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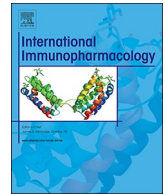
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Factors affecting tumor responders and predictive biomarkers of toxicities in cancer patients treated with immune checkpoint inhibitors

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ABSTRACT

Cancer immunotherapy has brought a great revolution in the treatment of advanced human cancer. Immune checkpoint inhibitors (ICIs) that target cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and the programmed cell death protein 1 pathway (PD-1/PD-L1) have been widely administrated in the past years and demonstrated promising in a variety of malignancies. While some patients show benefit from ICIs, others do not respond or even develop resistance to these therapies. Among the responders, the treatments are consequently accompanied with immune-related adverse effects (irAEs), which are diverse in their effected organs, degree of severity and timing. Some of the toxicities are fatal and result in discontinuance of immunotherapy. The toxicity profile from anti-CTLA-4 to anti-PD-1/PD-L1 immunotherapies is distinct from those caused by conventional anticancer therapies, though their presentation may be similar. In order to better help clinicians recognize, monitor and manage irAEs in a growing population of cancer patients who are receiving ICI therapy, this article summarizes the FDA approved ICIs and focuses on (1) existing toxic evidence related to ICIs, (2) occurrence of irAEs, (3) factors influencing tumor responders treated with ICIs, (4) predictive biomarkers of irAEs, and (5) new potential mechanisms of resistance to ICI therapy.

1. Introduction

Cancer patients at their early disease stage are more likely to well respond to most of conventional regimens with a favorable prognosis, whereas those with an advanced disease do not. Immunotherapy is a novel approach developed in the last decade and approved by the United States Food and Drug Administration (FDA) to treat advanced malignancies, which eradicates cancer cells by reinvigorating immune system, activating tumor antigen-specific cytotoxic T lymphocytes (CTLs) with improved clinical outcome in cancer.

Emergence of immune checkpoint blockades opens a new era of cancer therapy. 2018 Nobel Prize in Physiology or Medicine in the discovery of cancer therapy by inhibition of negative immune regulation further ignites enthusiasm to immunotherapeutic for more cancer types [1]. Monoclonal antibodies (mAbs) targeting CTLA-4, PD-1, or

PD-L1 have received considerable and broad interest because of their ability to exert enduring clinical response in many first- and second-line regimen-refractory cancers [2–4]. Some patients generated abiding responses and therefore their survival was improved [5]. Anti-PD1/PD-L1 mAbs increase overall survival (OS) compared to the standard of care in different tumor types. These immune checkpoint inhibitors (ICIs) truly revolutionize the treatment of cancer patients particularly with an advanced disease [6,7]. Nonetheless, the response rates still remain relatively low [4,8]. Some patients initially respond to ICI therapy, but develop resistance after relapse [9–11]. A certain proportion of patients still have a progressive disease in spite of initial response [12], and others do not respond at all [9]. The proportion of patients in the United States who are eligible for these ICIs is approximately 44%, while around 13% respond to these ICIs [4].

Presently, ICIs in combination are being extensively evaluated for

Abbreviations: APCs, Antigen-presenting cells; CR, complete response; CTLA-4, cytotoxic T lymphocyte-associated antigen 4; DCs, dendritic cells; irAEs, immune-related adverse effects; LAG-3, lymphocyte-activation protein 3; NK, natural killer cells; OS, overall survival; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PFS, progression free survival; TIM-3, T-cell immunoglobulin and mucin-domain containing-3; TILs, tumor-infiltrating lymphocytes

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potential clinical benefit in a large number of tumor histology. Due to positive outcomes in preliminary trials, nivolumab plus ipilimumab is one of the most enthusiastically investigated combined immunotherapy regimens. Among many cancer types, melanoma is the one with a high response rate to the ICI therapy [13,14]. On Oct. 22, 2018, Bristol-Myers Squibb (BMS) Company announced that the four-year Phase III CheckMate-067 clinical trial with three arms showed more enduring, long-term survival benefits with the combination of Nivolumab and Ipilimumab versus either Nivolumab or Ipilimumab alone in patients with advanced melanoma [15,16]. With a minimum follow-up of 48 months, the four-year OS rates were 53% for the Nivolumab plus Ipilimumab in combination, 46% for Nivolumab alone, and 30% for Ipilimumab alone. Additionally, the percentage of the patients experiencing a complete response (CR) was 21% for Nivolumab plus Ipilimumab, 18% for Nivolumab alone, and 5% for Ipilimumab alone [16]. This result suggests that inhibiting both CTLA-4 and PD-1/PD-L1 could synergistically reactivate T cells and were beneficial to patients [17]. Consequently, the combination of the two regimens enhances the efficacy. Indeed, the more recently published results revealed that the combination of Ipilimumab and Nivolumab had a significantly better Progression Free Survival (PFS) compared to chemotherapy in NSCLC [18], with a 42.6% PFS rate for the immunotherapy combo compared to a 13.2% for chemo. Accumulating clinical trial data validate the benefit and rationale of this combinatorial immunotherapy as a powerful new treatment modality for the treatment of cancer.

The most successful immunotherapy to date is the blockade of the immune checkpoints PD-1 and CTLA-4 [19]. However, immune checkpoint blockade can lead to immune-related adverse events (irAEs) that affect any organ system with various clinical presentations. Most of the irAEs are mild, but some are severe and could result in the withdrawal of immunotherapy, morbidities or even death. A meta-analysis of 112 clinical trials involving 19,217 patients who received ICIs showed the toxicity-related fatality rates of 0.36% (anti-PD-1), 0.38% (anti-PD-L1), 1.08% (anti-CTLA-4), and 1.23% (PD-1/PD-L1 plus CTLA-4), respectively [20]. There's been a lot of fanfare about the efficacy of the ICIs, but less attention has been paid to the occurrence, prevention, prediction of irAEs, and the resistance mechanisms. Early recognition and treatment of these irAEs represent an important clinical challenge for Oncology physicians. Clinicians across disciplines should not be unaware of these uncommon lethal complications. This article presents the existing toxic evidence related to ICIs, factors influencing tumor responders, prediction and treatment of these irAEs, as well as the new mechanisms of resistance to ICI therapy.

2. T cell exhaustion and T cell anergy

The immune system consists of innate and adaptive immunity. The innate immune cells target invaders at the first sign of infections or inflammation. Adaptive immune cells, including T cells, recognize and attack specific antigens. Immune evasion is a strategy used by cancer cells to escape host's immune attack, which involves a number of mechanisms, including such as T-cell exhaustion, immunosuppressive cytokines that “cool down” the immune system. T cell exhaustion is a state of T cell dysfunction that arises from long-term antigen stimuli in cancer and chronic infections. It is defined by poor effector function with sustained expression of inhibitory receptors [21]. The silence of effector T cells is T cell exhaustion, which is different from anergy. T cell anergy is a tolerance mechanism in which the lymphocytes are intrinsically functionally inactivated following an antigen encounter [22]. The role of anergy is an acquired state of T cells in functional unresponsiveness [23], but they still remain alive for an extended period in a hyporesponsive state [22].

Many different molecular and cellular mechanisms contribute to the failure of T cells to eradicate cancer cells. Via immune suppressive networks, ongoing T cell functions are impaired and enable tumor immune escape [24]. In order to avoid autoimmunity and

immunopathology, immune system also induces self-tolerance. To prevent destruction of the immune system, some effector T cells functionally silence. Cancer cells normally find ways to engage as checkpoint molecules to inactivate T cells for avoiding immune attack. The inhibitory mechanisms can be abused by cancer cells that evade immune responses [25].

