Immunotherapy against endocrine malignancies: immune checkpoint inhibitors lead the way

Lucas Leite Cunha¹, Marjory Alana Marcello¹, Vinicius Rocha-Santos² and Laura Sterian Ward¹

¹Laboratory of Cancer Molecular Genetics, Faculty of Medical Sciences, University of Campinas (FCM-Unicamp), Campinas, São Paulo, Brazil

²Liver and Gastrointestinal Transplant Division, Department of Gastroenterology, University of São Paulo, School of Medicine, São Paulo, São Paulo, Brazil

Correspondence
should be addressed
to L S Ward

Email
ward@fcm.unicamp.br

Abstract

Immune checkpoint inhibitors are agents that act by inhibiting the mechanisms of immune escape displayed by various cancers. The success of immune checkpoint inhibitors against several tumors has promoted a new treatment strategy in clinical oncology, and this has encouraged physicians to increase the number of patients who receive the immune checkpoint therapy. In the present article, we review the main concepts regarding immune checkpoint mechanisms and how cancer disrupts them to undergo immune escape. In addition, we describe the most essential concepts related to immune checkpoint inhibitors. We critically review the literature on preclinical and clinical studies of the immune checkpoint inhibitors as a treatment option for thyroid cancer, ovarian carcinoma, pancreatic adenocarcinoma, adrenocortical carcinoma and neuroendocrine tumors. We present the challenges and the opportunities of using immune checkpoint inhibitors against these endocrine malignancies, highlighting the breakthroughs and pitfalls that have recently emerged.

Key Words

- ▶ immune checkpoint inhibitors
- ▶ endocrine neoplasms
- ▶ immune escape
- ▶ thyroid cancer
- ▶ ovarian cancer
- ▶ pancreatic adenocarcinoma
- ▶ neuroendocrine tumors
- ▶ adrenocortical carcinoma

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Introduction

Immune checkpoint inhibitors are agents that act by inhibiting immune escape through various mechanisms. In other words, immune checkpoint inhibitors act on the immune system (not necessarily on tumor cells), enabling it to detect, target and destroy cancer cells. By doing so, immune checkpoint inhibitors also activate immune memory, leading to a sustained antitumor response. Additionally, since these drugs primarily act on the host, it may be possible to use them against many tumor types, independent of the origin of cancer. The cost of enhancing the immune response is mirrored in adverse events, which are largely related to immune-mediated destruction of healthy tissues and uncontrolled inflammation.

Endocrine malignancies are a heterogeneous group of diseases that is composed of tumors with different biological features. Endocrine tumors are usually indolent, and their low proliferative capacity is one of their known signatures. On the other hand, very aggressive tumors such as anaplastic thyroid cancer and adrenocortical carcinomas are also included among endocrine neoplasms. Data derived from The Cancer Genome Atlas (<https://cancergenome.nih.gov/>) demonstrated that these tumors present a low mutation burden, markedly lower than lung, bladder and upper gastrointestinal cancers (Agrawal *et al.* 2013, Bates 2016, Bongiovanni *et al.* 2017). Despite their low mutational load, many endocrine tumors

harbor driver mutations that are commonly implicated in aggressiveness, making the endocrine neoplasms a very intriguing field for exploration.

In the present article, we review the main concepts regarding immune checkpoint mechanisms and how cancer disrupts them to undergo immune escape. We describe the most essential points related to the pharmacology and clinical aspects of immune checkpoint inhibitors. Finally, we critically review the literature on preclinical and clinical studies of immune checkpoint inhibitors as a treatment option for some endocrine malignancies.

Concepts in immune checkpoints and immune checkpoint inhibitors

The immune system avoids autoimmunity by turning off immune responses against cells of the host organism. This means that there are several mechanisms responsible for the inactivation of effector T cells that prevent damage to healthy tissues despite the continuing clearance of

infectious microorganisms. Cancer takes advantage of this property of the immune system to avoid its own elimination by immune cells by using a process called immune escape (Ramsay 2013). One mechanism of immune escape is the hijacking of immune cell-intrinsic checkpoints that are induced by T-cell activation (Fig. 1) (Pardoll 2012, Ribas 2012).

Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and anti-CTLA-4 antibody

T-cell recognition of antigen through the T-cell receptor (TCR) is regulated by a balance between costimulatory and inhibitory signals. T-cell recognition of antigen through the TCR is regulated by a balance between costimulatory and inhibitory signals.

One of the immune checkpoints is mediated by CTLA-4. CTLA-4 is active in the initial stages of the immune response, is expressed by T cells after 48 h of activation and participates in dominant negative signaling by competing with the CD28 costimulatory receptor for the binding of B7-1 (CD80) and B7-2 (CD86) (Ribas 2012). CTLA-4-deficient mice develop a phenotype characterized by immune hyperactivation and lymphoproliferative disease with multiorgan lymphocytic infiltration and tissue destruction (Tivol et al. 1995). Similarly, the *in vivo* blockage of CTLA-4 in animal models augments immune responses, resulting in the rejection of tumors, including pre-established tumors. Interestingly, this also results in immunity against secondary exposure to tumor cells, suggesting that the memory component of immune response can be evoked by anti-CTLA-4 antibodies (Leach et al. 1996).

Ipilimumab Ipilimumab is a recombinant fully humanized monoclonal IgG class 1 antibody that is directed against CTLA-4 (Fig. 2). This antibody is currently approved by the FDA for the treatment of metastatic melanoma and as an adjuvant therapy for patients with stage III melanoma (Hodi et al. 2010, Eggermont et al. 2016). Ipilimumab binds to CTLA-4 expressed on T cells and inhibits the CTLA-4-mediated downregulation of T-cell activation, leading to a cytotoxic T-cell-mediated immune response against cancer cells. The detailed mechanisms of action of CTLA-4 blockade with ipilimumab are not fully understood, but it is estimated that they can be classified into two main types: cell-intrinsic mechanisms acting in cis and cell-extrinsic mechanisms acting in trans (de Coana et al. 2017). The cis mechanisms involve

Figure 1

Immunological synapse, showing tight contact between a T cell and an antigen-presenting cell. In the lower part of the image, the T-cell interacts with a dendritic cell, and the peptide is presented by MHC and recognized by TCR. In the upper part of the image, the tumor cell performs an analogous presentation of the tumor-associated antigen. When antigen presentation occurs in the context of inhibitory molecules (CTLA-4/B7-1/2 or PD-1/PD-L1), a negative signal is triggered in the cytoplasm of the T-cell. This negative signal leads to the downregulation of effector functions, inhibition of survival, growth and proliferation and stimulation of an exhausted phenotype in T cells. Cancer cells disrupt immune responses by hijacking this mechanism, thereby avoiding tumor destruction and elimination.

Figure 2

Immunological synapse after the administration of immune checkpoint inhibitors. Immune checkpoint inhibitors are antibodies directed against co-inhibitory molecules. By blocking the interaction between CTLA-4 and B7-1/2 (e.g., ipilimumab) or PD-1 (e.g., nivolumab and pembrolizumab) and PD-L1 (e.g., durvalumab, atezolizumab and avelumab), immune checkpoint inhibitors inhibit the immune response mediated by immune escape mechanisms and encourage the immune system to destroy tumor cells.

T cells that express CTLA-4, which remain suppressed (Leach *et al.* 1996). In this case, the blockage of CTLA-4 activates the cells, allowing them to proliferate and perform their functions. The trans mechanisms involve other cell types, including regulatory T cells and myeloid-derived suppressive cells (Walker & Sansom 2011). In this case, the anti-CTLA-4 treatment reduces the suppressive potential of these cells (Pico de Coana *et al.* 2013). In both cis and trans mechanisms, ipilimumab acts to favor the breakdown of the tumor immune escape, leading to an antitumor immune response.

