

New insight in endocrine-related adverse events associated to immune checkpoint blockade

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Abstract

Anticancer immunotherapy, in the form of immune checkpoint inhibition (ICI), is a paradigm shift that has transformed the care of patients with different types of solid and hematologic cancers. The most notable improvements have been seen in patients with melanoma, non-small-cell lung, bladder, renal, cervical, urothelial, and colorectal cancers, Merkel cell carcinoma, and Hodgkin lymphoma. Monoclonal antibodies (mAbs) targeting immune checkpoints (i.e., anti-CTLA: ipilimumab; anti-PD-1: nivolumab, pembrolizumab; anti-PD-L1: durvalumab, atezolizumab, avelumab) unleash the immune system against tumor cells targeting mainly T cells. Treatment with ICIs is associated with a variety of diverse and distinct immune-related adverse events (irAEs), reflecting the mechanistic underpinning of each target (i.e., CTLA-4, and PD-1/PD-L1 network). The most frequent endocrine irAEs associated with anti-PD-1 mAb treatment are thyroid dysfunctions, whereas hypophysitis is mostly linked to anti-CTLA-4 treatment. Type 1 diabetes mellitus and adrenalitis are rare irAEs. Combination therapy (anti-CTLA-4 plus anti-PD-1/PD-L1) can be associated with an increased risk and prevalence of endocrine irAEs. In this paper we discuss the pathophysiological and clinical aspects of irAEs with specific emphasis on endocrine irAEs associated with ICIs. With a growing number of patients treated with ICIs, a tight collaboration among oncologists, endocrinologists and immunologists appears necessary when the circumstances are more challenging and for better management of severe endocrine irAEs. Further investigations are urgently needed to better understand the mechanisms by which different ICIs can induce a variety of endocrine irAEs.

Keywords: CTLA-4; diabetes; endocrine disorders; immune checkpoint inhibitors; cancer immunotherapy; PD-1; PD-L1; thyroid; tumor microenvironment.

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1. Introduction

Seeking and destroying the non-self/cancer cells expressing neoantigens are major roles of cells of the immune system. Tumor cells can produce several neoantigens which are captured by antigen presenting cells (APCs) and presented to T cells through the complex antigen class II major histocompatibility complex (MHC)-T cell receptor (TCR) (1). However, recognition of antigen-MHC complexes by TCR is not sufficient for activation of naïve T cells. Additional costimulatory signals are required and are provided by the engagement of CD28 on T cell surface with B71 (CD80) and B72 (CD86) molecules on professional APC (2,3). By the mid-1990s, it became clear that in addition to initiating proliferation, T cell activation also induced an inhibitory pathway that could eventually attenuate and terminate the response. Cytotoxic T-lymphocyte antigen 4 (CTLA-4), which is normally located in cytoplasm of both CD4⁺ and CD8⁺ T cells, is induced on the cell surface and binds to both CD80 and CD86 with higher affinity and avidity than CD28 (4,5). The fact that CTLA-4 operates at the cell surface suggested a strategy for boosting T cell immunity by using a CTLA-4 inhibitory antibody. Allison and collaborators first demonstrated that administering CTLA-4 blocking antibodies in mice prevented tumor establishment and induced the rejection of preestablished tumors (6). They also demonstrated that rejection depended on immunogenicity of the tumor (7). The preclinical success led to the development and testing of a fully humanized monoclonal antibody (mAb) against CTLA-4 (ipilimumab) in humans with advanced melanoma (8,9). Tremelimumab, another anti-CTLA-4 monoclonal antibody (mAb), is under evaluation in a trial in combination with durvalumab (anti-PD-L1) for the treatment of metastatic non-small cell lung cancer (NSCLC) (10).

A second inhibitory receptor expressed mainly on activated CD8⁺ T cells, programmed cell death-1 (PD-1), also involved in limiting their activation, was discovered by Honjo and coworkers (11). PD-1 is triggered by PD ligand 1 (PD-L1) and PD-L2 that constitutively reside on tumor and on certain immune cells and can be up-regulated by IFN- γ and TFN- α (12-14). PD-L1 can suppress

the function of effector T cells by binding to PD-1, leading to the adoption of an exhausted T cell phenotype (14). Signaling *via* PD-1/PD-L1 can also convert T helper cells into Treg cells (15). Interestingly, PD-L1 can also activate pro-survival signaling pathways in cancer cells upon PD-1 binding, rendering cancer cells resistant to T cell-mediated cytotoxicity (16). mAbs targeting PD-1 (nivolumab, pembrolizumab, cemiplimab) (17-20) and PD-L1 (atezolizumab, durvalumab, avelumab) (21-29) have also shown clinical response in multiple solid and hematologic tumors.

