New developments in the mechanism and application of immune checkpoint inhibitors in cancer therapy (Review)

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Abstract. The use of immune checkpoint inhibitors (ICIs) has been demonstrated in the treatment of numerous types of cancer and ICIs have remained a key focus of cancer research. However, improvements in survival rates only occur in a subset of patients, due to the complexity of drug resistance. Therefore, further investigations are required to identify predictive biomarkers that distinguish responders and non-responders. Combined therapeutics involving ICIs and other modalities demonstrate potential in overcoming resistance to ICIs; however, further preclinical and clinical trials are required. Concurrently, prompt recognition and intervention of immune-related adverse events are crucial to optimize the use of ICIs in clinical treatment. The present study aimed to review the current literature surrounding the mechanisms and application of ICIs, with the aim of providing a theoretical basis for clinicians.

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Contents

1. Introduction

Cancer is a major public health concern worldwide. According to the American Cancer Society, it was estimated that 1.9 million new cases and 610,000 cancer-related deaths occurred in the United States in 2022, posing a serious threat to human health (1). Conventional cancer treatments include surgery, radiotherapy, chemotherapy and targeted therapy; however, certain types of advanced or metastatic cancer are difficult to cure using traditional treatments, and novel tools and approaches are required. Research has shown that the immune system serves a pivotal role in maintaining the stability of the internal environment (2); however, cancer cells often escape surveillance and destruct the host immune system (3). Therefore, research has focused on the development of immunotherapy that inhibits tumor-induced immune tolerance and restores the immune response against tumor cells. Unlike conventional cancer treatments which mainly act on cancer cells, immunotherapy indirectly promotes cancer control through activating the antitumor immune responses of the patient (4,5). The concept of antitumor immunotherapy has been around for over a century, starting with Coley's toxins and Erlich's hypothesis that the immune system suppresses cancer development (4), but it has developed rapidly in the past two decades and is currently a major focus in cancer therapy.

Among the different types of cancer immunotherapy, immune checkpoint inhibitors (ICIs) demonstrate a broad impact. ICIs are monoclonal antibodies (mAbs) that specifically target the inhibitory receptors on T cells, known as immune checkpoint molecules. These act as negative co-regulators that inhibit further T-cell activation and are essential for the maintenance of self-tolerance (4). Tumor cells often escape host immunity through immune checkpoint dysregulation, and ICIs are immunomodulators that reinforce antitumor immune responses (6-10). Moreover, ICIs have demonstrated notable outcomes in multiple tumor types (11), either as a single treatment or combined with conventional treatments. ICIs may be used in both advanced and metastatic cancer, either as adjuvant or neoadjuvant treatment in the early stage of cancer (12). Notably, ICIs are considered revolutionary in cancer treatment, highlighted by the 2018 Nobel Prize in Physiology or Medicine awarded jointly to James Allison and Tasuku Honjo, for their discovery of cancer treatment via suppression of negative immune regulation (12). The present review aimed to summarize the current knowledge and novel advances regarding the mechanisms and applications of various ICIs. This study may provide a theoretical basis for further associated research and the application of immunotherapy.

2. Tumor-immune interactions

During the tumor immune response process, T lymphocytes act as the final effector cell, and the activation of T cells requires two signals. The first signal originates from the specific recognition of antigen-major histocompatibility complex (MHC) complexes by T-cell receptors (TCRs). The second signal originates from the interaction between co-stimulatory molecules on the surface of T cells with the corresponding ligands. Co-stimulatory signals are required to enhance the antigen receptor signals that induce transcription factor activation and PI3K activation, thereby ensuring full activation of T cells (13). Compared with these activating co-stimulatory molecules, certain inhibitory molecules exist on the surface of T cells that downregulate activation signals. These inhibitory receptors function to prevent T-cell proliferation and cytokine production, in order to prevent excessive immune responses that can lead to destructive inflammatory or autoimmune conditions (14,15). Moreover, the strict regulation between co-stimulatory and inhibitory molecules serves a critical role in immune homeostasis. Co-stimulatory molecules include CD28, 4-1BB and inducible co-stimulator (CD278), and inhibitory molecules include cytotoxic T lymphocyte associated antigen-4 (CTLA-4) and programmed death-1 (PD-1), which are both categorized as immune checkpoint molecules (15). Notably, immune checkpoint molecules refer to 'brake' proteins that inhibit the activation of immune cells (Fig. 1). Tumor cells often inhibit the effects of T cells through the immune checkpoint pathway, leading to immune escape.

3. ICI therapy for the treatment of cancer

CTLA-4 and anti-CTLA-4 therapy. In 1987, the CTLA-4 gene was initially discovered in mice by Brunet *et al* (16) and the human CTLA-4 gene was cloned the following year (17). Krummel and Allison (17) confirmed the function of CTLA-4 as a negative regulator of T-cell activation; this was the first immune checkpoint molecule described (17). CTLA-4, also known as CD152, is a transmembrane receptor on T cells. In the early stages of T-cell activation, CTLA-4 is induced and binds to the same co-stimulatory ligands (B7-1 and B7-2) expressed on antigen-presenting cells (APCs) as CD28. Compared with CD28, CTLA-4 possesses a higher avidity and affinity for the ligands, thereby preventing the CD28-dependent co-stimulation signal required for T-cell activation (18-20). CTLA-4 has been shown to interact with the serine/threonine phosphatase PP2A, which blocks Akt activation and downstream signals of T cells, and CTLA-4 may also reduce the formation of the ζ chain of TCR associated protein kinase 70 kDa (ZAP-70) microcluster, thus limiting T-cell activation. In addition, CTLA-4-associated SHP-2 has phosphatase activity toward the RAS regulator p52, thereby affecting the downstream RAS pathway. All of these effects can lead to the suppression of T-cell activation, which is critical for immunologic tolerance in physiological conditions (21). Notably, the biallelic genetic deletion of Ctla4 leads to lymphoproliferation disorders with early lethality in mice (22,23).