The pharmacokinetics of ipilimumab were investigated in 499 patients with unresectable or metastatic melanoma. Studies were performed by administering ipilimumab every 3 weeks, in 4 cycles. Patients received the drug at three doses, namely, 0.3, 3 or 10mg/kg, and the third dose allowed a steady-state concentration. Within the dose range examined, the plasma peak, trough and area

under the curve concentrations of ipilimumab were proportional to the dose (Wolchok *et al.* 2010, Fellner 2012). The maximum CTLA-4 blockade occurred at the concentration of 20µg/mL, and 30% of patients in the group receiving 3 mg/kg experienced this effect (Wolchok *et al.* 2010). No dosage adjustment was indicated for high body weight, preexisting mild-to-moderate renal insufficiency (creatinine clearance of 29 mL/min or above) or various degrees of hepatic dysfunction at baseline because these factors did not show a meaningful effect on the pharmacokinetics of ipilimumab. Other factors that did not appear to significantly impact the clearance of ipilimumab include age, gender, concomitant use of budesonide, performance score, HLA-A2*0201 status, anti-ipilimumab antibody positivity, prior history of systemic anticancer therapy and baseline lactate dehydrogenase levels (Wolchok *et al.* 2010, Trinh & Hagen 2013).

Programmed cell death protein 1 (PD-1) and programmed cell death protein ligand (PD-L1) axis and PD-1/PD-L1 blockade

The PD-1/PD-L1 axis is involved in multiple stages of the immune response. The activation of T cells triggers the expression of PD-1, which is maintained after T cells migrate to peripheral tissues. The cytokines produced in peripheral tissues provide a microenvironment that induces the expression of PD-L1 and PD-L2. The signals through the PD-1/PD-L1 axis are inhibitory in nature, resulting in decreases in the expression of cell surface activation markers, the proliferation of T cells and the production of cytokines (Butte *et al.* 2007). This produces a precise regulation of the T-cell response, preventing uncontrolled inflammation and autoimmunity (Keir *et al.* 2006, 2008).

Notably, both the PD-1/PD-L1 axis and regulatory T cells are necessary for the maintenance of peripheral tolerance. Francisco and coworkers derived both of these concepts while studying regulatory T-cell plasticity (Francisco *et al.* 2009). The authors demonstrated that the expression of PD-L1 by antigen-presenting cells is essential for the induction of differentiation of regulatory T cells from naïve CD4 T cells. Additionally, PD-L1 enhances and sustains FOXP3 expression and the suppressive function of induced Treg cells (Francisco *et al.* 2009). PD-1 is also expressed by B cells and natural killer-cell effectors; PD-L1 is expressed by B cells, T cells and macrophages, whereas PD-L2 is mainly expressed by antigen-presenting cells and epithelial cells (Keir *et al.* 2008, Gravelle *et al.* 2017).

The molecule PD-1 interacts with PD-L1 and PD-L2. The relative contribution of PD-L2 is not completely understood. PD-L2 is expressed later in the maturation of dendritic cells and at lower levels. Shin and coworkers have shown that PD-L2, but not PD-L1, elicits direct activating effects on dendritic cells (Shin *et al.* 2005). However, the concurrent presence of PD-L1 on the same cell might prevent this activating effect of PD-L2 due to competition for PD-1. Indeed, Ghiotto and coworkers observed that PD-L1 and PD-L2 competed for PD-1 binding and that conversely, an antagonist PD-1 monoclonal antibody blocked both PD-L1 and PD-L2 from binding to PD-1 and strongly enhanced T-cell proliferation (Ghiotto *et al.* 2010).

Many chronic infections are characterized by the functional impairment of antigen-specific T cells by a process called T-cell exhaustion, and it has been demonstrated that PD-1 is involved in this process. Barber and coworkers reported that PD-1 was selectively upregulated by exhausted T cells in mice chronically infected with lymphocytic choriomeningitis virus (Barber *et al.* 2006). Blocking the interaction of PD-1 with PD-L1 restored the ability of the T cells to undergo proliferation, secrete cytokines, kill infected cells and decrease viral load, suggesting that the PD-1/PD-L1 axis is a key mechanism for anergy (Barber *et al.* 2006). This mechanism might also contribute to the immune response against tumors. Notably, hematological malignancies and solid tumors are frequently enriched with tumor-infiltrating lymphocytes in which PD-1 is markedly upregulated, whereas the ligands PD-L1 and PD-L2 are expressed by various types of tumor cells, reinforcing the hypothesis that the PD-1/PD-L1 axis is a mechanism co-opted by tumors in order to evade the immune system (Ahmadzadeh *et al.* 2009, Cunha *et al.* 2013b, Wu *et al.* 2015, Gravelle *et al.* 2017).

Nivolumab Nivolumab is a fully humanized IgG class 4 antibody that binds to PD-1 with high affinity, preventing its interaction with both PD-L1 and PD-L2 (Fig. 2). The blockage of these interactions results in the loss of inhibitory signals in T cells and tumor recognition by cytotoxic T cells, stimulating the memory response to tumor antigen-specific T-cell proliferation (Wang *et al.* 2014, Guo *et al.* 2017). This antibody is currently approved by the FDA for the treatment of metastatic melanoma, advanced non-small-cell lung cancer, advanced renal cell carcinoma, recurrent or metastatic squamous cell carcinoma of the head and

neck after previous treatment, recurrent classical Hodgkin lymphoma after an autologous stem cell transplantation and previously treated advanced bladder cancer. The peak concentration of nivolumab is achieved by 1–4 h after the start of infusion, and its serum half-life is 12 (0.3, 1.0 or 3.0 mg/kg) to 20 days (10.0 mg/kg) (Brahmer *et al.* 2015). The binding of nivolumab with PD-1 was analyzed by PD-1 occupancy, which was found to be dose independent, with a mean peak occupancy of 85% at 4–24 h and an average plateau occupancy of 72% at 57 days and beyond (Brahmer *et al.* 2010). Bajaj and coworkers demonstrated that sex, performance status, baseline estimated glomerular filtration rate, age, race, baseline lactate dehydrogenase, mild hepatic impairment, tumor type, tumor burden and PD-L1 expression had significant but not clinically relevant (<20%) effects on nivolumab clearance (Bajaj *et al.* 2017).

Pembrolizumab Pembrolizumab is a humanized recombinant monoclonal IgG class 4 kappa-isotype antibody to PD-1, and it results in an increased immune reactivity that can overcome immune tolerance, thus enabling its use in immunotherapy (Fig. 2) (Raedler 2015). Pembrolizumab is indicated for the treatment of unresectable or metastatic melanoma, metastatic non-small-cell lung cancer with high PD-L1 expression and recurrent or metastatic head and neck squamous cell carcinoma with disease progression on or after platinum-containing chemotherapy, and it has recently been approved by the FDA for use in adult and pediatric patients with refractory classical Hodgkin lymphoma or patients who have relapsed after three or more prior lines of therapy. Pharmacokinetic studies have revealed that the steady-state concentration of pembrolizumab was reached by 19 weeks of repeated dosing with administration every 3 weeks, and the systemic accumulation was 2.2-fold. Age, sex, ethnicity, renal impairment, mild hepatic impairment and tumor burden had no clinically important effect on the clearance of pembrolizumab (Merck 2017). Phase I, II and III clinical trials have assessed different pembrolizumab regimens administered every 2 or 3 weeks (Garon *et al.* 2015, Ribas *et al.* 2015, Robert *et al.* 2015). These studies helped to establish that the approved pembrolizumab dose of 2 mg/kg every 3 weeks without dose adjustment in a variety of patient subpopulations is probably the best regimen available at present. (Ahamadi *et al.* 2017). The authors found no clinical significance for variations in sex, baseline performance status, renal and hepatic function,

tumor type and burden or prior ipilimumab treatment on pembrolizumab exposure (Ahamadi et al. 2017).