There is compelling evidence that distinct cellular mechanisms underlie the mechanisms of action of mAbs anti-CTLA-4 and anti-PD-1 (30) (Table 1). The primary site of action of CTLA-4 and its ligand is in draining lymph nodes where naïve T cells, primed by exposure to tumor antigens presented by APCs, become activated. In contrast to CTLA-4, the primary site of activation of PD-1 and its ligands is in peripheral tissues, including the tumor microenvironment (TME). Over the past several years, immune checkpoint inhibitors (ICIs), which target inhibitory receptors (i.e., CTLA-4 and PD-1/PD-L1 pathways) on T cells and reinvigorate anti-tumor immune responses have revolutionized clinical cancer care (31). Any cancer with high load of genetic defects can respond this type of immunotherapy (32,33).

Figure 1 schematically illustrates the mechanisms of action of anti-CTLA-4 and anti-PD-1/PD-L1 pathway checkpoint blockade.

The remarkable successes of immunotherapy of cancer with mAbs targeting CTLA-4 and PD-1/PD-L1 network stimulated great interest in a new generation of ICIs. Emerging targets of immune checkpoint blockade for cancer immunotherapy include lymphocyte activation gene-3 (LAG3) (34), T cell immunoglobulin mucin domain 3 (TIM-3) (35), B and T lymphocyte attenuator (BTLA) (36), V-domain immunoglobulin suppressor of T cell activation (37) and T cell immunoglobulin and ITIM domain (TIGIT) (38). Recent preclinical studies have shown that the above checkpoints represent emerging targets for novel immunotherapies of cancers.

Upregulation of immune checkpoint molecules, such as CTLA-4 and PD-1, can also occur during infections including HIV and hepatitis B virus (HBV) (39). While still in early stages, basic and clinical data suggest that blockade of CTLA-4 and PD-1 may be beneficial in the treatment of HIV, HBV, and HCV infections (40,41).

Activated immune cells in TME secrete several cytokines and chemokines which foster the proliferation of tumor cells (42-45). The role of cytokines/chemokines and their possible modulation in TME by ICIs is largely unknown (33,45-47).

Clinical trials with nivolumab showed a complete or partial responses in a percentage of patients with melanoma (17,19), NSCLC (18), and in urothelial cancer (48). It received the U.S. FDA approval for metastatic melanoma treatment in 2014 (49), and it is actually used also in several solid (NSCLC, renal cell and hepatocellular carcinoma, colorectal cancer, cervical cancer) and hematologic cancer (Hodgkin lymphoma) (50) (Table 2).

Pembrolizumab, was first approved in 2014 by the U.S. FDA for the treatment of metastatic melanoma and subsequently for the treatment of different solid (NSCLS, SCLC, urothelial carcinoma, Merkel cell carcinoma, gastric and cervical cancers, hepatocellular carcinoma) and hematologic (Hodgkin lymphoma) tumors (50,51). Cemiplimab is a human mAb anti-PD-1 which has been approved in 2018 by the U.S. FDA for the treatment of patients with cutaneous squamous cell carcinoma (CSCC) based on the results of two studies (52) (Table 2). In 2018, another anti-PD-1 antibody, toripalimab, obtained conditional approval in China for the treatment of melanoma not responding to systemic therapy (53). Other anti-PD-1 drugs are under investigation, such as pidilizumab (CT-011, Cure Tech) and BMS-936559 (Bristol Myers Squibb) (54).

PD-L1, expressed on several immune cells, appears to play a crucial role in the suppression of the immune system. Atezolizumab has been approved in 2016 by the U.S. FDA for the treatment of urothelial carcinoma (23), and clinical trials showed efficacy in NSCLC (28), small-cell lung cancer (SCLC) (27), and triple-negative breast cancer (TNBC) (29). Durvalumab has been approved by the U.S. FDA in 2017 for urothelial carcinoma (22) and NSCLC (24). Avelumab has been approved by

the U.S. FDA in 2017 for the treatment of urothelial carcinoma (25), Merkel cell carcinoma (21) and renal cell carcinoma. Table 2 summarizes the ICIs and the indications approved by the U.S. FDA.

2. Combined Strategies

There is compelling evidence that CTLA-4 and PD-1 can produce non redundant effects on effector functions T cells (55). These findings prompted to examine the effects of targeting these different pathways in combination. In a murine model of melanoma, Allison and coworkers demonstrated that the combination of CTLA-4 and PD-1 blockade significantly increased tumor rejection compared to either molecule alone (56). Importantly, there is now evidence that the combination of nivolumab and ipilimumab in patients with melanoma can result in remarkable synergistic effects (57).

Building upon the remarkable activity seen in patients with melanoma, several studies are exploring various combinations of different ICIs (57,58). Other combined strategies include ICIs plus chemotherapies (59), ICIs plus radiotherapy (60), ICIs and antiangiogenic agents (61), and ICIs plus oncolytic viruses (62) in different types of solid and hematologic tumors.