CTLA-4 not only prevents the activation of self-reactive T cells, but also other T cells, by binding to the ligand for CD28. In addition, CTLA-4 exerts inhibitory effects that are mediated through regulatory T cells (Tregs). Tregs express high levels of CTLA-4 on the cell surface, and specific loss of CTLA-4 leads to an increased susceptibility to autoimmune diseases. This indicates that Treg-derived CTLA-4 is required to maintain immunologic tolerance (24,25). Moreover, Tregs directly remove the co-stimulatory ligands, B7-1 and B7-2, from the surface of APCs via trans-endocytosis. CTLA-4 promotes T-cell motility through antagonizing the formation of microclusters, thus reducing the T cell/APC dwell times (26). In addition, CTLA-4 negatively regulates the immune response through inhibiting the maturation and antigen presentation of APCs (27), and inducing the production of indolamine-2,3-dioxygenase (IDO) by APCs (28). As CTLA-4 was the first immune checkpoint molecule to be discovered, the associated regulatory mechanisms have been extensively studied. However, further investigations into the function of downstream signal components are crucial, and future studies should focus on evaluating CTLA-4-mediated regulation of T-cell activity.

Following the discovery of the role of CTLA-4 in immune suppression, research has focused on restoring the antitumor function of T cells through inhibiting the binding of CTLA-4 to B7. Using animal models (29), anti-CTLA-4 immunoglobulin (Ig)G1 mAb was developed in 1999, and later named ipilimumab. In 2010, ipilimumab was successfully used in the treatment of metastatic melanoma in a phase III clinical trial. Results of this study demonstrated that the median overall survival (OS) among patients receiving ipilimumab alone was 10.1 months (30). Subsequently, the United States Food and Drug Administration (FDA) approved ipilimumab as a first-line drug in the treatment of advanced melanoma in 2011; this was the first approved immunotherapy drug (Table I). After 4 years, ipilimumab was approved as an adjuvant treatment for stage III melanoma. In addition, the efficacy of ipilimumab has been demonstrated in other solid tumors, including lung, renal, pancreatic and prostate cancer (31-33).