Durvalumab Durvalumab is an Fc-optimized monoclonal antibody directed against PD-L1 with potential immune checkpoint inhibitory and antineoplastic activities (Fig. 2). On May 1, 2017, the U.S. FDA granted accelerated approval to durvalumab for the treatment of patients with locally advanced or metastatic urothelial carcinoma who have disease progression during or following platinum-containing chemotherapy or within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy. By binding to PD-L1, durvalumab can reverse T-cell inactivation and encourage the immune system to exert a cytotoxic T-lymphocyte-mediated response against PD-L1-expressing tumor cells. Steady-state concentration of durvalumab was achieved at approximately 16 weeks (AstraZeneca 2017). The steady-state clearance of durvalumab was 8.24 mL/h, and the geometric mean terminal half-life was approximately 17 days. The pharmacokinetics of durvalumab were not clinically significantly affected by age, body weight, sex, albumin levels, lactate dehydrogenase levels, creatinine levels, soluble PD-L1, tumor type, race, mild renal impairment, moderate renal impairment, mild hepatic impairment or Eastern Cooperative Oncology Group (ECOG) status (AstraZeneca 2017).

Atezolizumab Atezolizumab is an Fc-engineered, fully humanized monoclonal antibody of an IgG1 isotype against PD-L1 (Fig. 2). Atezolizumab is FDA approved for locally advanced or metastatic urothelial carcinoma and metastatic non-small-cell lung cancer during or following platinum-containing chemotherapy (Ning et al. 2017). Similar to durvalumab, atezolizumab binds to PD-L1 and inhibits its interaction with PD-1. This releases the PD-1/PD-L1-mediated inhibition of the immune response, resulting in the reactivation of the antitumor immune response (Ning et al. 2017). Based on a population analysis that included 472 patients given various doses, the typical population clearance of atezolizumab was 0.20 L/day, the volume of distribution at steady state was 6.9 L and the terminal half-life was 27 days. The population pharmacokinetics analysis suggests that the steady state is obtained after 6–9 weeks (2–3 cycles) of repeated dosing. Atezolizumab clearance was found to decrease over time, with a mean maximal reduction from baseline value of approximately 17.1%. The systemic exposure of atezolizumab was not clinically significantly affected

by age, body weight, gender, positive anti-therapeutic antibody status, albumin levels, tumor burden, region or race, mild or moderate renal impairment, mild hepatic impairment, level of PD-L1 expression or ECOG status (Genentech 2017, Ning et al. 2017).

Avelumab Avelumab is an investigational fully humanized anti-PD-L1 IgG1 monoclonal antibody. In 2017, the U.S. FDA granted accelerated approval to avelumab for the treatment of patients aged 12 years and older with metastatic Merkel cell carcinoma (Kim 2017). Avelumab is also included in the international JAVELIN clinical trial, as both monotherapy and combination therapy in more than 16 different tumor types (Chin et al. 2017). Avelumab binds PD-L1 and blocks the interaction between PD-1 and PD-L1 (Fig. 2). By inhibiting PD-L1 interactions, avelumab is thought to enable the activation of T cells and the adaptive immune response. By retaining a native Fc region, avelumab is thought to engage the innate immune response and induce antibody-dependent cell-mediated cytotoxicity, prompting the restoration of antitumor immune responses (Disis et al. 2016, Chin et al. 2017, Kim 2017). The steady-state concentration of avelumab was achieved after approximately 4–6 weeks (2–3 cycles) of repeated dosing. In patients with solid tumors, the total systemic clearance was 0.59 L/day, and the terminal half-life was 6.1 days in patients receiving 10 mg/kg (Serono 2017).

New immune checkpoint inhibitors

Inspired by the success of checkpoint inhibitors, several other checkpoints are now under investigation as targets for monotherapy or as combination therapy, including lymphocyte activation gene-3 (LAG-3), T-cell immunoglobulin mucin-3 (TIM-3) and indoleamine-2,3-dioxygenase (IDO) (Brignone et al. 2010, Wang-Gillam et al. 2013, Beatty et al. 2017).

LAG-3 Also called CD223, LAG-3 is expressed on activated T cells, NK cells, B cells and tumor-infiltrating lymphocytes (Grosso et al. 2007, Assal et al. 2015). LAG-3 acts as a negative regulator of T-cell activation and homeostasis (Grosso et al. 2007). LAG-3 exhibits its effects on immunity through several mechanisms: induction of functional unresponsiveness of T cells, inhibition of TCR-induced calcium ion flux, inhibition of cytokines and promotion of the suppressive activity of Treg cells (Huang et al. 2004). LAG-3 and PD-1 are co-expressed in malignant

mouse and human tissues, and in these malignant microenvironments, it leads to immune tolerance by inhibiting APC and T-cell function (Woo *et al.* 2012, Lee *et al.* 2013). Similar to anti-PD-1 immunotherapy, anti-LAG-3 has been shown to reduce tumor growth of colorectal adenocarcinoma and fibrosarcoma in mice, and it produces a synergistic effect when co-administered with anti-PD-1 (Woo *et al.* 2012). In the last few years, studies have investigated the use of IMP321, a LAG-3-Ig recombinant fusion protein that antagonizes the normal function of LAG-3. These studies were focused on renal cell carcinoma (Brignone *et al.* 2009), metastatic breast carcinoma (Brignone *et al.* 2010) and advanced pancreatic adenocarcinoma (Wang-Gillam *et al.* 2013). All these studies showed promising results regarding the effect of LAG-3 in reducing tumor size. In addition, these studies demonstrated that LAG-3 is well tolerated by patients, making it a suitable candidate for immunotherapy.

TIM-3 T-cell immunoglobulin mucin-3 was first identified as a molecule specifically expressed on IFN- γ -secreting CD4 T helper 1 and CD8 cytotoxic T cells in both mice and humans (Zhu *et al.* 2011). TIM-3 acts as a negative regulatory molecule by binding with one of its ligands, Galectin-9 (Gal-9). When this binding occurs, Gal-9 leads to cell death, especially in T cells whose activities have been suspended. TIM-3 can also impair immune responses by promoting the expansion of myeloid-derived suppressor cells (Sakuishi *et al.* 2011). Tumor-infiltrating CD4 and CD8 cells co-express TIM-3 and PD-1 in murine models of colon adenocarcinoma, melanoma and mammary adenocarcinoma (Assal *et al.* 2015). In humans, TIM-3 is expressed on tumor-infiltrating lymphocytes or T cells in the peripheral blood of patients with various types of cancer such as hepatocellular cancer, cervical cancer, colorectal cancer, ovarian cancer, non-small-cell lung cancer, head and neck cancer, renal cell carcinoma, gastric cancer, esophageal cancer, prostate cancer and non-Hodgkin lymphoma (Yang *et al.* 2012, Jie *et al.* 2013, Yan *et al.* 2013, Cheng *et al.* 2015, Japp *et al.* 2015, Ji *et al.* 2015, Thommen *et al.* 2015, Cai *et al.* 2016, Xie *et al.* 2016). Preclinical models show conflicting results for the administration of TIM-3-targeting monoclonal antibody. While administration of the antibody alone did not show promising results in colon adenocarcinoma, the combination of anti-TIM-3 and anti-PD-1 antibodies demonstrated a considerable antitumor effect (Sakuishi *et al.* 2010). The same occurred with the co-administration of anti-TIM-3 and anti-CTLA-4

antibodies in a mouse model (Ngiow *et al.* 2011). Taken together, these studies suggest that the combination of anti-TIM-3 with anti-PD-1 or anti-CTLA-4 shows promise for the improvement of current immunotherapy.

IDO Indoleamine 2,3-dioxygenase (IDO) is an IFN-inducible enzyme that suppresses adaptive T-cell immunity by catabolizing the essential amino acid tryptophan from the cellular microenvironment (Mellor 2005, Wingender *et al.* 2006). When tryptophan levels are decreased, the cell cycle is arrested with the inactivation of mTOR pathway, and tryptophan metabolites lead to T-cell apoptosis and Treg cell differentiation (Assal *et al.* 2015). One of the IDOs, IDO1, is expressed in mature dendritic cells in lymphoid tissues, some epithelial cells of the female genital tract and placental and pulmonary endothelial cells, and IDOs can also be found in lymphoid tissues of tumors such as cervical, colorectal, gastric tumors, as well as vascular cells in renal cell cancer (Theate *et al.* 2015). Tumors expressing IDO1 have a more aggressive phenotype and lead to poorer prognosis than tumors that do not express this protein (Godin-Ethier *et al.* 2011). There are several ongoing studies investigating the effect of the IDO inhibitor indoximod both alone and in combination with other antitumoral agents (Assal *et al.* 2015, Jiang *et al.* 2017, Meng *et al.* 2017, Tomek *et al.* 2017).

