti-PD-1 (nivolumab, pembrolizumab) (n=103); 3. a combination of ipilimumab and anti-PD-1 (n=59). The presence of endocrinopathies was then evaluated in these patients. Thirty-one patients (18%) developed an endocrine irAE (14%, thyroid dysfunction; 6%, hypophysitis; 0.6%, autoimmune diabetes). Combination immunotherapy more frequently resulted in a single or multiple endocrinopathy in comparison to anti-PD-1 monotherapy (27% vs 9% and 7% vs 0% respectively, P<0.01). Endocrinopathies occurred after a median of 8 weeks from

the beginning of treatment, with a significantly earlier onset in comparison to the treatment with ipilimumab alone (median: 30 vs 76 days, P=0.046) [**72**].

5 Management of thyroid disorders

The Society for Immunotherapy of Cancer (SITC) has recently established a multidisciplinary Toxicity Management Working Group, to develop consensus guidelines to evaluate and manage toxicities associated with ICI [**73**]. Then, the American Society of Clinical Oncology (ASCO) and the National Comprehensive Cancer Network (NCCN) published clinical practice guidelines on the management of ICI toxicities [**74, 75**].

Clinical signs and symptoms vary according to the influenced target organ. Thyroid disorders are usually asymptomatic and are monitored by routine laboratory tests, even if sore throat, other symptoms of hyperthyroidism, tachycardia and palpitations, can be present in the case of thyroiditis [**35**].

Endocrinopathies can often be managed by the treating oncologist. In more severe cases (i.e. in the presence of insulin-dependent diabetes, adrenal insufficiency, or disorders of gonadal hormones, or for long-lasting management of hypophysitis) an endocrinologist should be considered [**35**].

The treatment algorithms are useful to manage ICI-related endocrinopathies, but also the clinical experience of physicians are determinant. In the case of asymptomatic TSH abnormalities, if TSH is <0.5 x lower limit of normal (LLN) or >2 x upper limit of normal (ULN), FT4 levels should be evaluated. According to the clinical presentation, it could be necessary to assess the cortisol level besides FT4 and T3. Thyroid hormone replacement therapy is started when FT4 levels fall beneath normal range and can be changed as required, usually starting with 50 mcg of levothyroxine, excluding a possible contemporaneous adrenal insufficiency by a cortisol level dosage [**35**].

During treatment with ICI, thyroid function should be evaluated at baseline and next every 2 months [8, **76**]. ICI can be carried on for grade 1–2 hyperthyroidism, and the therapy is started in the case of hyperthyroidism symptoms [**77, 78**]. If hyperthyroidism is symptomatic (grade 3), ICI should be stopped and corticosteroids begun (oral prednisolone 1–2 mg/kg/day) [**77**]. From necessity, anti-thyroid drugs (propilthyouracil, methimazole, or carbimazole) may be recommended. In case of severe hyperthyroidism (grade 4), ICI should be stopped at all, and methylprednisone (1–2 mg/kg/day; IV; for 3 days) and then oral prednisolone (1–2 mg/kg/day) should be administered. Prednisolone is then decreased over at least 1 month [**77**]. Beta-blockers can be used to treat hyperthyroidism symptoms, as tachycardia and tremor. Steroids should be administered if the patient is very symptomatic [26].

To evaluate the pathogenesis of hyperthyroidism, thyroid ultrasound and vascularization should be evaluated, in fact, hypervascularity is associated with Graves' disease, while hypovascularity with destructive thyroiditis [8].

In the course of treatment thyroid function should be regularly monitored to permit the early detection of hypothyroidism, and in the case the patient is already symptomatic, the clinical state should be evaluated to decide whether ICI should be stopped [**33, 77, 79**].

6 Conclusion

The immune system recognizes and destroys tumor cells, through the antigens recognition by T-cells. Cancer therapies lead cancer cells to die and release tumor antigens, presented by dendritic cells in the tumor-draining lymph nodes in order to activate tumor immunity. In this way, tumor-specific T cells enter the circulation towards tumors, infiltrating the tumor

mass. Cancer cells lysis, mediated by T-cells, leads to a higher release of tumor antigens, perpetuating the cycle [8]. However cancers inhibit “immune checkpoints” during tumor progression, suppressing the immune response.

ICI are drugs that inhibit the “inhibitory checkpoint molecules”. Different types of cancer immunotherapies have been approved recently: CTLA-4 monoclonal antibodies (as ipilimumab); anti-PD-1 monoclonal antibodies (as pembrolizumab and nivolumab); and anti-PD-L1 monoclonal antibodies (as atezolizumab, avelumab, and durmalumab).

The increased immune response induced by these agents leads to irAEs, that can vary from mild to fatal, according to the organ system and severity [27]. Immune-related endocrine toxicities by ICI include thyroid dysfunction, hypophysitis, adrenal insufficiency and T1DM, and are usually irreversible, with studies showing recovery of the pituitary–thyroid axis in up to 50% of patients, and in 50–60% for the pituitary–gonadal axis. For this reason, immune-related endocrine toxicities need to be immediately recognized, and appropriately treated [8].

Of note, the appearance of anti-thyroid autoantibodies, thyroiditis, or thyroid dysfunction, in patients treated with ICI has been associated with a prolonged survival [**71, 80**], leading to hypothesize that these could be markers of a more potent immune activation. This could be investigated also in other cancer patients (not only with thyroid cancer).

The induction of an autoimmune reaction could be assessed also in other types of cancer showing specific antigens, or overexpressing an antigen (i.e. calcitonin, or precursor protein preprocalcitonin in medullary thyroid cancer, or carcinoembryonic antigen in colon cancer, etc.), in association with ICI therapy.

Consensus guidelines to evaluate and manage irAEs associated with ICI have been recently published [**73-75**].

Clinical signs and symptoms vary according to the influenced target organ. Endocrinopathies can often be managed by the treating oncologist. However in more severe cases (i.e. in the presence of insulin-dependent diabetes, adrenal insufficiency, or disorders of gonadal hormones, or severe hyperthyroidism, or hypothyroidism, or for long-lasting management of hypophysitis) an endocrinological evaluation, and a prompt therapy, are needed [**35**].

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