3. Mechanism of CTLA 4 and PD-1/PD-L1 inhibition

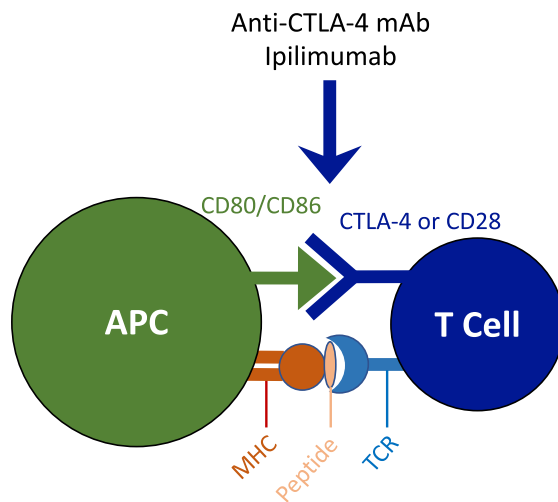
The immune system produces an appropriate immune response against pathogens and cancer. T cell activation plays a pivotal role in killing pathogens and tumor cells. We have reported that T cell activation score, which summarizes weighted average of exhaustion- and activated-related gene expression, is positively associated with patient survival in breast cancer [26]. T cell exhaustion, which is characterized by progressive loss of T cell function, leads to poor prognosis. Recent evidence suggests that the suppressive signals within the tumor microenvironment induce intratumoral T cells in dysfunctional states [27]. The key features of T-cell exhaustion include increased expression levels of inhibitory receptors (e.g., PD-1 and CTLA4), reduced levels of cytokines (e.g., IFN- γ , CCL3 and CCL4), weakened cell proliferation potential, and altered transcriptional program/epigenetic landscape. These suppressive signals including PD-1, CTLA-4, T-cell immunoglobulin mucin-domain containing-3 (TIM-3) and lymphocyte-activation gene 3 (LAG-3, CD223) affect the normal functions of T cells that consequently cannot produce effector cytokines (e.g., PRF1, GZMA, and GZMB).

Both CTLA-4 and PD-1 have similar inhibitive effects on T-cell activity. However, the differences exist between them in the timing of downregulation, the responsible signaling mechanisms, and the anatomic locations of immune inhibition. CTLA-4 is thought to regulate T-cell proliferation at early stage in immune response, primarily in lymph nodes, whereas PD-1 suppresses T cells later, primarily in peripheral tissues or at the tumor site. Unlike CTLA-4 expression confined to T cells, PD-1 is more broadly expressed on activated T cells, B cells, and myeloid cells [28]. While CTLA-4 functions during the priming phase of T-cell activation, PD-1 functions during the effector phase, predominantly within peripheral tissues [28]. To overcome these issues, highly effective immunotherapies that simultaneously target these inhibitory receptors expressed by exhausted CD8⁺ T cells (Tex cells), such as PD-1 or CTLA-4, have shown dramatic effects among patients with melanoma, and also showed potential therapeutic effects on patients with breast cancer [29], ovarian cancer, and other cancer such as glioblastoma [30].

Excitingly, the successful reinvigoration of T cell function by blockade of CTLA-4 [31,32], PD-1, or its ligand PD-L1 [33] highlights the importance of the PD-1/PD-L1 axis in T cell dysfunction. Therapeutic reinvigoration of tumor antigen-specific T cells greatly improved clinical outcome of cancer patients.

4. Immune checkpoints on activation of anti-tumor immune response

Both the CTLA-4 and PD-1 can participate in T cell dysfunction, but they do not have exactly the same impact on immune system homeostasis, as demonstrated in murine models [34]. CTLA-4 and PD-1 act at different times in the cancer-immunity cycle. CTLA-4 limits T-cell responses early in immune response, primarily in lymphoid tissue; PD-1 limits T-cell response later in immune response, primarily in peripheral tissues. CTLA-4 expressed by T cells and PD-1 expressed by both T cell and other immune cells. Similarly, CTLA-4 ligands expressed by professional APCs, PD-1 ligands expressed by both APCs and other immune cells, and also can be inducibly expressed on tumor cells [35]. CTLA-4 blockade allows the activation and proliferation of more T-cell clones and reduces regulatory T cells (Treg)-mediated immunosuppression. PD-1 pathway blockade restores the activity of antitumor T cells that



APC: antigen presenting cell; CTLA-4: Cytotoxic T-lymphocyte-associated protein 4;
CD: Cluster of differentiation

have become quiescent. A dual pathway blockade could have a synergistic effect, resulting in a larger and longer lasting antitumor immune response.

CTLA-4 is constitutively expressed on Treg or induced as following T-cell activation via CD28 and T-cell receptor (TCR) signaling, but it is only upregulated in conventional T cells after activation. This accompanying expression of CTLA-4 on activated T cells may provide a 'balance' system to avoid over-reaction-induced tissue injury. CTLA-4 and CD28 receptors share two ligands, CD80 and CD86, but CTLA-4 has a higher affinity and avidity for CD80 than CD86. The levels of CTLA-4 expression in most resting T cells are extremely low or absent, and CTLA-4 predominantly appears following T-cell activation. CTLA-4 acts as an "off switch" when bound to CD80 or CD86, which are on the surface of APCs. Thus, as a brake, CTLA-4 downregulates immune responses. By utilizing this mechanism, cancer cells sometimes can avoid the attack of the immune system. When CTLA-4 signaling outweighs CD28 signaling, the T-cell will become anergic or undergo apoptosis. However, Ipilimumab can reverse the inhibitory signals transmitted to T-cells, and thus activate the immune system by targeting CTLA-4 (Fig. 1).

Programmed cell death protein 1 (PD-1) is a co-inhibitory molecule of T-cell receptor signaling [36]. It is more widely expressed than CTLA-4 and can be detected not only on activated T cells, but also B cells and natural killer (NK) cells. In addition, it lasts a longer time than CTLA-4 (6–12 h for PD-1 vs 1 h for CTLA-4) [37]. PD-L1, a ligand of PD-1, is expressed on both cancer cells and tumor-infiltrating lymphocytes (TILs) [38]. The engagement of PD-1/PD-L1 suppresses T cell function and the axis is considered one of the major pathways involved in tumor immune evasion [39] (Fig. 2A). PD-1 and its ligands PD-L1/PD-L2 negatively regulate immune responses. These immune checkpoint pathways downregulate T-cell activation to maintain peripheral tolerance, allowing cancer cells to escape immune surveillance. These cancer cells carry accumulated genetic and epigenetic alterations and use several mechanisms to be the victorious of the immunoediting process. Antibodies of either PD-1 or PD-L1 can block inhibitory signals transmitted to T cells and allow the metabolic reprogramming of T cells (Fig. 2B Activated anti-tumor immune response-PD-1 and PD-L1 signaling).

Among the immune checkpoints, CTLA-4 and PD-1 are the most extensively investigated. Others are still in pipeline such as adenosine A2A receptor (A2AR) [40], indole 2,3-dioxygenase (IDO) [41], LAG3 [42], Siglec7 [43], and the new Siglec-15 that was recently reported by Lieping Chen group as a potential target for normalization cancer immunotherapy [44], TIM3 [45], and VISTA [46], etc.