Combination of therapies

Radiotherapy utilizes the DNA-damaging properties of ionizing radiation to kill tumor cells, promoting the control of tumor growth. Preclinical investigations demonstrated that radiotherapy can induce an immunogenic cell death and promote the activation of the T-cell response, establishing a proimmunogenic milieu (Golden *et al.* 2014). Immunogenic cell death is defined as the cell death that occurs associated with an improvement of molecular signals that culminate in maturation of antigen-presenting cells. Once antigen-presenting cells are mature, they can cross-present tumor antigens to T cells, leading to an effective immune response (Tesniere *et al.* 2008, Pilonis *et al.* 2015). By removing the obstacles hindering the activation and function of antitumor immune cells, inhibitors of immune checkpoints would benefit patients with antitumor immunity activated by radiotherapy (Pilonis *et al.* 2015).

Lenvatinib is an oral, multi-targeted tyrosine kinase inhibitor that impairs several signaling networks implicated in tumor growth and maintenance. This drug

is indicated for treatment of locally recurrent or metastatic progressive, radioiodine-refractory differentiated thyroid cancer (Frampton 2016). The rationale for the use of lenvatinib plus pembrolizumab is not fully understood. However, it seems reasonable that actions occurring via different mechanisms may synergize to produce the antitumor activity. In particular, preliminary results in patients with selected solid tumors demonstrated that the combination of lenvatinib and pembrolizumab has antitumor activity with partial responses, manageable toxicities and no new safety signals identified (Taylor et al. 2016).

Lenvatinib, as an angiogenic inhibitor targeting vascular endothelial growth factor receptor (VEGFR) and fibroblast growth factor receptor (FGFR), may synergistically act with immune checkpoint inhibitors (Tartour et al. 2011, Kuusk et al. 2017). This rationale is supported by the observation that proinflammatory cytokines indirectly induce angiogenesis mediated by VEGF expression (Neufeld & Kessler 2006). Reciprocally, antiangiogenic agents have an effect by decreasing myeloid-derived suppressor cell (Ko et al. 2009), regulatory T cells (Finke et al. 2008), immunosuppressive cytokines in the tumor microenvironment (Ozao-Choy et al. 2009) and the expression of negative costimulatory molecules CTLA4 and PD-1 in both CD4+ and CD8+ cells (Ozao-Choy et al. 2009). In addition, antiangiogenic agents may increase tumor-specific CD8+ cells, favoring tumor regression (Manning et al. 2007).

The prediction of response to immune checkpoint inhibitors

The success of the immune checkpoint inhibitors against several types of tumors has promoted a new treatment strategy in clinical oncology, and this has encouraged physicians to increase the number of patients who receive the immune checkpoint therapy. However, the cost of these drugs and their adverse effects indicate that a predictor of clinical response is urgently needed.

One attempt at prediction involves the histopathological assessment of the tumor microenvironment, which could hypothetically identify the tumors that would be more or less vulnerable to checkpoint blockade (Taube et al. 2012, Teng et al. 2015). According to this proposal, the tumor microenvironment could be classified into four distinct categories: type I, PD-L1-positive and presence of tumor-infiltrating lymphocytes; type II, PD-L1-negative and absence of tumor-infiltrating lymphocytes; type III,

PD-L1-positive and absence of tumor-infiltrating lymphocytes and type IV, PD-L1-negative and presence of tumor-infiltrating lymphocytes. Type I reflects a tumor microenvironment in which the gamma interferon released by tumor-infiltrating lymphocytes probably mediates the upregulation of PD-L1 in tumor cells. Thus, the type I tumors are most likely to benefit from single-agent anti-PD-1/L1 blockade, since this therapy would restore the activation of tumor-infiltrating lymphocytes by shutting down the immune escape mechanism (Teng et al. 2015). By contrast, the type II tumor microenvironment completely lacks cellular immune responses, and a poorer prognosis is thought to be associated with these tumors. At this point, it is worthwhile to note that CTLA4 blockade frequently induces increases in the intratumoral infiltration of CD8+ cells in biopsy samples (Huang et al. 2011). Hence, one could believe that the combination of anti-CTLA-4 and anti-PD-1 therapy would benefit patients with type II tumor microenvironment (Teng et al. 2015). The type III microenvironment is probably a consequence of constitutive PD-L1 expression due to oncogenic signaling (Azuma et al. 2008, Teng et al. 2015). The type IV microenvironment is likely associated with extrinsic mechanisms of immune escape, such as infiltration of myeloid-derived suppressor cells, regulatory T cells and M2 macrophages (Teng et al. 2015).

This model has some limitations. Most observations used in this model were made and validated in patients with melanoma, and few studies could replicate the same model in patients with non-melanoma solid tumors and hematological malignancies (Taube et al. 2014, Velcheti et al. 2014, D'Incecco et al. 2015). Even if the classification of the microenvironment can be obtained by simple laboratory methods, it is plausible that a mere slice of the tumor cannot fully reflect the complexity inherent in the biology of the immune-tumor interaction. Furthermore, adding to this complexity, we must consider the tumor heterogeneity and focal expression of PD-L1. Thus, a negative immunohistochemical result may not be representative of the expression of all malignant cells presented in tumor microenvironment (Taube et al. 2014). In addition, even when PD-L1 is expressed, the definition of true positivity is not completely safe from subjectivity (Taube et al. 2014, Teng et al. 2015).

Although PD-L1 expression in tumor cells correlates with the response to PD-1/PD-L1 blockade therapy (Taube et al. 2014, Daud et al. 2016), the clinical benefit is not limited to patients whose tumors are positive for PD-L1 (Herbst et al. 2014), suggesting that the expression of PD-L1

in tumor cells cannot be fully translated to the clinic as an isolated criterion (biomarker) for the indication of PD-1/PD-L1 blockage. It is possible that other characteristics of the immune microenvironment may also be important in predicting the effect of PD-L1 blockage. Tumors that respond to anti-PD-L1 therapy present elevated expression of IFN as well as of IFN-inducible genes (Herbst et al. 2014). In addition, the activation of PD-1/PD-L1 axis is a dynamic process and PD-L1 blockage may subject tumors to changes in their immune microenvironment. Patients treated with anti-PD-L1 antibody experienced a decrease in tumor size, accompanied by an increase in PD-L1 expression on tumor-infiltrating immune cells and tumor cells (Herbst et al. 2014). Additionally, unidentified factors may contribute to the response observed in patients whose tumors are negative for PD-L1. These considerations may help explain why patients whose tumors show weak or absent PD-L1 staining may still benefit from PD-1/PD-L1 blockage.

Challenges and opportunities for immune checkpoint inhibitors in ovarian cancer

Ovarian cancer is the most lethal gynecologic malignancy, and it is also considered an endocrine neoplasm. In the USA, 22,400 new cases of ovarian cancer are expected during 2017, resulting in 14,800 estimated deaths in the same year (Siegel et al. 2017). The mortality can be partially explained by advanced tumor stages at diagnosis. In particular, 60% of patients are diagnosed with distant metastasis, 20% with tumors spread to regional lymph nodes and a minority (14.8%) with disease confined to the primary site (with the remaining patients being of unknown stage) (Surveillance, Epidemiology, and End Results (SEER) database; <https://seer.cancer.gov/>). A great majority (95%) of ovarian cancers are derived from epithelial cells and other ovarian cell types, which include germ cell tumors and sex cord-stromal tumors (Berek et al. 2015). High-grade serous carcinoma is the most common ovarian neoplasm, typically bearing *TP53* and *BRCA* mutations and accounting for the majority of deaths (Kurman 2013). More than 60% of patients with epithelial ovarian cancer will relapse after platinum-based initial therapy, especially those patients diagnosed with stage III or IV disease (Jayson et al. 2014). They will eventually stop responding to systemic treatment, contributing to the very high mortality rates for this cancer. These statistics have prompted the physicians to investigate immune responses in ovarian cancer, seeking the development of new immunotherapeutic approaches.