It is important to note that although the resulting clinical activity/efficacy of combined cancer immunotherapies is unprecedented, the occurrence and the severity of immune-related adverse events (irAEs) also tend to increase.

3. Toxicities associated with ICIs

As a result of the pivotal roles played by immune checkpoints (ICs) in the maintenance of self-tolerance, their therapeutic blockade with ICIs can cause irAEs (63-66).

The development of irAEs in patients treated with ICIs has biological plausibility (67). In fact, experimental deletion of CTLA-4 and PD-1 axes can induce several autoimmune disorders (68). In addition, genetic deletion of PD-L1, as well as treatment with anti-PD-L1, can transform transient myocarditis into lethal disease (69). These experimental findings indicate that ICs play a central role in the prevention of autoimmunity.

It is well known that traditional and even targeted chemotherapies can be associated with a wide spectrum of adverse events (70-72). However, the irAEs associated with ICIs are clinically and pathophysiologically different from those caused by chemotherapies. The precise mechanisms by which different ICIs (CTLA-4 *versus* PD-1/PD-L1 inhibitors) can cause a variety of irAEs is not completely understood. There is recent evidence that the activation of the immune system caused by ICIs can stimulate the production of autoantibodies against autoantigens (73).

Inhibitors of the PD-1 axis only activate a restricted subset of T cells in the TME and in the circulation. Accordingly, the irAEs associated with these agents tend to be more limited in severity and incidence but have an earlier onset than the adverse effects of CTLA-4 inhibition (74). However, several fundamental questions still remain unanswered and there is the possibility that irAE associated with ICIs manifest in patients with subclinical autoimmune alterations. Moreover, it is unclear whether the immune cells mainly responsible for irAEs are the same as those involved in the potentiating of anti-tumor immune response. Finally, there is the possibility that different pathophysiological mechanisms underly various types of irAEs.

irAEs associated with ICIs emerged from initial clinical trials with ipilimumab (75,76). It was found that the risk of irAEs augmented with increasing of the dose (77). A wide spectrum of dermatological (75), gastrointestinal (78), pulmonary (79), endocrinological (33,80,81), cardiological (63,64), neurological (82), etc. of adverse events have been described. Myocarditis in patients treated with ICIs is associated with high mortality (63,64,83). In the majority of patients, irAEs can be successfully managed by the administration of glucocorticoids (84-86).

4. Endocrine irAEs

A significant percentage of cancer patients treated with ICIs manifest endocrine irAEs (84-86). Hypophysitis and thyroid dysfunctions are the most common endocrine irAEs while cases of T1DM and adrenal insufficiency are rare (81,86-88). In up to 50-60 % of patients a recovery of the pituitary-thyroid and pituitary-gonadal axis can occur (80,89,90). By contrast, improvement of the pituitary-adrenal axis was observed only in few cases (90). The Society for Immunotherapy of Cancer (SITC) (85), the National Comprehensive Cancer Network (NCCN) and the American Society of Clinical Oncology (ASCO) recently issued guidelines for the evaluation and management of ICI toxicities (86).

4.1 Hypophysitis

Hypophysitis is a very rare inflammatory disorder of the pituitary gland, characterized by an infiltration of immune cells, mainly lymphocytes and plasma cells, which leads to expansion and subsequent atrophy of the pituitary (91,92). A recent study showed that T cells infiltrate the pituitary gland in patient affected by autoimmune hypophysitis (93). Primary hypophysitis is characterized by inflammatory infiltrate limited to pituitary tissue, whose autoimmune pathogenesis is still unclear, whereas secondary hypophysitis, derives from a local or systemic disease or iatrogenic etiology (90,94). Hypophysitis inflammation resulting from autoimmune reaction generally determines structural changes and the swelling of the gland whose consequence is headache, one of the first symptoms, visual impairment and endocrine dysfunctions (95,96).

Initial studies reported the occurrence of hypophysitis in patients treated with ipilimumab. The incidence of hypophysitis varies on the basis of the dose of ipilimumab and on the use of adjuvant therapy (89), ranging from 1.8-3.3% in those who received low doses (< 3 mg/kg) (97) to 4.9–17% for doses > 3 mg/kg (98). High rates of severe hypophysitis (grade 3 or 4 toxicity)

occurred with the combined treatment of ipilimumab plus a prostate cancer cell vaccine (99) or bevacizumab (100).

The prevalence, the course and the outcomes of hypophysitis were analyzed in 154 melanoma patients treated with ipilimumab (101). The prevalence of hypophysitis was significantly higher in old male patients and the authors concluded that both old age and male gender could be considered as risk factors (101). The incidence of hypophysitis associated with ipilimumab is higher in men, whereas idiopathic hypophysitis is more common in women (89,102,103). A systematic review and meta-analysis found a higher risk of hypophysitis (OR 5.30, 95% CI 1.71-16.46) in patients treated with ipilimumab compared to control therapies (104). A recent study highlighted the relationship between hypophysitis and ICIs by reporting 191 cases related to ipilimumab, 22 cases to nivolumab, 11 to pembrolizumab and 21 related to the combination of nivolumab plus ipilimumab (94,105).