Fig. 1. Targeting CTLA-4 to activate immune system. CTLA-4 is constitutively expressed on Tregs or induced following T-cell activation via CD28 and TCR signaling. CTLA-4 and CD28 receptors share both CD80 and CD86 ligands. When CTLA-4 signaling outweighs CD28 signaling, the T cells become anergic or undergo apoptosis. Ipilimumab (Anti-CTLA-4) can reverse inhibitory signals and thus activate immune system.

5. Mechanisms of the ICIs against cancer

Immune checkpoints are actually a normal part of the immune system with the role of preventing immune response from being too strong to destroy healthy cells in the body. Certain protein receptors located on the surface of T cells help distinguish healthy cells from cancer cells. Current ICIs target CTLA-4 and PD-1 receptors. The PD-1 and PD-L1 is a receptor-ligand system and in tumor microenvironment, their engagement results in the blockade of anti-tumor immune responses [47]. PD-1 is mainly expressed on T cells, whereas PD-L1 is expressed on both cancer cells and APCs. The PD-1 receptor seeks out the PD-L1 protein on other cells (e.g. tumor cells) to determine if those cells are healthy. Cancer cells are able to disguise themselves as healthy cells by sending a signal to PD-1 using the protein PD-L1. Pembrolizumab, nivolumab and atezolizumab target PD-1 receptor and disrupt the signal from PD-L1, exposing cancer cells for attacking. Like PD-1, CTLA-4 receptor helps the immune system target tumor cells, but CTLA-4 does not need to link with a specific protein to regulate an immune response. Ipilimumab rewires the CTLA-4 receptor to stimulate an immune system attack. All these ICIs work by blocking checkpoint proteins from binding with their partner proteins. These prevent the "off" signal from being sent, allowing the T cells to kill cancer cells. ICIs may be used in combination or in conjunction with other cancer treatments, such as chemotherapy or/and radiotherapy, which generate 'inflammation' or 'hot' tumors and neoantigens. It has been reported that the deficiency of DNA damage repair-related genes could increase the effect of T cell activation on patient survival in breast cancer [48]. ICIs have been expanded from melanoma to other solid cancers in clinic, including breast, bladder, cervical, colorectal, head and neck cancer, Hodgkin lymphoma, liver, lung, renal cell and stomach cancer.

6. ICIs and their adverse effects

To date, the US FDA has approved seven ICIs as shown in Table 1. Ipilimumab, the first one approved by the US FDA in 2011, targets CTLA-4. From then, several ICIs have been granted marketing authorization. The US FDA approved the second ICI Nivolumab in 2014, which targets PD-1. Combination of Ipilimumab and Nivolumab was approved in 2015. Atezolizumab is the third ICI approved by the US FDA in 2016, which target PD-L1. Pembrolizumab is the fourth ICI that target PD-1 with the US FDA approval in 2017. Avelumab is a PD-L1 blocking antibody, the fifth ICI approved by the US FDA in 2017. The sixth ICI that target PD-L1 is Durvalumab, which was approved by the

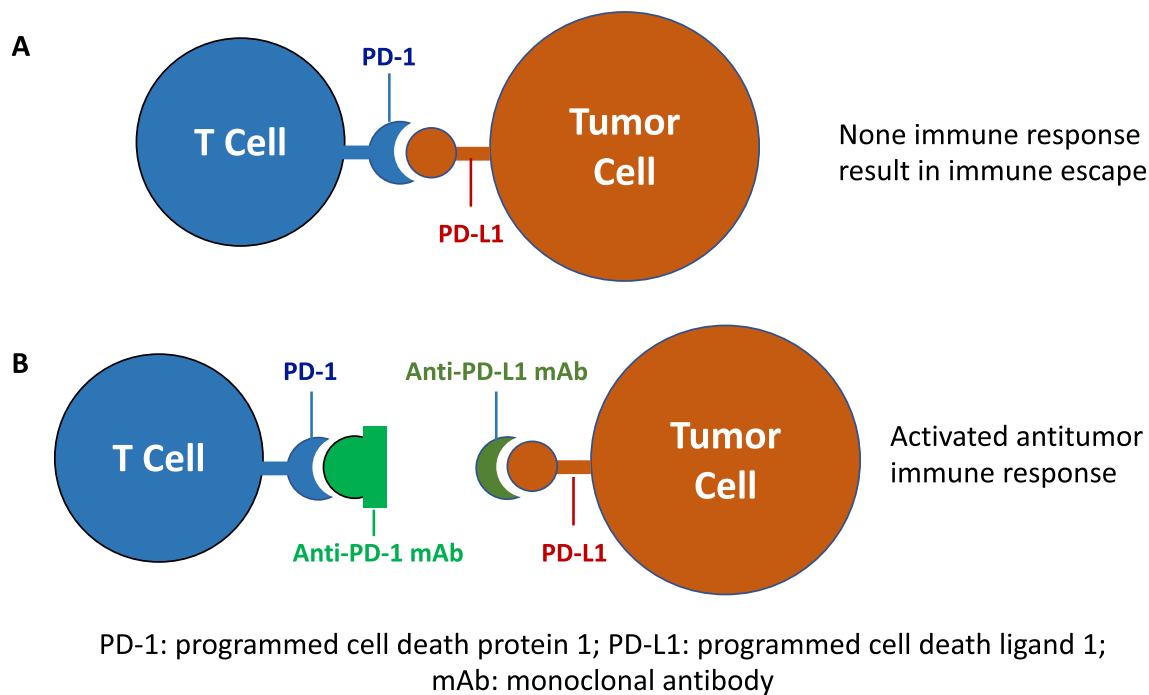


Fig. 2. Activated anti-tumor immune response on PD-1/PD-L1 signaling inhibitors. PD-1 is a co-inhibitory molecule of TCR signaling, which can be detected on activated T cells. PD-L1, a ligand of PD-1, is expressed on cancer cells. The engagement of PD-1/PD-L1 suppresses T cell function, this result in tumor escape (Fig. 2A). Antibodies of either PD-1 or PD-L1 can block inhibitory signals transmitted to T cells and consequently activate anti-tumor immune response (Fig. 2B).

US FDA in 2017. The latest one is Cemiplimab that target PD-1, approved by the US FDA in 2018.

There are three mAbs that target PD-1 including Pembrolizumab (Keytruda), Nivolumab (Opdivo) and Cemiplimab (Libtayo). Both Pembrolizumab and Nivolumab were approved in 2014, but Nivolumab was approved in Dec. 2014, only 3 months later than the approval time of Pembrolizumab in Sept. 2014. Both of them can be used for unresectable or metastatic melanoma and disease progression following therapy with Ipilimumab and BRAF inhibitor in patients who carry a BRAF V600 mutation. Nivolumab can be used for metastatic squamous NSCLC with progress on or after platinum-based chemotherapy as well. It should be noted that *BRAF* is a human gene, which encodes BRAF protein. A specific mutation in *BRAF* gene (e.g. V600) may be found in some cancer types including melanoma and colorectal cancer [49]. Because BRAF V600 mutation may increase the growth and spread of cancer cells, checking for BRAF mutation in tumor tissues may help clinicians to tailor cancer treatment with targeted therapy of BRAF inhibitor [50]. As for the three PD-L1 mAbs, their clinical usage is different. Atezolizumab had FDA approval in May 2016 for second-line treatment of NSCLC and urothelial carcinoma. Avelumab was approved in March 2017 for rare skin cancer Merkel cell carcinoma, and Durvalumab was approved in May 2017 for second-line urothelial carcinoma. The results of a meta-analysis on nineteen randomized clinical trials involving 11,379 patients showed that anti-PD-1 exhibited superior OS (HR, 0.75; 95% CI, 0.65–0.86; $p < 0.001$) and PFS (HR, 0.73; 95% CI, 0.56–0.96; $p = 0.002$) compared with anti-PD-L1. No significant difference was observed in their safety profiles [51].