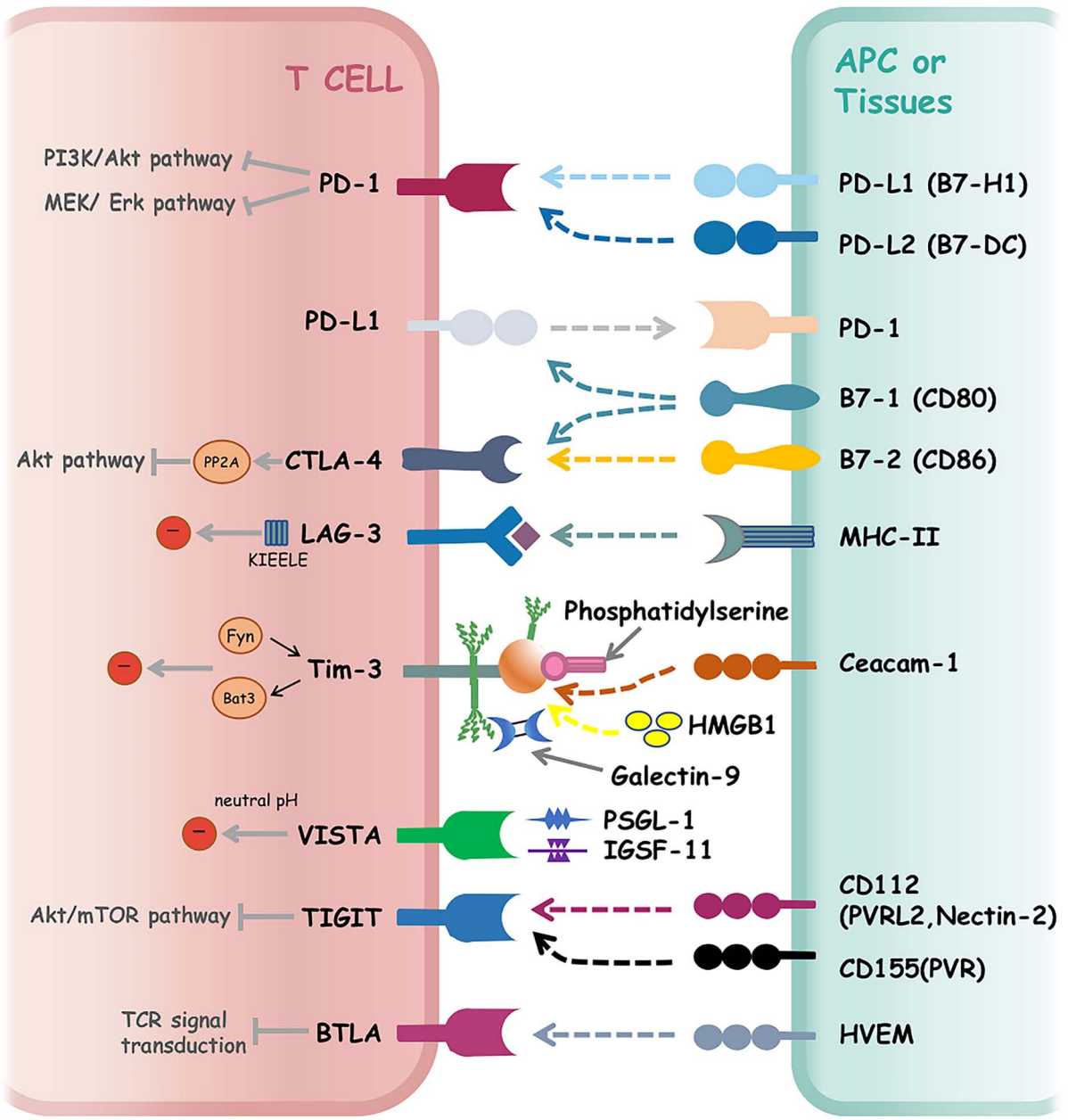

Figure 1. Summary of the T-cell coinhibitory molecules and downstream pathways. '-' in red circles indicates the inhibition of T-cell proliferation, survival and cytokine production. APC, antigen-presenting cell; PD-1, programmed death-1; PD-L1, programmed cell death-ligand 1; CTLA-4, cytotoxic T lymphocyte associated antigen-4; LAG-3, lymphocyte activation gene-3; MHC-II, major histocompatibility complex-II; Tim-3, T-cell immunoglobulin mucin-3; Ceacam-1, carcinoembryonic antigen cell adhesion molecule 1; HMGB1, high-mobility group box 1; VISTA, V-domain immunoglobulin suppressor of T-cell activation; PSGL-1, P-selectin glycoprotein ligand-1; IGSF-11, immunoglobulin superfamily member 11; TIGIT, T-cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domain; BTLA, B- and T-lymphocyte attenuator; HVEM, herpes virus entry mediator.

However, the effects of ipilimumab in these solid tumors have been reported to be limited, and this may be due to low tumor immunogenicity and a potently immunosuppressive tumor microenvironment (TME) (34). In addition to ipilimumab, tremelimumab is currently undergoing clinical trials in a variety of tumors as an additional CTLA-4 blockade (11,35). In 2015, tremelimumab was granted orphan drug qualification by the FDA in the treatment of malignant mesothelioma. In 2020, in combination with durvalumab, tremelimumab was also used to treat hepatocellular carcinoma (HCC). As well as regulating T-cell activation, CTLA-4 blockade may limit the penetration of Tregs into the TME, to prevent Tregs from inhibiting the activity of cytotoxic T cells and enhancing antitumor activity. However, associated research is limited at present (36,37).

PD-1 and anti-PD-1 therapy. In 1992, PD-1 was initially identified by Ishida *et al* (38) at Kyoto University. In 1999, preclinical data established the central role of PD-1 in immune suppression (39). In the same year, Dong *et al* (40) discovered the third member of the B7 family, B7-H1. Freeman *et al* (41) later confirmed that B7-H1 is programmed cell death-ligand 1 (PD-L1) that binds to PD-1. PD-1, which belongs to the CD28 Ig superfamily, is induced transiently on activated

Table I. FDA-approved immune checkpoint inhibitors.

Drug	Target	FDA approval year	Indications
Ipilimumab	CTLA-4	2011	•Unresectable or metastatic melanoma •Adjuvant therapy stage III melanoma •Intermediate or poor-risk, previously untreated advanced RCC, in combination with nivolumab •MSI-H or dMMR metastatic CRC, in combination with nivolumab
Nivolumab	PD-1	2014	•Unresectable or metastatic melanoma, single or in combination with ipilimumab •Adjuvant therapy for lymph node-positive or metastatic melanoma •Metastatic NSCLC with progression after platinum drugs •Metastatic SCLC with progression, 3rd line •Advanced RCC after antiangiogenic therapy •Previously untreated advanced RCC, in combination with ipilimumab •Hodgkin lymphoma, refractory to auto-HSCT, brentuximab or three other treatments •Recurrent or metastatic HNSCC •Locally advanced or metastatic UC, cisplatin refractory •MSI-H or dMMR metastatic CRC, single or in combination with ipilimumab •HCC refractory to sorafenib •Unresectable advanced, recurrent, or metastatic esophageal squamous cell carcinoma •Advanced RCC, first line in combination with cabozantinib
Pembrolizumab	PD-1	2014	•Advanced/unresectable melanoma •Advanced NSCLC (PD-L1$^+$) •Metastatic SCLC, 3rd line •Metastatic or recurrent HNSCC •Hodgkin lymphoma, 4th line •Refractory primary mediastinal large B-cell lymphoma, 3rd line •Recurrent or cisplatin-intolerant UC, PD-L1$^+$ •Unresectable or metastatic, MSI-H or dMMR solid tumors refractory to prior treatment, and CRC refractory to chemotherapy •Recurrent locally advanced or metastatic gastric cancer, PD-L1$^+$, 3rd line •Recurrent locally advanced or metastatic esophageal cancer, PD-L1$^+$, 2nd line •Recurrent or metastatic cervical cancer, PD-L1$^+$, 2nd line •HCC (sorafenib refractory) •Recurrent locally advanced or metastatic MCC •Advanced RCC, in combination with axitinib, 1st line •Advanced endometrial carcinoma, without MSI-H or dMMR, in combination with lenvatinib •Locally recurrent unresectable or metastatic TNBC with tumor PD-L1 combined positive score ≥10 (in combination with chemotherapy)

Table I. Continued.