Melichar and coworkers noted the presence of costimulatory molecules (CD80 and CD86) in lymphocytes in the ascitic fluid from patients with ovarian carcinoma and other types of peritoneal carcinomatosis (Melichar et al. 2000). Although CD28 (receptor for CD80 and CD86) was detected in the majority of CD3 cells, CTLA-4 expression on intraperitoneal tumor-infiltrating lymphocytes or peripheral blood mononuclear cells was infrequent, suggesting that this immune checkpoint is not a key mechanism by which lymphocytes promote tumor escape for ovarian tumors with peritoneal dissemination. In contrast, CTLA-4, in addition to PD-1, seems to be an important mechanism of suppression of antitumor immune response by myeloid-derived suppressor cells. Indeed, the blockage of PD-1, CTLA-4 or both in myeloid-derived suppressor cells could significantly reduce arginase I activity, a potent mechanism of local immune suppression, supporting the notion that CTLA-4 is important for immune escape led by myeloid-derived suppressor cells (Liu et al. 2009).

One *in vivo* study with a syngeneic murine ovarian cancer model demonstrated that the efficacy of CTLA-4 blockage was potentiated by combined treatment with decitabine, a DNA methyl transferase inhibitor (Wang et al. 2015). Additionally, improvement in survival was achieved by treating an immunocompetent model of *BRCA1*-deficient murine ovarian cancer with CTLA-4 blockage combined with targeted cytotoxic therapy using a PARP inhibitor (Higuchi et al. 2015). The same experiments demonstrated that the favorable outcome was mediated by an increase in the proportion of cytotoxic cells with an effector/memory phenotype (Higuchi et al. 2015). This leads to the question of how we can translate the blockage of CTLA-4 for human patients with ovarian cancer.

Table 1 summarizes the results obtained from clinical trials testing immune checkpoint inhibitors in patients with ovarian cancer. The first attempt to use ipilimumab in patients with ovarian cancer was reported by Hodi and coworkers (Hodi et al. 2003). They infused ipilimumab into two female patients with metastatic ovarian cancer who were previously vaccinated with irradiated, autologous granulocyte-macrophage colony-stimulating factor-secreting tumor cells. The authors observed a reduction or stabilization of CA-125 levels in the two patients. The authors proceeded with the investigation and treated another nine stage IV ovarian carcinoma patients by using the same antibody after the same vaccination protocol (Hodi et al. 2008), but significant antitumor effects were

observed only in a minority of patients. The authors demonstrated that the safety profile of the treatment was positive, and only two patients experienced grade 3 adverse effects with gastrointestinal toxicities. One of the patients achieved a dramatic reduction in CA-125 levels several months after the initial dose of ipilimumab, along with a substantial regression of a large, cystic hepatic metastasis and the complete resolution of mesenteric lymphadenopathy and thickening of the gastrocolic ligament. Three additional patients achieved stable disease for at least 2 months.

A phase II, open-label clinical trial (NLM 2017) investigated ipilimumab as monotherapy for the treatment of women with platinum-sensitive ovarian cancer and recurrent disease. Forty patients were intravenously injected with 10 mg/kg of ipilimumab once every 3 weeks for a total of 4 doses (induction phase), followed by additional doses once every 12 weeks (maintenance phase) until disease progression or unacceptable toxicity occurred. The primary outcome measure was the number of participants with drug-related adverse events of grade 3 (severe) or higher. The secondary outcome measure was the best overall response rate, defined as the proportion of patients who received treatment and, during the study, experienced complete or partial response confirmed by RECIST or CA-125 levels. The median age of the patients in the study was 61.5, ranging from 42 to 74 years. Two patients completed the induction phase, and 38 did not (17 due to drug toxicity, 14 due to disease progression, 1 due to adverse events not related to the drug, 1 due to death and 5 not reported). Of the 2 patients admitted to the maintenance phase, 1 experienced drug toxicity, and the other decided to withdraw. The best overall response rate was observed in 10.3% of patients according to RECIST and in 11.1% according to CA-125 levels. Twenty-six patients (65%) presented with serious adverse events, such as pneumonitis (10%), diarrhea (10%), small intestinal obstruction (10%), adrenal insufficiency (7.5%), hyponatremia, colitis, pleural effusion and incisional hernia (5% for each event). Among all adverse events, diarrhea (65%), fatigue (52.5%), pruritus (52.5%), rash (42.5%) and nausea (42.5%) were the most frequent. Notably, the dose used by the authors (10 mg/kg) was higher than that approved by the FDA for metastatic melanoma (3 mg/kg) but similar to the phase III clinical trial that investigated ipilimumab as an adjuvant therapy after complete resection of high-risk stage III melanoma (Eggermont et al. 2015). Comparing both studies that used 10 mg/kg of ipilimumab, adverse events leading to

discontinuation occurred in 17/40 (42.5%) of patients with ovarian cancer, while the same outcomes were observed in 245/471 (52%) of patients with stage III melanoma. Larger clinical trials using ipilimumab for ovarian cancer are still ongoing.

Another important immune checkpoint mechanism in ovarian cancer is mediated by the PD-1/PD-L1 axis. Abiko and coworkers by studying ovarian cancer cell lines, observed that tumor cell lysis by cytotoxic T cells was attenuated when PD-L1 was overexpressed, whereas it was promoted when PD-L1 was silenced (Abiko et al. 2013). They demonstrated that PD-L1 overexpression inhibited aggregation and degranulation of cytotoxic T cells, suggesting that PD-1/PD-L1 activation may also promote the exhaustion and dysfunction of cytotoxic T cells. On the other hand, PD-L1 on tumor cells may be induced by IFN- γ from cytotoxic T cells, and it may expel cytotoxic T cells from the tumor epithelium to the stroma, especially in ovarian cancer with peritoneal dissemination (Abiko et al. 2015). This supports the idea that complex crosstalk between tumor-infiltrating lymphocytes and ovarian tumor cells may mediate the PD-1/PD-L1 axis activation. It is not surprising that patients with ovarian cancer who were stratified based on high levels of tumor-infiltrating lymphocytes (tumors enriched with CD4+, CD8+ and PD-1+ cells) and low expression of immune regulatory molecules (TGFB1, PD-L1, PD-L2, COX-1 and COX-2) had significantly better prognosis than patients in the other groups (Hamanishi et al. 2011). Taken together, the preclinical results led scientists to pursue the blockage of the PD-1/PD-L1 axis as a therapy for patients with ovarian cancer.

In a single-center, open-label, phase II trial, Hamanishi and coworkers published interesting results (Hamanishi et al. 2015). They enrolled 20 patients with platinum-resistant recurrent or advanced ovarian cancer to receive intravenous infusion of nivolumab every two weeks at a dose of 1 mg/kg (10 patients) or 3 mg/kg (10 patients), and the primary outcomes (best overall response assessed by RECIST) and secondary outcomes (drug safety, progression-free survival, overall survival, disease control rate and adverse events) were evaluated (Hamanishi et al. 2015). The most common treatment-related adverse events were increased serum hepatic enzymes, hypothyroidism, lymphocytopenia, decreased albumin, fever, rash, arthralgia, arrhythmia, fatigue and anemia. Grade 3 or 4 treatment-related adverse events occurred in eight patients, and treatment-related serious adverse events occurred in two patients. Among all patients, the

Table 1 Results from clinical trials that have investigated immune checkpoint inhibitors in patients with ovarian carcinoma.