ICIs enhance anti-tumor immunity and reinvigorate immune system to fight cancer. However, the irAEs arising from ICIs treatment may occur in any part of the body. The incidence of irAEs varies depending on the administration of ICI types and doses, and the type of cancer. These side effects are graded by degree. Grade 1 (mild severity) or Grade 2 (moderate severity) events generally are managed symptomatically without stopping treatment. But, patients with persistent Grade 2 of irAEs need to discontinue the treatment, until the symptoms improved. For patients with Grade 3 or Grade 4 symptoms, treatment will

be typically stopped, and a specialist referral need to be arranged. The reported incidence of any-grade irAEs associated with single ICI treatment ranges from 15% to 90% [52]. The most common and well-documented types of side effects are rash (24% in patients treated with CTLA-4 inhibitors, 15% in patients treated with PD-1/PD-L1 inhibitors), diarrhea (27–54% in patients treated with CTLA-4 inhibitors, and it is very low in patients treated with PD-1/PD-L1 inhibitors), fatigue (36% in patients with early fatigue, 42.9% in patients with delayed fatigue) [53], colitis (18.8% in patients treated with CTLA-4 inhibitors) [54], liver toxicity (3% to 9% for Ipilimumab and 0.7% to 1.8% for PD-1/PD-L1 inhibitor) [55], pneumonia (very low in patients treated with CTLA-4 inhibitors, 3–6% in patients treated with PD-1/PD-L1 inhibitors) [56], and hypothyroidism (6.7% in patients with PD-1 inhibitors) [57]. However, combined PD-1 plus CTLA-4 blockade triggers substantially more irAEs (55–60%) than monotherapy alone (10–20%) [15,20,58].

Besides common irAEs, other rare but life-threatening irAEs were also observed. These include cardiac failure (1.4%, 3/214), cardiopulmonary arrest (0.37%, 2/537), myocardial infarction (0.5%, 2/402), stroke (1.48%, 2/135), pancreatitis (0.14%, 5/700), severe skin reaction (1.6%, 11/682), sepsis (3.39%, 2/59), pulmonary embolism (0.35%, 1/287), respiratory failure (0.54%, 1/184), and myocarditis (0.48%, 1/207), etc. [59,60]. In terms of the time of occurrence, most irAEs appear in the first few months of immunotherapy. Generally, dermatologic irAEs occur early (within the first month), followed by gastrointestinal and hepatic toxicities (the second month), and endocrine toxicities (the third month and beyond). Usually, patients receiving CTLA-4 therapy more commonly present with colitis and hypophysitis, while patients receiving PD-1 therapy more commonly present with pneumonitis and thyroiditis [56,61], while thyroid abnormalities including hypothyroidism, thyrotoxicosis, painless thyroiditis, or even “thyroid storm” are more frequently associated with anti-PD-1-antibodies [62], and these endocrine toxicities are usually irreversible in 50% of cases [63]. According to Common Terminology Criteria for Adverse Events (CTCAE) v5.0, main irAEs and their grading are summarized in Table 2.

There are few reports on the long-term irAEs. Some patients die from the irAEs. Among the long-term irAEs, endocrine system is the

Table 1

Summary of currently approved immune checkpoint inhibitors and their clinical indications.

Generic Name	Trade Name	Target	Type	Time approved by US FDA	Developed by	Market manufacturers	Cancer types and indications
Ipilimumab Pembrolizumab	Yervoy Keytruda	CTLA-4 PD-1	IgG1 mAb IgG4 mAb	2011 2014	Medarex Schering-Plough	Bristol-Myers Squibb Merck & Co.	Melanoma; RCC, prostate cancer, lung cancer, bladder cancer, etc. Melanoma, NCCLC and SCLC, SCCHN, cHL, primary large B-cell lymphoma, urothelial carcinoma, gastric cancer, etc.
Nivolumab	Opdivo	PD-1	IgG4 mAb	2014	Medarex & Ono Pharmaceutical	Bristol-Myers Squibb	Metastatic NSCLC, metastatic melanoma, advanced RCC, SCLC, SCCHN, HCC, urothelial carcinoma, metastatic CRC, cHL, Melanoma, CRC, RCC, NSCLC
Combination of Ipilimumab & Nivolumab	Yervoy + Opdivo	CTLA-4 & PD-1	IgG1 & IgG4	2015	–	–	Metastatic cutaneous squamous cell carcinoma, myeloma, lung cancer
Cemiplimab	Libtayo	PD-1	IgG1 mAb	2018	Regeneron Pharmaceutical Co.	Regeneron and Sanofi Genzyme	NSCLC, urothelial carcinoma, breast cancer, melanoma, RCC, CRC, SCCHN
Atezolizumab	Tecentriq	PD-L1	IgG1 mAb	2016	Genentech/Roche	Genentech/Roche	Merkel-cell carcinoma, RCC, NSCLC
Avelumab Durvalumab	Bavencio Imfinzi	PD-L1 PD-L1	IgG1 mAb IgG1 mAb	2017 2017	Merck KGaA & Pfizer Medimmune/AstraZeneca	Merck KGaA Medimmune/AstraZeneca	Urothelial carcinoma, NSCLC

Abbreviations: cHL: classic Hodgkin lymphoma; CRC: Colorectal cancer; HCC: Hepatocellular Carcinoma; NSCLC: Non-small cell lung cancer; RCC: Renal Cell Carcinoma; SCCHN: Squamous cell carcinoma of the head and neck.

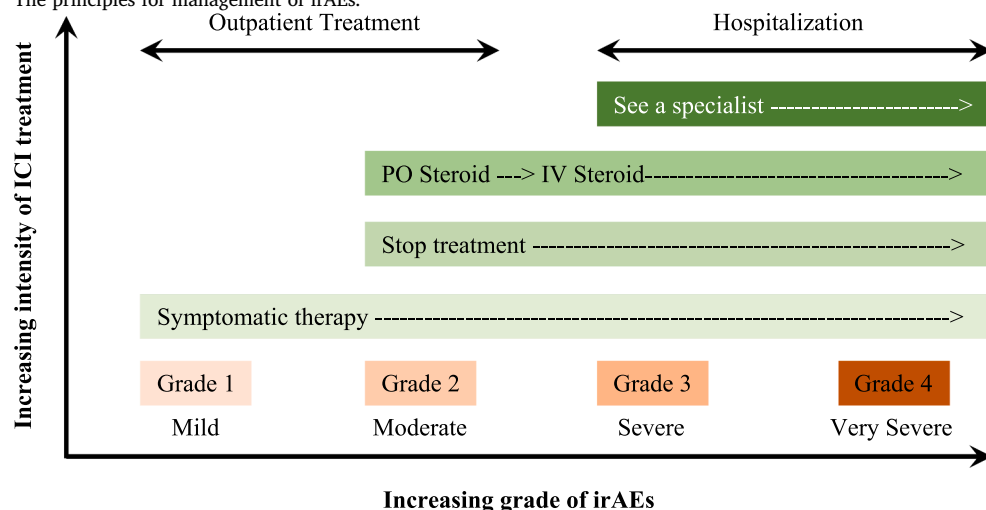
Table 2

Main irAEs and their grading according to Common Terminology Criteria for Adverse Events (CTCAE) v5.0.