Drug	Target	FDA approval year	Indications
Atezolizumab	PD-L1	2016	•Locally advanced or metastatic UC, PD-L1^{+}, cisplatin ineligible or refractory •Metastatic NSCLC with progression after platinum drugs •Unresectable locally advanced or metastatic TNBC, PD-L1^{+}, in combination with protein-bound paclitaxel •Extensive stage SCLC, in combination with carboplatin and etoposide •Metastatic or unresectable HCC, first line (in combination with bevacizumab) •BRAF^{V600} mutation-positive unresectable or metastatic melanoma in combination with cobimetinib and vemurafenib
Avelumab	PD-L1	2017	•Metastatic MCC •Locally advanced or metastatic UC, platinum refractory •Advanced RCC, in combination with axitinib, 1st line
Durvalumab	PD-L1	2017	•Advanced or metastatic UC, cisplatin refractory •NSCLC, stage III non-platinum/radiation refractory •Locally advanced or metastatic biliary tract cancer, in combination with chemotherapy
Cemiplimab	PD-1	2018	•Metastatic or locally advanced unresectable cutaneous squamous cell carcinoma

FDA, Food and Drug Administration; CTLA-4, cytotoxic T lymphocyte associated antigen-4; RCC, renal cell carcinoma; MSI-H, microsatellite instability-high; dMMR, mismatch repair deficient; CRC, colorectal cancer; PD-1, programmed death-1; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer; HSCT, hematopoietic stem cell transplantation; HNSCC, head and neck squamous cell cancer; UC, urothelial carcinoma; HCC, hepatocellular carcinoma; PD-L1, programmed cell death-ligand 1; MCC, Merkel cell carcinoma; TNBC, triple-negative breast cancer.

T, B, natural killer (NK) T and myeloid cells (42,43). PD-1 binds to the B7 family ligands, PD-L1 (B7-H1) and PD-L2 (B7-DC), and inhibits the proliferation and effector functions of immune cells. Specifically, the ligation of PD-L1 and PD-1 leads to tyrosine phosphorylation of the immunoreceptor tyrosine-based inhibitory motif (ITIM) and immunoreceptor tyrosine-based switch motif (ITSM) of PD-1. Binding of the ITSM by SHP-1 or SHP-2 results in the inhibition of casein kinase II, the induction of PTEN phosphatase activity and thus the suppression of the PI3K/Akt pathway (43). Other signaling pathways initiated by TCR ligation are also inhibited by PD-1, including ZAP70 and protein kinase Cθ activation. PD-1 ligation can also inhibit the activation of phospholipase C-γ1 and downstream Ras signaling, resulting in decreased activation of the MEK/Erk pathway (44,45). Furthermore, PD-1 signaling may lead to a decrease in T-cell proliferation, survival and protein synthesis, which is essential for maintaining peripheral tolerance. Notably, the genetic loss of Pdcd1, which encodes PD-1, may cause autoimmune pathologies (39,46).

Unlike CTLA-4, PD-1 expression is transient in the early stage of T-cell activation and then decreases when the activating antigen is removed. However, PD-1 expression is high if the antigen is present for a prolonged period of time; for example, during chronic infection or in a tumor (47,48). It has previously been demonstrated that PD-1 expression is increased on the majority of tumor-infiltrating T lymphocytes (TILs) in various tumor types, and this is an important cause of tumor immune escape (49,50). The two ligands of PD-1 are comparable with other B7 immune regulatory ligand family members, and the affinity of PD-L2 to PD-1 has been reported to be higher than that of PD-L1 (46,51). The expression levels of PD-Ls are different in different human cells. Notably, PD-L1 is expressed on hematopoietic cells, such as B cells, T cells, macrophages, dendritic cells (DCs) and mesenchymal stem cells (MSCs) (52), and is also expressed on nonhematopoietic cells, including vascular endothelial cells, fibroblastic reticular cells, astrocytes, liver cells, pancreatic cells and neurons (43). By contrast, PD-L2 is mainly expressed on APCs, such as macrophages and DCs, and peritoneal B1 cells (53). In addition, the main PD-1 ligand expressed on solid tumor cells is PD-L1 (54,55). Results of previous studies have suggested that PD-L1 is upregulated in multiple cancer types, including melanoma, ovarian cancer and lung cancer (56-58). Activated T cells also produce cytokines that promote the expression of PD-L1 on cancer cells (56). When PD-L1 on cancer cells binds to PD-1 on T cells, immunosuppression occurs. In addition, PD-L1 specifically interacts with the B7-1 (CD80) co-stimulatory molecule to inhibit T-cell responses (59).

Collectively, these mechanisms cause cancer cells to evade the immune system.

A previous study found that the CTLA-4 inhibitor was effective only for certain cases of advanced melanoma, and serious side effects may occur in treating other types of tumors (34). Notably, ICIs were not widely accepted until the use of PD-1/PD-L1 antibodies was established in the clinic in 2014. Although PD-1/PD-L1 antibodies mainly function through upregulating T-cell activation, they also block the host-derived PD-L1 signals from non-tumor cells in the microenvironment, as well as the interactions between PD-L1 and B7-1 (11). At present, a total of six PD-1/PD-L1 inhibitors have been approved by the FDA, including nivolumab, pembrolizumab, atezolizumab, avelumab, durvalumab and cemiplima. The associated indications include melanoma, Hodgkin's lymphoma, non-small cell lung cancer (NSCLC), renal cell cancer (RCC), gastric cancer, liver cancer, colorectal cancer (CRC), cutaneous squamous cell cancer and urothelial cancer (UC). These indications are displayed in Table I.