Agent	Mechanism	Dose (mg/kg)	Patients	No. of patients	Phase
BMS-936559	Anti-PD-L1	0.3–10	Advanced ovarian cancer	17	I
Ipilimumab	Anti-CTLA-4	3	Metastatic ovarian cancer	9	I
Ipilimumab	Anti-CTLA-4	10	Platinum-sensitive ovarian cancer	40	II
Nivolumab	Anti-PD-1	1 or 3	Platinum-resistant ovarian cancer	20	II
Pembrolizumab	Anti-PD-1	10	Advanced ovarian cancer, failure to prior treatment, positive for PD-L1	26	Ib
Avelumab	Anti-PD-L1	10	Advanced ovarian cancer	124	Ib
Durvalumab + Olaparib	Anti-PD-L1 + PPAR inhibitor	10 mg/kg (durvalumab) + 200 or 300 mg (olaparib) 2×/day; 1500 mg (durvalumab) + 300 mg (olaparib) 2×/day	Ovarian cancer and triple-negative breast cancer	10 patients with ovarian cancer + 2 patients with triple-negative breast cancer	I/II
Durvalumab + Cediranib	Anti-PD-L1 + VEGFR inhibitor	10 mg/kg + 20 or 30 mg daily	Ovarian cancer, cervical cancer and uterine leiomyosarcoma	4 patients with ovarian cancer + 2 patients with cervical cancer + 1 patient with uterine leiomyosarcoma	I/II

best overall response rate was 15%, and the disease control rate was 45%. Interestingly, four patients had a durable and evident antitumor response, and two of them (both in the 3 mg/kg cohort) demonstrated complete response to treatment. For both cohorts, the median progression-free survival time and median overall survival time were 3.5 and 20 months, respectively.

Pembrolizumab is also being investigated in ovarian cancer. In a phase Ib clinical trial, 26 patients with PD-L1-advanced solid tumors were enrolled to receive pembrolizumab at 10 mg/kg, intravenously, every 2 weeks for up to 24 months (Varga et al. 2015).

The primary outcome measure was the best overall response using RECIST. The secondary outcome measures were progression-free survival, overall survival and duration of response in the participants who achieved partial response or better. The key eligibility criteria for the ovarian cancer cohort included advanced epithelial ovarian, fallopian tube or primary peritoneal carcinoma, failure of prior therapy and PD-L1 expression, and twenty-six patients were enrolled. Three patients responded (one patient with complete response and 2 patients with partial response), and 6 patients had stable disease. The best overall response rate was 11.5%.

Clinical activity

PR	CR	ORR	SD	PD	PFS (median)	OS (median)	Safety (% of grade 3/4 AE)	References
6% (24 weeks)	0%	6% (24 weeks)	18% (24 weeks)	NR	NR ^a	NR	9 ^b	Brahmer et al. (2012)
11.1% (≥4 years)	NR	11.10%	33.3% (at least 2 months)	66.70%	NR	NR	22.20	Hodi et al. (2008)
NR	NR	BORR of 10.3% by RECIST, 11.1% by CA-125 ^c	NR	NR	NR	NR	50	NLM (2017)
5% (at least 350 days)	10% (at least 350 days)	15% (at least 350 days)	30% (one pt with almost 400 days)	50%	3.5 months	20 months	40	Hamanishi et al. (2015)
7.7% (≥24 weeks)	3.8% (≥24 weeks)	11.5% (≥24 weeks)	23% (≥24 weeks)	NR	NR	NR	3.80	Varga et al. (2015)
9.70%	0%	9.70%	44.40%	NR	11.3 weeks	10.8 months	6.50	Disis et al. (2016)
11.1% (6 months)	NR	NR	55.5% (≥4 months)	NR	NR	NR	25%	Lee et al. (2016)
28.5% (4 months)	NR	NR	28.5% (4 months)	NR	NR	NR	NR ^d	Lee et al. (2016)

^aPFS of 22% at 24 weeks. ^bConsidering all patients in the study (both ovarian and non-ovarian cancer patients). ^cCA-125 level criteria of [Rustin et al. \(1996\)](#). ^dTotal number of patients with grade 3/4 not provided.

AE, adverse events; BORR, best overall response rate; CR, complete response; NR, not reported; ORR, objective response rate; OS, overall survival rate; PD, progressive disease; PFS, progression-free survival rate; PR, partial response; SD, stable disease.

All patients experienced at least one adverse event, and the most common adverse events were fatigue, anemia and decreased appetite.

Anti-PD-L1 antibodies have been tested in patients with ovarian cancer. Brahmer and coworkers conducted a phase I clinical trial investigating the safety and clinical activity of intravenous anti-PD-L1 antibody in patients with advanced cancer (patients who had tumor progression after at least one previous course of tumor-appropriate therapy for advanced or metastatic disease) ([Brahmer et al. 2012](#)). An escalating dose regimen was adopted, ranging from 0.3 to 10mg/kg of body weight.

Anti-PD-L1 antibody was administered every 2 weeks in 6-week cycles for up to 16 cycles or until the patient had a complete response or confirmed disease progression. Seventeen women with ovarian cancer were enrolled. The objective response rate, stable disease for more than 24 weeks and progression-free survival rate at 24 weeks were 6, 18 and 22%, respectively. In the cohort, almost all patients presented with adverse events of some grade. Fatigue, infusion reactions, diarrhea, arthralgia, rash, nausea, pruritus and headache were the most common adverse events; 9% of patients presented with treatment-related grade 3 or 4 events.

The preliminary results of using avelumab in patients with ovarian cancer from the JAVELIN Solid Tumor phase Ib study were recently reported (Disis *et al.* 2016). One hundred and twenty-four patients with advanced ovarian cancer received avelumab at 10 mg/kg, intravenously, every two weeks until progression, unacceptable toxicity or withdrawal, and they were followed for a median of 54 weeks. The objective response rate was 9.7%, stable disease was observed in 44.4%, and the disease control rate was 54%. The majority of patients (66.1%) experienced treatment-related adverse events; the most common adverse events were fatigue, infusion-related reaction and diarrhea. Severe grade 3/4 treatment-related adverse events were reported in 8 patients. Another phase I trial studying durvalumab, another PD-L1 inhibitor, in combination with olaparib (PARP inhibitor) or cediranib (VEGFR inhibitor) in patients with gynecological malignancies is currently ongoing, and the preliminary results are available (Lee *et al.* 2016). The authors enrolled a total of 19 patients with ovarian cancer, triple-negative breast cancer, cervical cancer or uterine leiomyosarcoma. Durvalumab plus olaparib yielded a disease control rate of 67%, while durvalumab plus cediranib yielded a disease control rate of 57%.

Immune checkpoint inhibitors for treating thyroid cancer

Thyroid cancer is the most common endocrine malignancy. The incidence of thyroid cancer has increased by more than 200% in the United States in the last four decades, and papillary thyroid carcinoma is the histological subtype responsible for the majority of these cases (Lim *et al.* 2017, Siegel *et al.* 2017). In addition to the increase in the incidence of papillary thyroid cancer with small tumors, the incidence rates have increased for tumors that are larger (>5 cm) and tumors with regional or distant dissemination (Enewold *et al.* 2009). A recent study demonstrated that the thyroid cancer incidence-based mortality has increased by 1.1% annually between 1994 and 2013 on average (Lim *et al.* 2017), prompting scientists to find new strategies to treat patients with this disease. Moreover, the not-insignificant number of patients with radioiodine-refractory thyroid cancers, the clinical success of tyrosine kinase inhibitors for this indication as well as for medullary thyroid cancer, the success of immunotherapy against other tumors with different histological origin and the availability of basket trials to test the same experimental drug for various

different indications have recently fueled the field of tumor immunology in thyroid cancer.

The immune checkpoint has been investigated in the thyroid cancer microenvironment. We previously demonstrated that samples from thyroid cancer display more intense staining and higher mRNA levels of PD-L1 than those from benign tumors (Cunha *et al.* 2013a). In that study, we found a positive linear correlation between age at diagnosis and *PD-L1* mRNA levels. Additionally, tumors at advanced stages displayed higher *PD-L1* mRNA levels than tumors at early stages, suggesting that PD-L1/PD-1 is likely associated with the aggressive phenotypes of thyroid cancer (Cunha *et al.* 2013a).