irAEs	Grade 1	Grade 2	Grade 3	Grade 4
Colitis	Increase of more than 4 stools per day over baseline; mild increase in ostomy output compared with baseline	Increase of 4–6 stools per day over baseline; moderate increase in ostomy output compared with baseline	Increase of 7 stools or more per day over baseline; incontinence, hospitalization is indicated; severe increase in ostomy output compared with baseline; limiting self-care ADL	Life-threatening consequences, urgent intervention indicated
Pneumonitis	Asymptomatic, confined to one lobe of the lung or less 25% of lung parenchyma; clinical or diagnostic observations only	Symptomatic, involved more than one lobe of the lung or 25% to 50%; medical intervention indicated; limiting instrumental ADL	Severe symptoms, hospitalization required; involves all lung lobes or more than 50% of lung parenchyma; limiting self-care ADL; oxygen indicated	Life-threatening respiratory compromise, urgent intervention indicated (intubation)
Thyroiditis	Thyroid-stimulating hormone less than 10 mIU/L and asymptomatic	Moderate symptoms; able to perform ADL; thyroid stimulating hormone greater than 10 mIU/L	Severe symptoms, medically significant or life-threatening consequences, unable to perform ADL	Severe symptoms, medically significant or life-threatening consequences, unable to perform ADL

Table 3

The principles for management of irAEs.



most commonly affected with the development of type 1 diabetes mellitus or diabetic ketoacidosis. Because the majority of the cases developed pancreatic dysfunction within five cycles of therapy, hence, besides the evaluation of other systems, pancreatic functions should be closely monitored to minimize adverse impact on patients [64].

7. Occurrence of ICI-mediated toxicities and treatment

CTLA-4 and PD-1 immune checkpoints are negative regulators of T-cell immune function, and their expression on T cells leads to immune inhibition. Inhibition of these checkpoints results in an increased activation of the immune system. ICIs including the drugs Ipilimumab that targets CTLA-4 acts as a type of “off switch” on T cells, Pembrolizumab, Nivolumab and Cemiplimab against PD-1 that prevents T cells from recognizing and attacking inflamed tissues and cancer cells. Atezolizumab, Avelumab and Durvalumab act on PD-L1, a membrane protein acting as a ligand to induce anergy in PD-1-positive T cells, to reactivate the immune system that kills cancers. However, ICIs are non-specific and not exclusively restricted in tumor tissues, and the normal safeguards against autoimmunity within the body are also affected. These ICIs generally upregulate immune responses while working, but it can sometimes cause serious or even life-threatening side effects. When the use of these mAbs reactivates the immune system, activated T cells release inflammatory cytokines, which in turn activate immune cells, stimulating them to produce more cytokines in a positive feedback loop. In addition, direct binding of ICIs to targets expressed in normal tissues could lead to complement-mediated inflammation [65,66].

The irAEs develop during or after treatment with ICIs in the forms of systemic inflammatory response syndrome. The incidence of irAEs varies depending on the class of ICIs administered and the patient's cancer types. Adverse reactions of any severity that involve the immune system develop in about 54% of patients receiving a CTLA-4 inhibitor, 27% receiving a PD-1 inhibitor, and 17% receiving a PD-L1 inhibitor. The combination of CTLA-4 and PD-L1 inhibitors results in about 61% of patients developing irAEs [67]. For example, a woman with successful regression of lung cancer was reported to develop purpura fulminans after Nivolumab therapy, ultimately resulting in amputation of both lower extremities. Blood tests revealed thrombocytopenia with increased serum levels of soluble IL-2 receptor, ferritin, and triglyceride, suggesting hemophagocytic lymphohistiocytosis. Further detailed laboratory analyses revealed severe hypercytokinemia including increased levels of IL-1 β , IL-6, IL-10, TNF α , IFN- γ , and G-CSF [68].

Most recurrent/new irAEs were mild (58% Grade 1–2) and

manageable (84% resolved or improved to Grade 1) with an appropriate treatment according to a previous study enrolling 482 NSCLC patients on anti-PD-(L)1. But some can be serious or life-threatening. For example, 14% of the patients developed a severe irAE that requires treatment discontinuation [69]. While the management of irAEs varies according to the affected organ systems, in general, ICI therapy could be continued with close monitoring if Grade 1 toxicities occur, with the exception of some neurologic, hematologic, and cardiac toxicities. ICI therapy may be suspended if most Grade 2 toxicities emerge, with consideration of resuming when symptoms revert to Grade 1 or less. Corticosteroids may be administered for Grade 3 toxicities or above that generally warrant suspension of ICIs and the initiation of high-dose corticosteroids (prednisone 1–2 mg/kg/d or methylprednisolone 1–2 mg/kg/d) [70].

The treatment of irAEs depends on severity and type of the effect. Typically, irAEs that are moderate in severity (Grade 2–Grade 3) are treated with a course of steroids and interruption of the immunotherapy treatment until symptoms resolve or become mild. For Grade 3 or higher irAEs, high-dose corticosteroids or other immunosuppressive therapies may be needed, and immunotherapy will be interrupted or discontinued depending on the type of irAE. Current data suggest that the use of steroids or other immunosuppressive therapies to treat irAEs does not affect tumor response to ICIs [71]. Some studies indicated that the cancer patients whose treatment with ICIs was discontinued due to irAEs, a re-challenge with the same or another immune checkpoint inhibitor is still controversial [72,73].

The most important and effective strategy for managing irAEs is early identification and early intervention. Generally, grade mild toxicities can be managed by a temporal cessation of the offending medication and treatment can be resumed once the toxicities have been resolved. Oral antihistamine and Prednisone (0.5–1 mg/kg/day) are used for mild irAEs. But a specialist will be consulted for the severe irAEs. Accordingly, the treatment rules most commonly consisted of cessation of ICIs, initiation of steroids and supportive therapy. Table 3 is a summary that contains the principals of the irAEs treatment. Therefore, the patients should be carefully monitored during treatment for initial evidence of Grade 1 adverse events.

8. Response rates and biomarkers for responders to ICI therapy

As a clinician, it is important to know who will and who will not gain benefit from potential ICI therapy. To determine the percentage of cancer patients who could potentially gain benefit from an ICI drug, Alyson Haslam et al. used annual cancer deaths as a stand-in for annual

incidence of advanced or metastatic disease. The results showed that the estimated percentage of patients in the US with cancer eligible for ICIs was 26.86% (95% CI, 1.51–1.57%) in 2015 and increased to an estimated 43.63% (95% CI, 26.75–26.98%) in 2018 [4]. In addition, a study (n = 3089) recently published in JAMA Oncology showed that within four months of FDA approval of immunotherapies in various metastatic solid cancers, over 60% of eligible patients received anti-PD-1 agents treatment [74]. Another study recently presented data at the ASCO Genitourinary Cancers Symposium showed that by the beginning of 2018, approximately half of all patients with newly diagnosed advanced urothelial cancer received ICI therapy [75]. Furthermore, according to an analysis recently published in *JAMA Network Open*, 43% of patients with cancer in the US are eligible to receive these ICIs drugs [76].