In 2006, the first clinical trial of a PD-1 inhibitor, nivolumab, was conducted in refractory solid tumors. Notably, nivolumab was known as IgG4 anti-PD-1 mAb and Opdivo (MDX-1106, ONO-4538, BMS-936558), and was developed by Bristol-Myers Squibb. In December 2014, nivolumab was approved by the FDA for the treatment of metastatic melanoma, with the results of the CheckMate 037 clinical trial demonstrating an objective response rate of 31.7% in the nivolumab group, and 10.6% in the chemotherapy group (dacarbazine orcarboplatin) (54). At present, the indications have extended to various advanced solid tumors, including NSCLC, RCC, UC, squamous cell carcinoma of head and neck, Hodgkin's lymphoma, HCC, CRC and esophageal squamous cell carcinoma (59-63).

In September 2014, an additional PD-1 inhibitor, pembrolizumab (Keytruda, lambrolizumab, MK-3475), was approved by the FDA for the treatment of unresectable or metastatic melanoma. Pembrolizumab, a fully humanized IgG4 with high-affinity and high-selectivity, demonstrated an objective response rate of >30% in patients with advanced melanoma (NCT01866319) (63). At present, pembrolizumab is approved for use in >14 indications of 10 tumor types (64-69).

In September 2018, another PD-1 inhibitor, cemiplimab (Libtayo), was approved by the FDA for treatment of metastatic or locally advanced non-resectable cutaneous squamous cell carcinoma. Notably, this was the first drug approved by the FDA that was specifically for the treatment of patients with advanced cutaneous squamous cell carcinoma (70).

Atezolizumab (Tecentriq), a human IgG1 anti-PD-L1 mAb, was the first PD-L1 inhibitor approved by the FDA for the treatment of advanced or metastatic UC. Results of a phase II clinical trial demonstrated an objective response rate of 15% in all patients and 26% in patients with the highest levels of PD-L1 expression following treatment with atezolizumab (71). The indications of atezolizumab have subsequently extended to NSCLC, small cell lung cancer, breast cancer, HCC and melanoma (71-75).

An additional PD-L1 inhibitor, avelumab (Bavencio), not only functions via blocking PD-1/PD-L1 interactions, but also through antibody-dependent cell-mediated cytotoxicity (76). In March 2017, avelumab was approved by the FDA for the treatment of metastasis Merkel cell carcinoma in adolescents and adults >12 years of age, and the indication was subsequently expanded to UC and RCC.

In May 2017, the FDA-approved durvalumab (Imfinzi), an additional PD-L1 antibody, for the treatment of advanced UC, using accelerated approval. Results of a previous study demonstrated that durvalumab substantially improved the progression-free survival of patients with NSCLC, compared with the placebo (16.8 months vs. 5.6 months) (77). Subsequently, the FDA expanded the indications to include Stage III NSCLC in February 2018.

A novel PD-L1 inhibitor, envafolimab (KN035), was awarded Orphan Drug Designation by the FDA for the treatment of biliary tract cancer. Notably, the PD-1/PD-L1 inhibitors approved by the FDA may continue to be approved for additional indications. However, alternative anti-PD-1/PD-L1 axis-targeted therapies, such as pidilizumab (CT-011) and BMS-936559 (MDX-1105) are under investigation in clinical trials, and these treatment options may exhibit potential in a broad range of tumor types (78,79).

PD-1/PD-L1 inhibitors are used in the treatment of various types of cancer. However, results may differ between patients, and the mechanistic basis for the variation in response patterns are multifaceted. For example, a previous study reported that patients with melanoma that respond to these inhibitors had a higher proportion of BRCA2 mutations (80). In addition, innately resistant tumors display a transcriptional signature, indicating concurrent increased expression of genes involved in the regulation of mesenchymal transition, cell adhesion, extracellular matrix remodeling, angiogenesis and wound healing (80). Moreover, PD-1 inhibitor monotherapy for patients with NSCLC accompanied by EGFR mutations exhibit low response rates and unsatisfactory efficacy. PD-1 inhibitors may be ineffective in microsatellite stable type carcinoma (81), and PD-1/PD-L1 inhibitors exert minimal effects on certain types of tumors, such as multiple myeloma and uveal melanoma (78). However, further investigations into the specific molecular and cellular mechanisms of PD-1/PD-L1 inhibitors in antitumor immune enhancement are required.

Alternative immune checkpoint molecules and therapy. Following the discovery of CTLA-4 and PD-1/PD-L1 in antitumor treatment, further preclinical and clinical studies have focused on additional immune checkpoint molecules.

Lymphocyte activation gene-3 (LAG-3), also known as CD223, is a member of the Ig superfamily and a homologous protein of $CD4^+$, which can bind to MHC-II molecules with high affinity. LAG-3 is expressed on active NK cells, T cells, B cells, TILs, Tregs and DCs, and is required for immune homeostasis (82-84). However, persistent antigen stimulation in cancer may lead to chronic LAG-3 expression, promoting T-cell exhaustion. Results of previous studies have demonstrated that LAG-3 is highly expressed on the TILs of multiple tumors (85-88). The signaling pathways downstream of LAG-3 responsible for its inhibitory function are still unclear, but the KIEELE motif of the LAG-3 cytoplasmic tail has been shown to be essential for the inhibitory function (84). At present, research is focused on numerous therapeutic agents targeting LAG-3, for the treatment of multiple types of human cancer (89,90).