Tan and coworkers reviewed 451 cases of ICI-induced endocrinopathies and found 222 cases of hypophysitis/anterior hypopituitarism (88). The majority (65%) were male and in 200 cases was used ipilimumab. The median onset of clinical presentation was 12 weeks (range 3-76 weeks) after initiation of ICI. Headache was the prevalent symptom and pituitary magnetic resonance imaging (MRI/computerized tomography scan) showed enlarged/enhanced pituitary (88). High dose glucocorticoid was used in 69% of patients.

Two recent studies suggested that the frequency of hypophysitis during cancer treatment is related to the type of ICIs used and to the modality of administration as monotherapy or in combination with other forms of therapy (94,106). There were initial conflicting results regarding the incidence of hypophysitis induced from the combination of ipilimumab and nivolumab. Some authors found that combined therapy did not influence the incidence of hypophysitis compared to monotherapy (107), whereas other authors reported the opposite (102). Recently, de Filette and collaborators confirmed that hypophysitis is more common in patients treated with ipilimumab (5.6%) compared to nivolumab (0.5%) and pembrolizumab (1.1%). Moreover, combination therapy

was associated with a high incidence (8.8-10.5%) of hypophysitis (106). The onset of endocrine adverse events occurs within the 5-36 weeks, after the beginning of the therapy (103). It was also described a case of hypophysitis after 19 months from the first ipilimumab infusion (102). The latter observation suggests that a long-term monitoring should be recommended.

Recently, Sharma, Allison and collaborators demonstrated that auto-antibodies anti-guanine nucleotide-binding protein G (olf) subunit alpha (GNAL) and anti-integral membrane protein 2B (ITM2B) are associated with the development of IC-therapy-induced hypophysitis (73). Interestingly, GNAL and ITM2B are expressed in normal human pituitary gland tissue. Importantly, anti-GNAL, but not anti-ITM2B, autoantibody has the potential to act as both a predictive and on-treatment biomarker for IC-therapy-induced hypophysitis. These fascinating findings warrant further investigation in larger, prospective studies. If confirmed in larger studies, this autoantibody will serve an important role to enable early detection and time treatment of ICI-induced hypophysitis.

At the beginning of cancer immunotherapy, it is necessary to perform a careful evaluation of the basal hormonal assessment by doing a questionnaire focused on symptoms of hypophysitis and also by measuring glucose, cortisol, TSH, FT4, and electrolytes. Moreover, it should be considered that hypopituitarism and cancer can manifest with overlapping laboratory results and symptoms. Besides a whole endocrine assessment, the diagnosis of hypophysitis should be confirmed by a pituitary MRI (94). This procedure can also exclude the presence of brain metastases and assess the pituitary morphology, which can vary during the disease. It should be pointed out that a normal MRI cannot exclude the diagnosis of hypophysitis and the management should be performed according to the clinical picture and the endocrine assessment. The MRI changes of pituitary gland may precede the functional impairment, whose resolution could be obtained 1 to 8 weeks after the start of glucocorticoid treatment (108). Patients with a mild hypophysitis (grade 1) generally might continue ICI immunotherapy, whilst for those having higher grades of toxicities it is recommended to interrupt the therapy and to start systemic high-dose glucocorticoids. If a clinical improvement is

obtained and toxicity becomes \leq grade, the immunotherapy can be restarted combined with appropriate hormone replacement therapy (HRT) (84). The recovery of thyrotroph axis and gonadotroph function may occur, contrary to the corticotroph axis; thus, sometimes, permanent replacement dose of hydrocortisone or prednisolone are needed (84).