ICI therapy has achieved impressive success in the treatment of different cancer types. However, initiation of immune checkpoint therapy to treat cancers is obstructed by the low response rate and the occurrence of irAEs in some patients. Although there are about 50% of the patients with cancer are eligible for ICI therapy, only a subset of patients gains clinical benefit. Patients with melanoma exhibit the best response rates after treatment with ICIs [77]. About 20% of melanoma patients who received Ipilimumab exhibited a complete response 10 years after treatment initiation [78]; 33% of melanoma patients treated with pembrolizumab featured a 3-year response [79]. Combined treatment with anti-CTLA-4 and anti-PD-1 reached a higher response rate of 58%, but with severe toxicity [80]. However, the percentage estimated to respond to ICIs of the patients with cancer gradually improved with years, and was 0.14% in 2011, 5.86% in 2015, 12.46% in 2018 [4,76], and about 13% in 2019 [4]. Accordingly, it is critical to understand the determinants driving response, resistance and adverse effects, and thus it is urgent to develop biomarkers to predict which patients with cancer are responders to ICIs.

An increased PD-1/PD-L1/PDL-2 expression in PBMCs, particularly in T-cells, may be an additional mechanism that lead to tumor escape from immune control in lung cancer. Evaluating PD-1, PD-L1/2 expression in peripheral blood cells may serve as a potential biomarker for prognosis and response to therapy among the patients with lung cancer [81]. Increased PD-L1 expression has been correlated with immune response and is currently used as a biomarker for ICI therapy in NSCLC and urothelial carcinoma.

In addition to the above cell membrane biomarkers, some circulating or soluble biomarkers are also investigated. Elevated levels of soluble PD-L1 have been associated with worse prognosis in renal cell carcinoma and multiple myeloma as well [82]. Soluble PD-1, PD-L1, and CTLA-4 levels were increased compared to those of healthy controls and varied among tumor types [83]. The circulating exosomal PD-L1 may reflect the states of anti-tumor immunity in melanoma patients as responders to anti-PD-1 were characterized by the increase in circulating exosomal PD-L1 during early stages of treatment [84]. A recent study from Feun et al. indicated that high baseline plasma levels of anti-inflammatory cytokine TGF- β were significantly correlated with poor outcomes after anti-PD-1 treatment in patients with advanced, unresectable hepatocellular carcinoma [85].

NSUN2 (NOP2/Sun domain family, member 2) encodes a tRNA methyltransferase, adding the methyl group to cytidine in tRNAs and mRNAs, by which affecting the stability and translation of tRNA and mRNAs. A recent study shows that a significant negative correlation exists between *NSUN2* expression and T cell activation score, and that *NSUN2* expression are negatively correlated with the expressions of M2-like macrophage markers, and modifies the effect of T cell activation score on patient survival in head and neck squamous carcinoma [86]. Pathway analysis of *NSUN2* co-expressed genes showed that *NSUN2* might be involved in the regulation of immune system [86].

Although Nivolumab is applied on melanoma patients irrespective of PD-L1 expression level with overall response rate (ORR) of 57.5% for PD-L1-positive tumors and 41.3% for PD-L1-negative tumors [87], it is

known that the expression levels of immune checkpoint-associated molecules such as PD-1, PD-L1, and CTLA-4 are critical to evaluate the immune state of individuals. Specifically, in NSCLC, the combination of Durvalumab (anti-PD-L1) and Tremelimumab (anti-CTLA-4) in patients with low or negative expression of PD-L1 on tumor cells did not improve progression free survival and overall survival compared to standard-of-care chemotherapy [88]. Therefore, if a non-invasive approach can be used to know in advance who are suitable for ICI treatment, the response rate of ICI therapy can be greatly improved. However, the biopsy method is very limited because not all patients can be biopsied. It therefore seems important to screen patients in advance using non-invasive or minimally invasive approaches.

In Nov. 2018, a new technology with ^{18}F -BMS-986192 was developed to show whole body PD-1 and PD-L1 using positron emission tomography (PET) in patients with NSCLC in Cancer Center Amsterdam, The Netherlands. Tumor with PD-L1 and PD-1 expression can be quantified non-invasively using PET-CT in patients with NSCLC [89]. These results would be helpful to the patients with NSCLC while accepting ICI therapy.

Soon afterwards, BMS announced on May 26, 2019 that a novel approach was developed that allows for noninvasive same-day imaging of PD-L1 expression. These results can help doctors decide how to select the ICI drugs for patients with NSCLC (Fig. 3). Pembrolizumab is one of the ICIs, and it is currently recommended as the first-line treatment for patients with advanced NSCLC showing $\geq 50\%$ expression of programmed death-ligand 1 (PD-L1).

9. Mechanisms of resistance to ICIs

Mechanisms of resistance to ICI therapy including innate and acquired resistance are not fully understood. However, durable responses suggestive of long-lasting immunologic memory are commonly observed in large subsets of cancer patients treated with ICIs [90]. Failure of ICI therapy can result from the defects in any of the types as follows: (1) insufficient generation and infiltration of anti-tumor T cells, (2) inadequate function of tumor-specific T cells, (3) impaired formation and expansion of T-cell memory, or (4) insufficient antigens and/or presentation. In general, it is accepted that successful anti-tumor immune responses following PD-1/PD-L1 blockade require reactivation and clonal-proliferation of antigen-experienced T cells present in tumor microenvironment (TME) [91]. Inadequate T-cell function can arise through diverse tumor-intrinsic and tumor-extrinsic immunosuppressive components of the TME [92]. Tumor-intrinsic mechanisms of immune evasion include genetic and epigenetic alterations to influence neoantigen formation, presentation, and/or processing, as well as alterations in cellular signaling pathways that disrupt the action of CTLs. Immunotherapy resistance is associated with poor T cell infiltration into tumors. Tumor-cell-intrinsic oncogenic events that contribute to this defect include Wnt- β -catenin activation. p21-activated kinase 4 (PAK4) is now identified as an upstream modulator of this pathway, thus suggesting the potential of enhancing the efficacy of immunotherapy by targeting this druggable kinase [93]. Tumor-extrinsic mechanisms involve stromal cells or immune cells, or other systemic influences that can act in concert with cancer cells to promote growth and resistance to ICI therapy [90,94].

In addition, the response rates of different tumor types to PD-1/PD-L1 checkpoint blockade tend to be proportional to their corresponding tumor mutational burden (TMB). TMB is a genomic biomarker with the potential to make a significant impact on the landscape of cancer immunotherapy, which is presumably from the immunogenic neoantigens that are recognized as foreign by CTLs. This emerging marker measures the number of somatic mutations within a tumor genome and has already been shown to be associated with improved responses to ICI therapy in lung cancer, melanoma and bladder cancer [95]. However, tumors with similar TMB can have very different response to ICIs, indicating that response to immune checkpoint blockade (ICB) is