Ahn and coworkers observed that PD-L1 was more frequently expressed among anaplastic thyroid cancer, suggesting that PD-L1 expression was a late event in thyroid carcinogenesis (Ahn *et al.* 2017). However, the authors failed to demonstrate association between PD-L1 expression and clinicopathological variables of aggressiveness (Ahn *et al.* 2017). These results were confirmed by Zwaenepoel and coworkers, who found PD-L1 expression in 28.6% of anaplastic thyroid cancer (Zwaenepoel *et al.* 2017). PD-1 was not expressed on the tumor cells. Similarly, Bastman and coworkers identified PD-L1 expression in 13 out of 22 tumors and observed different immunohistochemical expression patterns in differentiated thyroid cancer (largely focal) and anaplastic thyroid cancer (more diffuse) (Bastman *et al.* 2016). Interestingly, they found that differentiated thyroid cancer was enriched with PD-1+CD4+ and CD8+ T cells (Bastman *et al.* 2016). We also observed that PD-L1 expression was correlated with intratumoral infiltration of CD4+, CD8+, CD20+ and FOXP3+ cells; tumor-associated macrophages and myeloid-derived suppressor cells (Cunha *et al.* 2013a). Notably, differentiated thyroid tumors are frequently found to be enriched with a mixture of immune cells (e.g., mast cells, lymphocytes, macrophages) that may be complexly related to prognosis (French *et al.* 2017). By contrast, almost no expression of PD-L1 was found in medullary thyroid carcinoma cells and accompanying inflammatory cells. Certainly, more studies are warranted to replicate these results and clarify these findings (Bongiovanni *et al.* 2017).

Angell and coworkers demonstrated that papillary thyroid carcinomas harboring the BRAF V600E mutation frequently express PD-L1 (Angell *et al.* 2014). Similarly, positivity for the BRAF V600E mutation was associated with the expression of HLA-G (a nonclassical and inhibitory MHC class I molecule). Those authors found that tumors

with BRAF V600E mutation presented lower CD8+/FOXP3+ cell ratios and were enriched with arginase-1+ myeloid populations (tumor-associated macrophages and myeloid-derived suppressor cells). These results demonstrate that a strongly immunosuppressive molecular profile may be elicited in the tumor microenvironment of papillary thyroid carcinomas positive for the *BRAF V600E* mutation, suggesting that the promotion of tumor immune escape may be a mechanism by which the *BRAF V600E* mutation may contribute to aggressive tumor behavior (Angell et al. 2014). Following this rationale, Brauner and coworkers thoroughly investigated the PD-1/PD-L1 immune checkpoint mechanism in a thyroid cancer model (Brauner et al. 2016). In their study, the authors demonstrated that thyroid cell lines and tumor specimens with the *BRAF V600E* mutation displayed higher baseline expression of PD-L1 mRNA compared with *BRAF WT* thyroid cells and *BRAF WT* tumor samples, respectively. They demonstrated that SCID mice injected with human thyroid cancer cells and treated with BRAF inhibitor for two weeks displayed significantly reduced *in vivo* expression of the PD-L1 mRNA. One week after tumor implantation, the mice were randomized for treatment with anti-PD-L1 antibody, PLX4720 (a selective inhibitor of oncogenic *BRAF V600E* (Tsai et al. 2008)) or a combination of the two agents. Treatment with anti-PD-L1 antibody alone was not able to reduce tumor volume. However, the authors showed that the combination of the *BRAF V600E* inhibitor and the anti-PD-L1 antibody acted synergistically, effectively reducing tumor volume compared to that in the other treatment groups. Notably, tumors from mice treated with anti-PD-L1 antibody or *BRAF V600E* inhibitor alone were markedly enriched with effector cytotoxic T cells, and the staining for granzyme B was increased in the combinatorial treatment group. These results suggest that immune checkpoint inhibitors that target PD-L1 along with *BRAF V600E* inhibitors would synergistically boost the cellular immune response of patients with thyroid cancer, promoting the effective elimination of tumor cells.

Severson and coworkers investigated the immune response triggered in metastasis in the lymph nodes of patients with differentiated thyroid cancer (Severson et al. 2015). They observed that PD-1+CD4+ cells and PD-1+CD8+ cells were enriched in 8/12 lymph node samples. The proliferative capacity of both CD4+ and CD8+ lymphocytes was maintained. Interestingly, the CD8+ cells from the PD-1+ lymphocyte-enriched lymph nodes were compromised in their ability to produce IL-2 and TNF α when compared with the control cells

from lymph node samples that lacked resident PD-1+ T cells. Additionally, the authors observed that although the CD8+ T cells were capable of degranulation, their cytotoxic ability was impaired. These results suggest that the lymph node likely provides an inflammatory microenvironment that fails to completely eliminate tumors cells, but it maintains a residual potential of effective antitumor immune response that likely impedes tumor growth and dissemination. The authors further suggested that immunomodulatory therapies that inhibit PD-1/PD-L1, such as pembrolizumab and nivolumab, may be an option for patients with differentiated thyroid cancer and persistent lymph node metastasis.

Clinical trials evaluating immune checkpoint inhibitors against thyroid cancer are ongoing (Table 2). Preliminary results were obtained from the study NCT02054806 (Mehnert et al. 2016). Twenty-two patients with papillary or follicular advanced thyroid cancer, who failed standard therapy and presented with PD-L1 expression in $\geq 1\%$ of tumor or stroma cells by immunohistochemistry, were treated with pembrolizumab at 10 mg/kg given every 2 weeks for up to 24 months or until confirmed progression, intolerable toxicity, death or withdrawal of consent. The median follow-up duration was 73.5 weeks. Eighteen patients had treatment-related adverse events; diarrhea and fatigue were the most common events, and none of the patients died or discontinued pembrolizumab because of adverse events. Two patients had a partial response, and the stable disease rate was 54.5%. The 6-month overall survival rate was 100%, and the 6-month progression-free survival rate was 58.7%, suggesting that more studies are warranted in order to clarify the clinical benefit of pembrolizumab in patients with advanced thyroid cancer.

Tracking immune checkpoint inhibitors in pancreatic cancer

Pancreatic adenocarcinoma is an aggressive malignancy that displays a high rate of treatment resistance (Bazhin et al. 2014). At the time of diagnosis, the majority of patients are in advanced stages, and surgery has little or no impact on their prognosis (Ryan et al. 2014). The 5-year survival of patients with metastatic or recurrent tumors remains lower than 5% in the most optimistic series, regardless of the diversity of treatment approaches, which has improved the therapeutic management (Conroy et al. 2011, Bazhin et al. 2014).

Table 2 Ongoing clinical trials registered in clinicaltrials.gov. Most of the clinical trials are recruiting patients with thyroid cancer to receive immune checkpoint inhibitors.

Intervention (s)	Identifier	Sponsor	Collaborator	Phase	Patients	Intervention model	Masking	Status
Ipilimumab+enoblituzumab	NCT02381314	MacroGenics	No	1	Thyroid cancer + tumors that progressed in spite of treatment	SGA	Open label	R
Nivolumab + ipilimumab	NCT02834013	NCI	No	2	Thyroid cancer + other solid tumors	SGA	No masking	R
Pembrolizumab + enoblituzumab**	NCT02475213	MacroGenics	No	1	Thyroid cancer + tumors that progressed in spite of treatment	SGA	Open label	R
Nivolumab + ipilimumab + lirilumab***	NCT01714739	Bristol-Myers Squibb	No	1/2	Thyroid cancer + other advanced solid tumors	PA (RD)	Participant, care provider, investigator	R
Ipilimumab + stereotactic body radiation therapy	NCT02239900	M.D. Anderson Cancer Center	Bristol-Myers Squibb	1/2	Thyroid cancer + other metastatic tumors*	PA (RD)	Open label	R
Pembrolizumab	NCT02688608	University of Texas Southwestern Medical Center	Merck Sharp & Dohme Corp	2	ATC	SGA	Open label	R
Pembrolizumab	NCT03072160	NCI	No	2	Medullary thyroid cancer	PA (non-RD)	No masking	Not yet R
Pembrolizumab + lenvatinib	NCT02973997	Academic and Community Cancer Research United	NCI	2	Advanced thyroid cancer	SGA	No masking	Not yet R
Pembrolizumab	NCT02721732	M.D. Anderson Cancer Center	Merck Sharp & Dohme Corp	2	ATC + other advanced solid tumors	PA (non-RD)	No masking	R
Pembrolizumab	NCT03012620	Unicancer	NCI, France; Ligue contre le cancer, France; Merck Sharp & Dohme Corp	2	Thyroid cancer + other advanced tumors	SGA	Open label	Not yet R
Pembrolizumab	NCT02628067	Merck Sharp & Dohme Corp.	No	2	Thyroid cancer + other advanced tumors	SGA	No masking	R
Durvalumab + tremelimumab + stereotactic body radiotherapy	NCT03122496	Memorial Sloan Kettering Cancer Center	AstraZeneca	1	ATC	SGA	No masking	R

Most of the clinical trials are still recruiting patients with advanced thyroid cancer of any histology to receive immune checkpoint inhibitors.