T-cell Ig mucin (Tim)-3, a member of the Tim family, is a type I transmembrane glycoprotein, which was initially recognized in $CD4^+$ T helper and $CD8^+$ T cytotoxic cells, and was subsequently shown to be expressed in Tregs, NK cells, monocytes and DCs (84,91). Several ligands of Tim-3 have been recognized, including galectin-9 (Gal-9), phosphatidyl serine (PS), high-mobility group box 1 (HMGB1) and carcinoembryonic antigen cell adhesion molecule 1 (Ceacam-1) (92). Notably, the binding of Tim-3 and Gal-9 induces T-cell death and reduces the immune response (92). The binding of Tim-3 and PS mediates the phagocytosis of apoptotic cells and cross-presentation (93). The binding of Tim-3 and HMGB1 inhibits antitumor immune responses mediated by HMGB1 (94). Ceacam-1 was identified as a novel ligand for Tim-3. In the absence of Ceacam-1, the negative regulatory function of Tim-3 is defective, indicating that the interaction between Ceacam-1 and Tim-3 is required for optimal Tim-3 function (95). Bat-3 and Fyn bind to the same region on the cytoplasmic tail of Tim-3, the binding of Gal-9 or Ceacam-1 to Tim-3 can trigger the dissociation of Bat-3 from the cytoplasmic tail of Tim-3, thus allowing Fyn to bind, which is implicated in the induction of T-cell anergy (84). Tim-3 expression is indicative of dysfunctional or exhausted T cells in cancer, and is elevated in various tumors (96-99). In addition, the co-blockade of Tim-3/PD-1 demonstrated increased efficacy in treating tumors, compared with blocking Tim-3 or PD-1 alone (100).

T-cell Ig and ITIM domain (TIGIT) is a member of the Ig superfamily, and is expressed on NK cells, activated T cells, memory T cells, follicular T helper cells and Tregs (101-104). The ligands of TIGIT, including CD155 (PVR) and CD112 (PVRL2, nectin-2), are expressed on APCs, T cells, nonhematopoietic cells and tumor cells (101,105). CD155/TIGIT can suppress the function of NK cells through inhibiting PI3K/MAPK and NF-κB signaling, and can suppress the function of T cells through inhibiting AKT/mTOR signaling. In addition, TIGIT can reduce the activity of the co-stimulatory molecule CD226 during the antitumor T-cell response (84). At present, three phase I/II clinical studies targeting TIGIT for cancer immunotherapy are ongoing (NCT05130177, NCT04354246 and NCT04995523; 106-108).

B- and T-lymphocyte attenuator (BTLA), also known as CD272, belongs to the CD28 Ig superfamily. The protein structure of BTLA is comparable with that of PD-1 and CTLA-4. BTLA is expressed on B cells, T cells, NK cells, DCs and macrophages (109,110). The ligand of BTLA is herpes virus entry mediator (HVEM), which belongs to the tumor necrosis factor receptor family. HVEM is widely expressed in B cells, T cells, NK cells, monocytes and DCs (109). Engagement of BTLA leads to the recruitment of SHP-1 and SHP-2 in T cells, thereby downregulating TCR signaling and the transmission of inhibitory signals (111). Results of previous studies have demonstrated that BTLA is highly expressed in melanoma, lung cancer, RCC, lymphoma, B-cell small lymphocytic lymphoma and chronic lymphocytic leukemia (109-112). At present, preclinical studies of BTLA or HVEM inhibitors are ongoing, and subsequent clinical studies will be developed (109-111).

V-domain Ig suppressor of T-cell activation (VISTA), also known as C10 or f54, is a member of the CD28 family. VISTA is a novel immune checkpoint expressed on myeloid cells and lymphoid cells, which is upregulated in various tumors (113). VISTA has two proven ligands, P-selectin glycoprotein ligand-1 (PSGL-1) and Ig superfamily member 11 (IGSF-11); PSGL-1 only functions at neutral pH and the affinity declines fourfold at pH 6.0 (113). VISTA can serve as both a ligand and receptor to suppress T cell-associated immune responses; however, the mechanism remains to be elucidated (113). Several clinical trials of VISTA inhibitors are ongoing for the treatment of multiple types of cancer (NCT02812875, NCT02671955 and NCT04475523; 114-116).

The FDA-approved ICIs classified by cancer type are summarized in Fig. 2. The characteristics of various immune checkpoint molecules and their associated roles in tumor immunotherapy differ. An increased understanding of the basic biological functions of these molecules is essential for the development of novel ICI therapies.

4. Biomarkers for ICIs

Although ICIs have demonstrated high levels of success in improving therapeutic efficacy in some patients, previous studies have demonstrated that only ≤20-30% of patients with NSCLC, melanoma or RCC benefit from ICIs (30,117-120). Non-responders include patients who do not respond to treatment at all and patients who relapse after remission to ICIs (121). These non-responders endure high treatment costs and associated levels of toxicity with little benefit from the treatment. Moreover, inappropriate application may cause disease progression (122). Therefore, the development of predictive biomarkers is required for prescribing ICIs in a personalized manner.