4.2 Thyroid disorders

Thyroid dysfunctions are reported as the first or second most frequent irAEs in patients treated with ICIs (106,109). Thyroid disorders can occur in patients treated with ipilimumab (1-6%) (80,89,90), in those treated with anti-PD-L1 (6-11%) and mostly in patients following anti-PD-1 therapy (up to 40%). It has been reported that at least one irAE occurred in 39–54% of patients treated with anti-PD-1 mAbs (80). Hypothyroidism was the most common event (5.9 %), whereas fewer patients reported hyperthyroidism. In another study, combination of nivolumab plus ipilimumab caused an increased incidence of hypothyroidism (17%) and hyperthyroidism (10%) (110). A more recent study comparing the incidence of endocrine irAEs induced by ipilimumab, anti-PD-L1 or anti-PD-1 drugs, showed higher incidence of thyroid dysfunctions, especially hyperthyroidism, with PD-1/PD-L1 inhibitors compared to ipilimumab (106). Thyroid irAEs include the whole spectrum of thyroid dysfunctions, from overt hyperthyroidism to hypothyroidism. Hyperthyroidism is often transient and may precede hypothyroidism (84). Painless thyroiditis and chronic autoimmune thyroiditis are the most common irAE. Occasionally, myxedema, thyroid storm, or glucocorticoid-responsive encephalopathy can occur. In one-third of patients with thyroid dysfunction an association between anti-peroxidase (TPO-Ab) or anti-thyroglobulin (Tg-Ab) antibodies and hypothyroidism can be found. Painless thyroiditis was observed in ten patients treated with an anti-PD-1 therapy; a transient thyrotoxicosis was observed in six out ten, while four had serum anti-thyroid antibodies. Thyrotoxicosis was treated with beta-blockers with remission of the disease, followed by hypothyroidism. The patients developed anti-thyroid antibodies and were

treated for 6 months with HRT (111). In another study two patients treated with nivolumab reported a worsening of the autoimmune thyroid disorder, with development of Hashimoto's thyroiditis (112). Hypo- and hyper-thyroidism have been also reported in patients with CSCC treated with cemiplimab (52). Therefore, primary hypothyroidism represents the most common irAE and may be severe (87).

Recently, it was reported an interesting case of a patient treated with pembrolizumab for carcinoma of the ovary (hypercalcemic type). Thirty days after a second cycle of immunotherapy, thyroiditis was diagnosed. The authors suggested that the patient had chronic thyroid inflammation associated with PD-L1 expression on follicular epithelial cells, which predisposed to the thyroid destruction after pembrolizumab administration (113).

In a study of 96 cancer patients treated with a cancer vaccine known as GVAX only (a cancer vaccine made by the transfection of the tumor cell with GM-CSF), GVAX plus ipilimumab, or ipilimumab only, Tg-Abs were measured before and after therapies. Tg-Abs specifically developed after GVAX only and co-treatment with ipilimumab. Interestingly, none of the patients receiving ipilimumab only, developed Tg-Abs (114).

Thyroid dysfunctions usually progress without symptoms and they are discovered occasionally by routine biochemical tests. New onset thyroiditis can cause sore throat, tachycardia and palpitations and other clinical features related to hyperthyroidism (115). In some cases, the management of irAEs requires the close collaboration between oncologists and endocrinologists (115). Guidelines recently issued for ICI toxicities management can help clinicians (84-86), but, when the circumstances are more challenging, a crucial role is provided by the specific expertise of specialists in this new field of medicine.

Early detection of symptomatic hypothyroidism is fundamental to decide an eventual withdraw of ICI therapy (90,116). Thyroid function should be evaluated prior to start ICI therapy and every 8 weeks throughout the treatment period (1). In the presence of asymptomatic TSH abnormalities values (higher or lower of the normal range), it's recommended to evaluate FT4

serum level. To rule out a hypoadrenalism concomitant with hypothyroidism, it is mandatory to assess serum cortisol level, prior to start the thyroid HRT. Therapy with Levo-thyroxine should start at low dosage, and then modified as needed (115).

ICI therapy should be modified on the basis of the grade of thyroid toxicities: 1) grade 1 or 2: ICI therapy should be continued with close follow-up and monitoring TSH and FT4; 2) grade 3: ICI therapy should be interrupted and oral prednisolone should be started (116,117); 3) grade 4: ICI should be withheld and therapy with methylprednisone should be started and followed by oral prednisolone (116,117). In case of hyperthyroidism, anti-thyroid therapies (i.e., propylthiouracil, methimazole or carbimazole) and eventually beta-blockers can be used (116).

4.3 Type 1 diabetes mellitus and adrenalitis

Type 1 diabetes mellitus (T1DM) and adrenalitis are rare irAEs (32,88,106). These conditions are more frequently associated with anti-PD-1/PD-L1 therapy compared to anti-CTLA-4 (87,118-121). After the first report of diabetes induced by ICI (122), several cases have been described (123,124). A recent systematic review identified 90 cases of T1DM of whom 79% in patients treated with anti-PD-1 or anti-PD-L1 as monotherapy, or in combination with ipilimumab (15%). The patients showed diabetic ketoacidosis (71%), elevated lipase levels (52%), anti-islet cell autoantibodies and anti-glutamic acid decarboxylase autoantibodies (53%), and susceptible HLA (mostly DR4) genotypes (65%). T1DM onset ranges from few weeks after the initiation of immunotherapy, up to more than one year (81).