*Patients with anaplastic thyroid cancer will be waived from the inclusion criteria given the rapid trajectory of their disease. **Enoblituzumab targets B7-H3. ***Lirilumab targets killer-cell immunoglobulin-like receptor, preventing the inhibition of natural killer cells.

ATC, anaplastic thyroid cancer; NCI, National Cancer Institute; PA, Parallel Assignment; R, recruiting; RD, randomized; SGA, Single Group Assignment.

Immune checkpoint therapies appear to be promising mainly in combination with vaccines, radiation or cytotoxic agents. Some authors have demonstrated encouraging results, such as delayed progression of pancreatic adenocarcinoma with 3.0 mg/kg of ipilimumab (Royal *et al.* 2010). Similar results were found for the blockage of the PD-1/PD-L1 axis, and the anti-PD-L1 antibody exhibited disappointing results in a phase I trial in patients with pancreatic adenocarcinoma (Brahmer *et al.* 2012). Although many studies are currently ongoing, it is reasonable to say that single immune checkpoint blockage is not a clinical option at this time.

Why immune checkpoint therapy is not efficient in pancreatic adenocarcinoma is not completely understood. It has been speculated that PD-L1 expression is associated with responses to PD-1/PD-L1 blockage therapy. Since the majority of pancreatic adenocarcinoma cases are negative for PD-L1 (Nomi *et al.* 2007), this might help explain the lack of success of anti-PD-1/PD-L1 therapy. Additionally, poor survival was observed in patients whose tumors were positive for PD-L1, probably due to an immunosuppressive microenvironment mediated by PD-L1 expression (Nomi *et al.* 2007). Indeed, Clark and coworkers thoroughly investigated the immune response to pancreatic cancer in a mouse model and observed the lack of effector immune cells in the tumor microenvironment (Fig. 3) (Clark *et al.* 2007). The authors observed a marked infiltration of Treg cells into the pancreas even before the development of invasive disease (Clark *et al.* 2007). In addition, myeloid-derived suppressive cells were observed at slightly elevated levels in pre-invasive lesions, but they became a prominent component of the leukocytic infiltrate in invasive pancreatic neoplasms (Fig. 3) (Clark *et al.* 2007). This markedly immunosuppressive microenvironment makes the use of immunotherapy in pancreatic adenocarcinoma particularly challenging.

An interesting strategy for overcoming the immunosuppressive tumor microenvironment may be

the use of different combinations of immune checkpoint inhibitors and other treatments. The combined treatment with gemcitabine and PD-L1 blockade displayed a synergistic antitumor effect on pancreatic cancer, resulting in complete response in treated mice (Nomi *et al.* 2007). Furthermore, immune checkpoint inhibitors can be used with chemotherapeutic agents, since a synergistic effect was observed with ipilimumab and cytotoxic drugs in solid tumor models (Lynch *et al.* 2012, Jure-Kunkel *et al.* 2013). Le and coworkers performed a phase Ib, open-label, randomized study to investigate combinations of different modalities of immunotherapy (Le *et al.* 2013). They demonstrated that patients who received 10 mg/kg of ipilimumab combined with GVAX vaccine had a slightly longer survival than patients who received 10 mg/kg of ipilimumab alone. Radiotherapy could be an excellent adjuvant for immune checkpoint inhibitors. Interestingly, response in distant metastasis is observed when the primary tumor is irradiated (Blanquicett *et al.* 2005). The mechanism underlying this observation is unclear, but it is likely mediated by immunologic reactions (Vatner *et al.* 2014), thereby supporting the idea that the combination of immune checkpoint inhibitors and radiotherapy warrants further investigation.

The initial assessment of immune checkpoints in other endocrine malignancies

Adrenocortical carcinoma

Adrenocortical carcinoma is a rare endocrine malignancy, and the SEER database states that its estimated incidence is 0.72 cases per million per year in the United States (Kebebew *et al.* 2006). Adrenocortical carcinoma is an aggressive disease, and the stage of the tumor at diagnosis is the factor with the greatest impact on prognosis (Kerkhofs *et al.* 2015). Fay and coworkers investigated the expression of PD-L1 in adrenocortical carcinoma tissues (Fay *et al.* 2015).

Figure 3

Immune microenvironment in pancreatic adenocarcinoma. Immune cells (e.g., T cells, macrophages, monocytes, mast cells, neutrophils and myeloid-derived suppressor cells) are frequently found in these tumors. Although some effector cells are sparsely observed in the leukocytic infiltrate, pancreatic cancer tissues are markedly infiltrated by immune cells with immunosuppressive functions, from early pre-invasive to advanced overt invasive stages. Additionally, invasive tumors are enriched in myeloid-derived suppressor cells.

They observed that PD-L1 was expressed in the membranes of tumor cells in 3/28 (10.7%) patients. By contrast, PD-L1 was expressed by tumor-infiltrating lymphocytes in 19/27 (70.4%) patients. PD-L1 in both tumor cells and tumor-infiltrating lymphocytes failed to predict prognosis in this set of patients. Although the severity of the disease demands new therapies, its rarity impairs the development of innovative approach in the field of immunotherapy. A small number of studies are registered in the clinical trials website.

Neuroendocrine tumors

Neuroendocrine tumors are typically slowly proliferating neoplasms. The standard treatment includes somatostatin analogues, IFN- α , mTOR inhibitor and chemotherapy (Kotteas et al. 2016). Kim and coworkers investigated PD-L1 expression in 24 foregut-derived and 8 hindgut-derived gastroenteropancreatic neuroendocrine tumors (Kim et al. 2016). They observed that only a minority (21.9%) of samples expressed PD-L1 (Kim et al. 2016). Expression of PD-L1 was associated with high-grade classification and unfavorable prognosis (Kim et al. 2016). More studies are needed to elucidate the clinical role of blockage the PD-1/PD-L1 axis.

Concluding remarks

The promising results of immune checkpoint inhibitors have prompted academics, cancer survivors and investors to accelerate and improve research regarding the development of these drugs. This has resulted in five approved checkpoint inhibitors and several that are currently in clinical pipelines. Endocrine neoplasms are increasingly being addressed by using this therapeutic modality, and several ongoing studies are being conducted on patients with endocrine cancers. Notably, some tumor types (e.g., pancreatic adenocarcinoma, adrenocortical carcinoma and neuroendocrine tumors) require further study because the available data in the literature are not sufficient for clinical translation.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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Author contribution statement

Lucas Leite Cunha: Conception and design, selection of notable articles for review, critical review of the literature, composition of the manuscript and final approval. Marjory Alana Marcello: Design, selection of notable articles for review, critical review of the literature, composition of the manuscript and final approval. Vinicius Rocha Santos: Design, selection of notable articles for review, critical review of the literature, composition of the manuscript, clinical and translational orientation and final approval. Laura Sterian Ward: Design, selection of notable articles for review, critical review of the literature, composition of the manuscript, clinical and translational orientation and final approval.

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