PD-L1. Results of a previous study suggested that PD-L1 expression in tumor cells and the tumor environment is positively associated with the response to PD-1/PD-L1-blocking antibodies (123). Immunohistochemistry (IHC) analyses performed on patients with metastatic melanoma, colon cancer, NSCLC, prostate cancer and RCC who received PD-1/PD-L1 targeted therapy demonstrated that PD-L1 upregulation acted as a potential biomarker (63,119,124-126). Different PD-L1 expression cutoffs and scoring systems have been used in different trials of FDA-approved drugs directed by PD-1/PD-L1 (123). However, PD-L1 may not be optimal as a potential biomarker, as the overall response rate of PD-1/PD-L1-blocking antibodies in patients with negative PD-L1 expression can also reach 0-20% (127,128). There are some limitations that must be considered when selecting PD-L1 as an immunotherapy biomarker. Notably, the expression of PD-L1 is induced and dynamic; thus, different treatment methods may impact the expression of PD-L1 in different stages of treatment (129,130). Besides, the expression between primary and metastatic tumors may be heterogeneous. As a result, the expression of PD-L1 at a certain time or location cannot accurately reflect the expression of PD-L1 in tumors (131,132). In addition, different commercially available PD-L1 IHC tests were used in different trials, and different cutoff scores were set to detect or quantify tumor PD-L1 expression, resulting in clinical failure to select patients according to a unified standard (133).

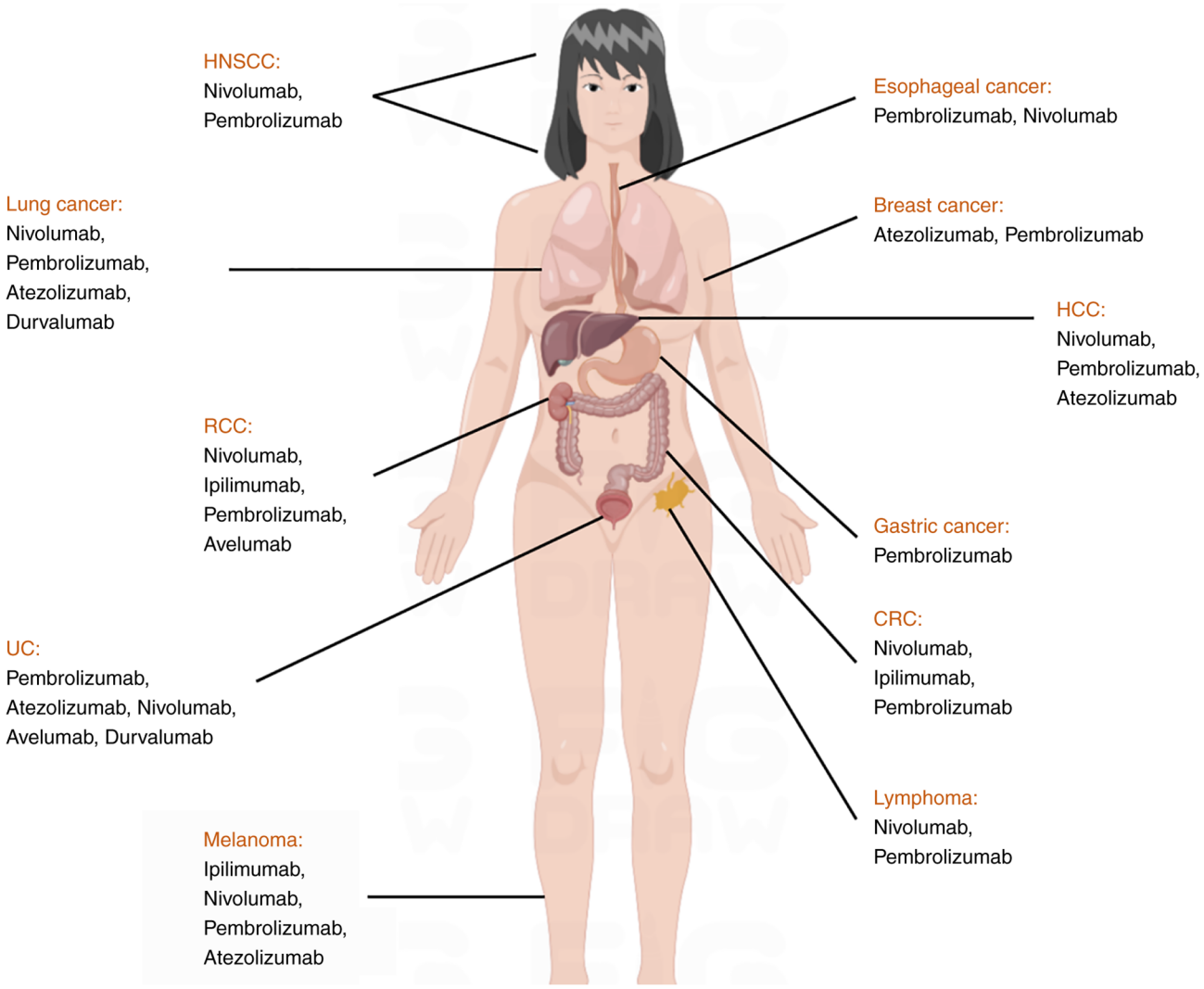

Figure 2. Summary of the Food and Drug Administration-approved immune checkpoint inhibitors classified by cancer type. HNSCC, head and neck squamous cell cancer; RCC, renal cell carcinoma; UC, urothelial carcinoma; HCC, hepatocellular carcinoma; CRC, colorectal cancer. This figure was drawn by FigDraw (https://www.figdraw.com/).