Another large study evaluated the clinical features, frequency, and follow-up of new-onset insulin-dependent diabetes in patients treated with ICIs (121). In 281 patients treated with ICIs, T1DM developed in 1.8% of those treated with anti-PD-1, while no cases were reported in patients treated with ipilimumab. New-onset insulin-dependent diabetes developed after a median of four cycles of ICIs or 4-5 month since initiation of therapy. Fifty percent of those patients developed

another irAEs, with the most common being thyroiditis (manifested as primary hyperthyroidism followed by hypothyroidism). Interestingly, patients with T1DM showed higher single-nucleotide polymorphisms in the PD-1 gene when compared to controls (121,125). It is important to note that diabetes caused by ICIs tends to be irreversible, despite pharmacological attempts with glucocorticoids (126). Development of polyuria, polydipsia, and weight loss should prompt investigation for possible development of T1DM. Diagnosis and management of T1DM is based on recognized guidelines (127). Test for antibodies (anti-glutamic acid decarboxidase, anti-insulin, anti-islet cell), C-peptide and insulin can distinguish T1DM and T2DM.

In 2015, a first case of adrenalitis was reported in a 79 years old patient treated with ipilimumab (119). Radiological studies showed the presence of bilateral hypermetabolic and enlarged adrenal glands and it was suggested that immunotherapy was the primary cause of adrenalitis. Adrenal insufficiency of unknown cause was shown in patients treated with tremelimumab or nivolumab, or the combination of ipilimumab and anti-PD-1 mAbs (128). Hyponatremia could be the first sign of primary adrenal failure or ACTH deficiency. Radiological evidence of adrenalitis (e.g., adrenal enlargement), in presence of normal endocrine function, suggests a subclinical form of the disease (119). In these cases, the adrenal function should be assessed by evaluating the levels of both cortisol and ACTH; moreover, to exclude primary adrenal failure, a Synacthen stimulation test should be performed.

4.3.1. Pathophysiology of the endocrine irAEs associated with ICIs blockade

CTLA-4 and PD-1 pathways play a fundamental role in autoimmunity, as well as in anti-tumor immunity (63,64). These pathways act in different immune microenvironments: CTLA-4 predominantly in lymph nodes, while the PD-1 pathway is involved within the TME (129). Table 1 summarizes some of the basic and clinical differences between anti-CTLA-4 and anti-PD-1 immune checkpoint blockade.

CTLA-4 is not only expressed on different types of immune cells, but also on pituitary adenomas and normal pituitary tissue (108). The low incidence of hypophysitis in patients treated with anti-PD-1/PD-L1 mAbs could be explained by the absence of PD-1/PD-L1 expression in normal hypophysis. Another important difference lies in the capacity to activate different effector T cells. Anti-CTLA-4 can generate new reactive effector T cells against the pituitary gland, whereas anti-PD-1/PD-L1 mAbs activate only existing effector T cells (94). The interaction between mAbs anti-CTLA-4 and CTLA-4 can activate antibody-dependent cell-mediated cytotoxicity (ADCC) or the complement-mediated lysis. Interestingly, in patients treated with ipilimumab (IgG1 antibody), there is evidence of complement activation lower than in those treated with tremelimumab (IgG2 antibody) (94,108). IgG1, the isotype of ipilimumab, has high affinity for Fc γ RIIIa, the Fc receptor mediating ADCC. Interestingly, patients with a polymorphism in this receptor, had greater responsiveness to ipilimumab (130).

Another interesting difference between CTLA-4 and PD-1/PD-L1 occurs during their activation. For instance, CTLA-4 induction in T cells occurs early in the antigen response in draining lymph nodes, whereas PD1/PD-L1 pathway is activated downstream of the immune response in peripheral tissues and in TME (131).

The activation of pre-existing low-grade autoimmunity to thyroid glands probably contributes to the pathogenesis of thyroid irAE. In fact, patients with TPO-Ab or Tg-Ab are more likely to develop thyroiditis associated to nivolumab therapy (108). Thyroid dysfunctions are more common upon treatment with anti-PD-1/PD-L1 compared to anti-CTLA-4. These findings could be explained by the PD-L1 and PD-L2 expression on the thyroid gland (132-134). Moreover, it has been shown that genetic mutations in PD-L1 and CTLA-4 may be involved in autoimmune thyroid disease development (135,136).

Several tumor epitopes can have a common amino acid sequence with thyroid antigens. Therefore, the cross presentation of those epitopes on HLA molecules could be associated with thyroid irAEs. The immunotherapeutic administration of NY-ESO-1, a tumor-associated antigen

which shares epitopic homologous regions with two major thyroid autoantigens (Tg, TSH-R) induced Graves' disease in a patient with risk allele of HLA (137). Interestingly, administration of ipilimumab in a melanoma patient caused a fulminant T1DM associated with anti-insulin antibodies (138). The patient was susceptible HLA-DR4 and the authors suggested a possible cross-reactivity between a melanoma antigen and insulin.