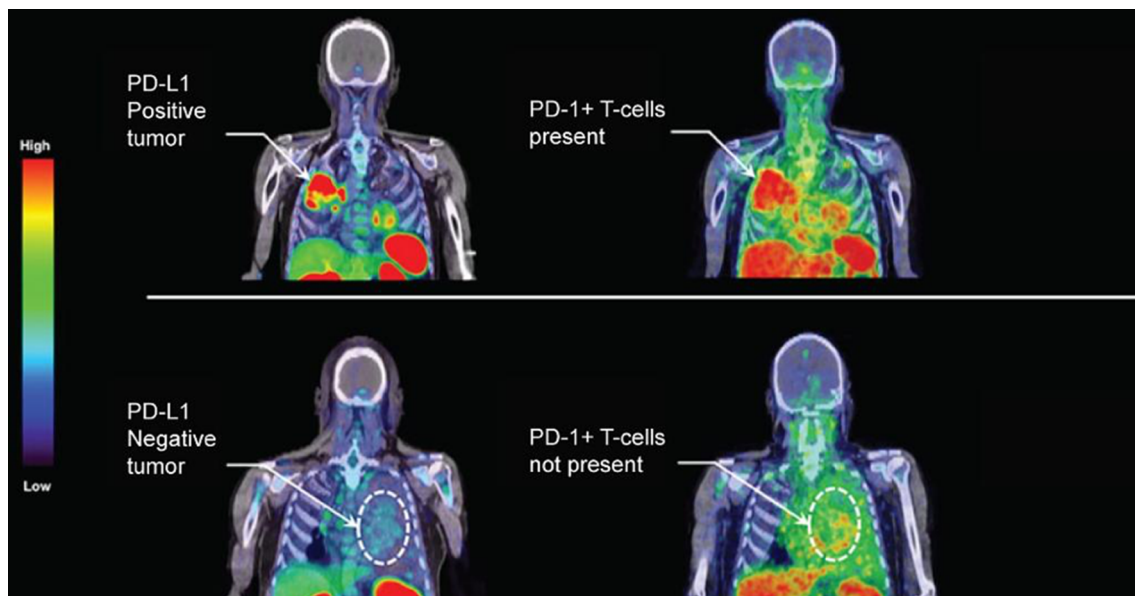


Fig. 3. Whole body PET scan quantifies expression of PD-1 and PD-L1 in patients with NSCLC. A new technology with ^{18}F -BMS-986192 shows whole body PD-1 and PD-L1 using PET scan in patients with NSCLC in Cancer Center Amsterdam, The Netherlands.

complex, heterogeneous, and inconsistent and that additional mechanisms are at play in a tumor context [10]. To those PD-L1-over-expressed tumors, the mechanism of resistance may result from non-reversible and severe T-cell exhaustion. In addition, co-expression of inhibitory receptors (LAG-3, TIM-3, TIGIT, VISTA and BTLA) [96], decreased ratio of TILs to Treg and myeloid derived suppressor cells (MDSC), altered metabolism through indoleamine 2,3-dioxygenase (IDO) and increased adenosine production and mutation in *PTEN*, *EGFR*, and *MYC* [97] may also contribute to resistance to ICIs.

In view of the different situations that caused resistance to ICI therapy, combination therapy can be generally used to overcome the resistance to ICIs. If the resistance caused by low mutation burden or lack of neo-antigens, radiation, chemotherapy and oncolytic virus can be combined with ICI therapy; Interferon can be combined with ICI therapy if the resistance is caused by defects in antigen processing/ β 2M; VEGF inhibitor can be used to the patients who have VEGF production by tumor, whereas VEGF-C could enhance the efficacy of anti-PD1/anti-PD-L1 in brain tumor by driving lymphatic drainage [98]; anti-TIM3 and anti-TIGIT can be used for secondary checkpoints; PI3K- γ selective inhibitor, HDAC inhibitor can be used to the patients with immunosuppressive cells in tumor microenvironment; IDO-1 inhibitor, anti-CD73 mAb and TGF- β receptor kinase can be used to the patients with inhibitory molecules and cytokines [99,100].

Besides the above, a few latest breakthrough findings were just published in Dec. 2019, which demonstrated new mechanisms of resistance to ICI therapy. Firstly, lack of immune cell infiltration into tumor is the main mechanism of primary resistance to PD-1 blockade therapies. A team led by Catherine Grasso and Antoni Ribas at University of California Los Angeles (Dec. 9, 2019) demonstrated that PAK4 was enriched in non-responding tumor biopsies with low T cell and dendritic cell infiltration [101], suggesting that PAK4 might inhibit the infiltration of immune cells. Secondly, Haydn Kissick and his team at Emory University recently reported that TILs are positively associated with survival benefit in several tumor types and with the response to immunotherapy. Thirdly, it has been shown that these stem cell-like T cells reside in dense antigen-presenting-cell niches within the tumor, and that tumors failing to form these structures are not extensively infiltrated by T cells [102]. Lastly, Professor Padmanee Sharma and her team as well as her husband Professor James Allison, a Nobel laureate in 2018, recently (Dec. 23, 2019) reported a unique population of CD73^{hi} macrophages in glioblastoma multiforme that

persists after anti-PD-1 treatment. These CD73^{hi} macrophages block the anti-PD-1 blockade immunotherapy and thus reduce the treatment benefit of glioblastoma. In addition, they demonstrated that the absence of CD73 improved survival in a murine model of glioblastoma multiforme treated with anti-CTLA-4 and anti-PD-1 [103].

10. Factors influencing tumor responders treated with ICIs

Some clinicopathological factors such as sex, age, tumor size, and stage, etc. are also predictive markers of ICI therapy. In general, male patients have significantly longer PFS and OS than those female patients [104], and cancer patients whose age between 60 and 75 correlated with better ORR to ICIs [105,106]. Tumor size is an independent factor that correlates with better ORR and OS for the cancer patients treated with ICIs [107]. In addition, there are some biomarkers that are helpful to predict if the cancer patients are responders. Higher density of CD8⁺ tumor TILs at the invasive margin correlates with a better response [108]. Tregs are the T cells with the markers of FoxP3⁺CD25⁺CD4⁺, which significantly suppress immune responses. High levels of circulating Tregs at baseline were associated with longer OS in ICI-treated melanoma patients [109]. Some increased cell surface molecules including PD-L1 [3], inducible T cell co-stimulator (ICOS) [110] and IDO [111] correlate with better ORR or improved OS. However, increased TIM3 expression is associated with a shorter OS [112]. A cell population of CD73^{hi} macrophages persists after anti-PD-1 treatment in glioblastoma multiforme [103]. Some soluble biomarkers such as higher serum level of CTLA-4 at baseline have both better ORR and OS [113], a significantly increased CD163 serum level was found in the responder cancer patients 6 weeks after ICI therapy compared to non-responders [114]. However, other soluble biomarkers such as higher levels of baseline PD-L1 in serum were associated with worse response, but increased PD-1 in serum after ICI therapy was a favorable sign for clinical response [82]. Increased level of NKG2D in serum was an independent factor for shorter OS [115].