4. Conclusions and Perspectives

Immune checkpoint blockade is a paradigm shift that has revolutionized the treatment of patients with different types of solid (31,34) and hematologic cancers (139,140). Unfortunately, this form of anticancer immunotherapy is effective only in a percentage of patients. Moreover, treatment with ICIs unleashes the immune system not only against tumor cells expressing neoantigens, but also against autoantigens (73). This results in the development of a variety of irAEs, occasionally severe in a significant percentage of patients (63,64). Patients undergoing immunotherapy with ICIs can also develop different types of endocrine irAEs (88,106,109,131).

Hypophysitis, thyroid dysfunctions, T1DM and adrenal insufficiency are immune-related endocrine toxicities associated with ICIs. The onset of irAEs usually occurs within 7 weeks from the start of ipilimumab treatment and 10-15 weeks for nivolumab (121,141). The onset of the endocrinopathies might change also according to the different mechanisms of action of the different ICIs. In fact, CTLA-4 expression is induced early in T cells in response to antigens, whereas the activation of PD-1/PD-L1 pathway in peripheral tissues and in TME occurs later (131). Recovery of both the gonadal- and thyroid-pituitary axes occur in up to 50-60 % of patients.

Patients with overt autoimmunity are usually excluded from clinical trials. Autoimmune disorders seem to affect more women than men. The correlation between sex, efficacy and irAEs in patients treated with ICIs is still unclear (142,143). Further studies should carefully investigate the issue of sex dimorphism in ICI-associated endocrine irAEs and efficacy.

It is important to note that, as other immunologically targeted agents progress through clinical development, such as those targeting LAG3, TIM-3, BTLA, VISTA, and TIGIT (34-38,144), clinical investigators must remain vigilant from the outset regarding not only the toxicity profiles of these agents as monotherapies, but also possible toxicities when used in combination with other treatments.

Interestingly, the development of thyroid dysfunctions, thyroiditis or the appearance of anti-thyroid antibodies, in subjects treated with ICI, was related to an extended survival (114). This suggests that these events might be considered as markers of a stronger immune activation (65).

Consensus guidelines have been recently released for the evaluation and management of irAEs associated with ICIs (84-86). Low grade endocrine irAEs are usually managed by oncologists. However, the collaboration among oncologists, endocrinologists and immunologists appears necessary when the circumstances are more challenging (i.e., gonadal hormones dysfunctions, adrenal insufficiency, hypophysitis, and new onset T1DM) and for better management of severe endocrine irAEs (115).

Further investigations are needed to better understand the mechanisms by which different ICIs can induce a variety of irAEs in both preclinical models and patients. A better knowledge of these complex immunological mechanisms should enable the development of more appropriate strategies to prevent or manage ICI toxicities without compromising the success of cancer immunotherapy.

Conflict of Interest

The authors have nothing to declare.

Role of the funding source

The authors have nothing to declare.

Summary

Different mAbs anti-CTLA-4, anti-PD-1, and anti-PD-L1 that are able to block “inhibitory checkpoint molecules”, have been approved for the treatment of an increasing number of solid and hematologic tumors. These agents are able to strength the immune response against cancer, but also can lead to irAEs, ranging from mild to fatal, according to the severity and organ system involved and severity (63,64,145).

A significant percentage of cancer patients undergoing immunotherapies with ICIs manifest endocrine irAEs (88,106,131). The most frequent irAEs associated with anti-PD-1-antibodies treatment are thyroid dysfunctions. Hypophysitis is mostly linked to anti-CTLA-4-antibody treatment. T1DM and adrenalitis are not common irAEs (106) and are more frequently associated to anti-PD-1 or anti-PD-L1 treatment compared to anti-CTLA-4. The development of thyroid dysfunctions, thyroiditis or the appearance of anti-thyroid antibodies, in patients undergoing ICI therapy, was related to extended survival (114). This suggests that these events might be considered as markers of a stronger immune activation (65).

Consensus guidelines have been recently released (84-86) for the evaluation and management of ICI-associated irAEs. Low grade endocrine irAEs are usually managed by oncologists. However, the collaboration among oncologists, endocrinologists and immunologists appears necessary when the circumstances are more challenging (115). Further investigations are needed to better understand the mechanisms by which ICI can induce endocrine irAE in both preclinical and clinical models. A better knowledge of these immunological mechanisms should enable the development of more appropriate strategies to prevent or manage endocrine irAEs caused by ICIs.

Practice points

- Immune checkpoint inhibitors (ICIs) unleash the immune system to eliminate cancer cells and have revolutionized the care of patients with different types of solid and hematologic cancers.
- Treatment with ICIs is associated with a variety of diverse and distinct immune-related adverse events (irAEs)
- A significant percentage of patients treated with ICIs manifest endocrine irAEs.
- The risk of hypophysitis is high in patients treated with ipilimumab.
- A high incidence of thyroid dysfunction and in particular hypothyroidism can be observed in patients treated with monoclonal antibodies targeting PD-1/PD-L1.
- T1DM and adrenalitis are rare irAEs, and more frequently associated with anti-PD-1/PD-L1 treatment compared to anti-CTLA-4.
- Cancer therapies with combinations of different ICIs are associated with increased risk of irAEs.

Research agenda

- Further investigations appear necessary to better understand the mechanisms by which different immune checkpoint inhibitors can induce various irAEs. A better knowledge of these mechanisms may contribute to the prevention and treatment of these adverse effects that may compromise cancer immunotherapy.
- Further investigations are needed to confirm the extended survival in patients undergoing ICI immunotherapy associated with the development of thyroid dysfunctions, thyroiditis or to the appearance of anti-thyroid antibodies.

Figure Legend

FIG. 1

A. Tumor cells synthesize and release several neoantigens (colored dots), which are captured by professional antigen presenting cells (APCs) and presented to naïve T-cells through the complex MHC-T cell receptor (TCR). CTLA-4 is a negative costimulatory receptor expressed on activated T cells which plays a critical role for maintaining immune homeostasis and for preventing autoimmunity. CTLA-4 binds to CD80 and CD86 and inhibits effector T cell activation by preventing binding of B7 ligands to the co-stimulatory receptor CD28. CTLA-4 overexpression is responsible for the absence of reactivity of T cells against tumor antigens. PD-1 is another inhibitory receptor, expressed on activated T cells, also involved in limiting the activation of T cells. PD-1-dependent T cell inhibition occurs following engagement of its ligand, PD-L1, which is expressed on tumor cells and on certain immune cells. Interactions between CTLA-4 and CD80/CD86 and between PD-1 and PD-L1 inhibit the effector functions of T cells and foster the proliferation of cancer cells.

B. mAbs targeting CTLA-4 (ipilimumab), PD-1 (nivolumab, pembrolizumab, cemiplimab), and PD-L1 (atezolizumab, durvalumab, avelumab) block immune checkpoints (CTLA-4, PD-1, and PD-L1, respectively) and restore antitumor immune response, resulting in tumor cell death *via* release of cytolytic molecules (e.g., granzyme B, TNF- α , INF- γ).

Table 1

Differences between anti-CTLA-4 and anti-PD-1 immune checkpoint blockade

ANTI-CTLA-4	ANTI-PD-1
<ul style="list-style-type: none">-Hard wired-Targets CD28 pathway-Works mainly during priming of naïve T cells-Works mainly in draining lymph nodes-Expand clonal diversity-Primarily effects CD4⁺ T cells-Can move T cells into “cold” tumors-Clinical responses are often slow-Adverse events are relatively frequent-Disease recurrence after response are rare	<ul style="list-style-type: none">-Induced resistance-Targets TCR pathway-Works mainly on exhausted T cells-Works mainly in peripheral tissues and in tumor microenvironment (TME)-Does not expand clonal diversity-Primarily effects CD8⁺ T cells-Does not move T cells into tumors-Clinical responses are usually rapid-Adverse events are less frequent-Disease recurrence after response are significant

Table 2

ICIs approved by the U.S. Food and Drug Administration*

Drug	Mechanism of Action	Approved Indications
Ipilimumab	Anti-CTLA-4	Melanoma, RCC, Colorectal cancer
Nivolumab	Anti-PD-1	Melanoma, NSCLC, RCC, HCC, Hodgkin's lymphoma, SCC of the head and neck, Urothelial carcinoma, Colorectal cancer with high MSI or mismatch-repair deficiency
Pembrolizumab	Anti-PD-1	Melanoma, NSCLC, SCLC, Hodgkin's lymphoma, SCC of the head and neck, Urothelial carcinoma, Gastric or GEJ cancer, Advanced solid tumors (MSI-H/d-MMR), PMBCL, HCC, Merkel cell carcinoma, Cervical cancer, RCC
Cemiplimab	Anti-PD-1	Cutaneous SCC
Atezolizumab	Anti-PD-L1	NSCLC, Urothelial cancer, SCLC, Triple negative breast cancer
Durvalumab	Anti-PD-L1	NSCLC, Urothelial cancer
Avelumab	Anti-PD-L1	Merkel cell carcinoma, Urothelial cancer, RCC

* As November 2019

CTLA-4: cytotoxic T-lymphocyte antigen 4; **dMMR:** deficient mismatch repair; **GEJ:** gastroesophageal junction; **HCC:** hepatocellular carcinoma; **MSI-H:** microsatellite instability-high; **NSCLC:** non-small cell lung cancer; **PD-1:** programmed cell death 1; **PD-L1:** programmed cell death ligand 1; **PMBCL:** primary mediastinal large B cell lymphoma; **RCC:** renal cell carcinoma; **SCC:** squamous cell carcinoma; **SCLC:** small cell lung cancer.

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