For some of the cytokines, IL-6 is a pleiotropic cytokine that is associated similarly with disease pathogenesis and metastasis and has already been reported as a serum marker for melanoma patients. Higher levels of serum IL-6 were not only correlated with shorter OS in melanoma [116], but also correlated with the treatment failure in CTLA-4 treated melanoma patients [117]. Increased IFN- γ among the cancer patients after ICI therapy was associated with a better outcome and

longer OS [118]; but higher serum levels of TNF- α and IL-6 were associated with a poor prognosis and shorter OS because TNF- α promotes tumor growth [117]. Other serum markers such as C-reactive protein (CRP), a marker that quickly reflects most inflammation, its lack of change in serum was significantly associated with longer OS in ICI-treated cancer patients [119]. High baseline lactate dehydrogenase (LDH) level is an independent factor for poor prognosis and poor response in cancer patients, and the melanoma patients with elevated baseline LDH had a significantly shorter OS [120]. Responders have an elevated HLA-A level before ICI therapy [121]. In addition, a few blood cell types are helpful predictive markers of the responders. Higher levels of CTLA-4 and PD-1-expressed lymphocytes at baseline were associated with longer OS [109]. Widely used CRISPR/Cas9 technology can be used to knock out the immune suppressor genes including PD-1, CTLA-4, TIM-3 and LAG-3, and this method might be an alternative in ICI therapy [122]. Higher blood eosinophil counts correlate with favorable outcomes and a longer OS and better ORR [109,123], but, neutrophil to lymphocyte ratio (NLR) was associated with a poor tumor response in the ICI-treated melanoma patients [124]. In fact, the importance of T-cell diversity is recognized in the development of tumor responses and toxicities in patients receiving ICIs therapies [125]. Recently, PD-L1 expression and TMB have emerged as important independent biomarkers of response to ICI therapy in most cancers [126]. More interestingly, a more recent report demonstrated that stem-cell-like CD8⁺ T cells exist in tumor niches that give rise to CTLs to boost anticancer immune responses [102].

11. Predictive biomarkers of irAEs

Current ICI therapy has significantly improved the outcome of cancer patients, although the efficacy of the ICI therapy still remains limited. In particular, the therapy only works for a small portion of the cancer patients. Importantly, ICI therapy is also associated with substantial toxicities. Therefore, in order to avoid overtreatment of ICIs and minimize the irAEs development, predictive markers of the irAEs occurrence are important and required [127].

Like the factors that influence tumor responders treated with ICIs, gender is also one of the factors. Male cancer patients are more favorable response to ICI therapy, and female cancer patients associated with higher rates of irAEs [128], suggesting that sex hormones may play a role in the development of irAEs.

Biomarkers in serum cytokines may help to predict the development of irAEs. IL-6 has been linked to IL-10 production within the melanoma tumor microenvironment [117]. Baseline IL-6 level in serum negatively correlates with the occurrence of irAEs in melanoma patients treated with Ipilimumab [128]. That means the lower level of baseline IL-6 in serum, the higher incidences of colitis-related irAEs [129]. Surprisingly, increased IL-6 levels after ICI therapy were significantly associated with occurrence of irAEs [130]. Therefore, these studies indicated that IL-6 may serve as a predictive marker for the occurrence of irAEs. In addition, IL-17 is a pro-inflammatory cytokine and is produced by a group of T helper 17 cells, which is in balance with Tregs to maintain homeostasis of intestinal tracts. IL-17 levels at baseline were significantly associated with the incidence of Grade 3 irAEs of diarrhea/colitis. Accordingly, an elevated IL-17 levels might be a reflection of cancer patients with colitis [131]. Interestingly, a recent research reported that the 11 circulating cytokine biomarkers (G-CSF, GM-CSF, fractalkine, fibroblast growth factor-2, IFN- α 2, IL12p70, IL-1 α , IL-1 β , IL1 α , IL-2, and IL-13) were significantly upregulated in patients with severe irAEs at baseline and early during treatment [132].

Furthermore, Fujimura, et al. measured soluble CD163 levels in serum and found that absolute change rate of CD163 in serum was significantly increased in patients with irAEs when compared to those without the symptoms of irAEs [133].

Moreover, autoantibodies in thyroid including thyroid stimulating hormone (TSH) and antithyroid peroxidase (TPO) antibodies were

significantly associated with higher incidence of irAEs in thyroid [134]. Autoantibodies including Glutamic Acid Decarboxylase (GAD-65), Insulin Autoantibodies (IAA), and Islet Antigen-2 (IA-2) are originally the markers for type 1 diabetes. However, these markers were found positive in the patients with solid tumor treated with anti-PD-1 or anti-PD-L1 mAbs, ICI therapy induced insulin-dependent diabetes [135].

In addition to cytokines and autoantibodies, immune cells including Treg cells, Th17 cells, CD8⁺ T cells, B cells and eosinophils that are involved in the pathophysiological process of autoimmune diseases may also play an important role in the development of irAEs. A multivariate study demonstrated that the absolute number of lymphocytes and eosinophils at baseline and at one month post initial treatment were independent factors, which were associated with a higher incidence of irAEs at the Grade ≥ 2 among the patients with solid tumors treated with anti-PD-1 antibodies [124,136]. More recently, abundant CD8⁺ T-cell infiltration of affected organs has been reported in patients with irAEs. This implies a central role of CD8⁺ T cells in the pathogenesis of certain types of irAEs, and each T-cell parameter significantly predicts the corresponding subtype of irAEs [137,138]. Memory cytotoxic (CD45RO⁺GzmB⁺Ki-67⁺) CD4⁺ T cells in irAEs has also been reported recently [139]. Besides T cells, early B-cell changes have also been suggested to predict irAEs following combined anti-PD-1 and anti-CTLA-4 therapy [140].

12. Concluding remarks and outlooks

Over the last decade, cancer immunotherapy has emerged as an attractive addition and alternative to conventional treatment strategies. ICI therapy opens an avenue of cancer immunotherapy with a potent clinical efficacy and prolongs survival in responder patients with cancer. Despite these promising long-term responses, the majority of cancer patients failed to respond to ICI-therapy with primary resistance. More importantly, some of cancer patients have to stop ICI therapy due to irAEs. Additionally, many of those initial responders to ICI-therapy eventually develop relapse. All the primary and secondary (acquired) resistance are a result of complex and constantly evolving interactions between cancer cells and the immune system. Numerous clinical trials for ICIs including adjuvant and neo-adjuvant therapies are still ongoing. Despite the improved outcome of the cancer treatment by ICIs, the efficacy still remains very limited and tumor regression have not been achieved in many cancer patients. Many factors can affect tumor responders and could be the predictive biomarkers of irAEs in cancer patients treated with ICIs. Although this information can help clinician to make decision in choosing or discontinuing ICI therapy, the consideration of multiple biomarkers simultaneously is strongly recommended.

The types of currently applied/investigated cancer immunotherapy include ICIs, co-stimulatory receptor agonists, adoptive cell transfer (ACT), chimeric antigen receptor-T cells (CAR-T), cancer vaccines, cytokines, monoclonal antibodies, and oncolytic virus therapy. Checkpoint inhibition therapies have been the highlight of immunotherapy studies in the last decade. Therapeutic strategies in combination may overcome immune checkpoint blockade resistance and improve the efficacy.

In addition, cyclin-dependent kinases (CDKs) play a role in regulating cell cycle, and CDK4/6 inhibitors currently have been used to treat metastatic breast cancer [141]. Because CDK4/6 inhibits tumor growth by acting on the cells in TME, either by affecting cell proliferation and maturation or by affecting antigen processing and other immunological features of the cells. Thus, a new strategy can be used to combine CDK4/6 inhibitors with ICIs or other types of immunotherapy [142]. Recently, CRISPR-Cas 9 is one of the newest tools for gene editing, capable of simultaneously knock-downing or inactivating multiple immune repressor genes (e.g. PD-1, CTLA-4, TIM-3 and LAG-3) from those T cells, which can be re-transfused back to patients for cancer treatment. This method provides an alternative strategy for ICI

therapy and the irAEs caused by ICIs can be minimized, but it is still under investigation. Furthermore, PAK4 and CD73 are the novel potential targets for targeted immunotherapies, and their corresponding new inhibitors are being consequently developed.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.intimp.2020.106628>.

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