Mutation signatures and microsatellite instability (MSI). Following the development of gene sequencing and bioinformatics, genomics technology is more frequently used for discovering biomarkers associated with ICIs. Previous clinical studies revealed that mismatch repair deficiency (dMMR) or MSI-high (MSI-H) are associated with response to ICIs (81,134). MMR is an important DNA repair mechanism that makes alterations in DNA mismatches, and dMMR may lead to MSI, which can be used for the clinical detection of dMMR (135). dMMR or MSI-H are often present in various types of cancer (136,137). The results of previous studies suggested that dMMR tumors exhibit high neoantigen load, tumor mutational burden (TMB), T-cell infiltration and upregulation of multiple immune checkpoints, including PD-1, PD-L1, CTLA-4 and LAG-3 (138-141), which may lead to high response rates to ICIs. Notably, MSI has been recognized by the FDA as a predictive biomarker for ICI responsiveness (142). Moreover, pembrolizumab was specifically approved in the treatment of multiple solid tumors with MSI-H or dMMR. This was the first FDA-approved tumor immunotherapy that was not based on tumor tissue type and instead based solely on genetic characteristics. Therefore, further investigations should focus on identifying MMR and MMR-like tumors. Further analysis of specific DNA repair gene sets, or bioinformatics analysis of specific DNA damage characteristics associated with specific DNA repair defects in the cancer genome, are required to assess the potential sensitivity to ICIs (143).

TMB. TMB refers to the total number of base substitution, insertion or deletion mutations in the coding region of exons of evaluated genes in tumor tissue samples. Notably, a higher TMB may affect the probability of immunogenic peptide generation; thus, affecting the response of patients to ICIs (144,145). As a result, the association between TMB and the efficacy of ICIs has been the focus of multiple studies. Notably, TMB may be associated with clinical benefits of ICIs in patients with melanoma, NSCLC, UC, squamous cell carcinoma of head and neck, and small cell lung cancer, while those with lower TMB, such as pancreatic cancer and prostate cancer, may exhibit poor responses to ICIs (71,146-152). In 2020, TMB became the second FDA-approved predictive biomarker for the efficacy of ICIs. However, there are

notable limitations. Firstly, different testing platforms are used in the clinical studies of TMB at present, and there is no standard definition of high TMB. Thus, the mutation load of different tumors is varied and different cutoffs must be established (146,147). Moreover, TMB alone may not distinguish all responders from non-responders. For example, the immunogenicity of tumor neoantigens may be improved by epigenetic modification in tumors with low TMB, leading to improved therapeutic responses to ICIs (62,153). However, for those tumors with high TMB, there may be other immunosuppressive molecules in the tumor immune microenvironment, such as IL-10 and metabolism-related enzyme IDO, which may affect the efficacy of ICIs (9,154). Consequently, TMB alone may be inadequate in predicting the efficacy of ICIs. Moreover, further investigations into cost control, application of dynamic biomarkers and blood-based TMB detection are required.

TILs. TILs are the effector cells of antitumor immunity and the target cells of ICIs. TILs act as a representative of tumor-immune system interaction; therefore, assessing the presence of TILs may aid in identifying patients who benefit from immunotherapy. In a study of pembrolizumab in the treatment of advanced melanoma, $CD8^+$ T-cell infiltration in the tumor tissue or invasion margin was revealed to be higher in responders than in patients who did not respond (155). Results of previous studies have also demonstrated that TILs may be used to predict the immunotherapeutic response and prognosis of numerous types of cancer, including breast cancer and CRC (156-160). Adding immunoscore based on TILs to the existing tumor, lymph node and metastasis classification system may improve the development of effective treatment plans and allow clinicians to provide more accurate prognoses (161). However, the widespread application of TILs as a predictive tool for immunotherapy response requires further validation and standardization.

Specific mutated genes. Specific gene mutations may exert effects on tumor cells that impact immune surveillance. Notably, several single gene biomarkers may impact treatment decisions with ICIs. In patients with melanoma, several gene mutations, including BRAF, JAK1/2, β2-microglobulin (β2M) and mutations in the interferon γ (IFN-γ) pathway, are associated with the efficacy of immunotherapies (162-167). SERPINB3 and SERPINB4 mutations have also been shown to be associated with the response to anti-CTLA4 immunotherapy in patients with melanoma, independent of tumor stage, TMB and patient age (168). In addition, inactivation of PTEN may be associated with resistance to ICIs in melanoma and uterine leiomyosarcoma (169,170). Patients with NSCLC and STK11/LKB1 or EGFR mutations, or ALK rearrangements, exhibited decreased efficacy and low response rates to ICIs. By contrast, KRAS/TP53 mutations were associated with improved clinical outcomes (171-173). In patients with RCC, PBRM1 mutations may be associated with clinical benefits of ICIs (174-178). Moreover, high-throughput clustered regularly interspaced short palindromic repeats screening has identified numerous genes associated with improved clinical benefits of ICIs, such as PTPN2, APLNR and SWI/SNF complex genes (179-181).

A collection of peptides presented to the cell surface by class I and class II human leukocyte antigen (HLA) molecules are referred to as the immunopeptidome. Cancer cells may have defective HLA-I functions, leading to abnormal tumor antigen presentation and the destruction of antigen-MHC binding; thus, evading immune surveillance and impacting the efficacy of immunotherapy (182,183). Results of a previous study demonstrated that loss of HLA expression impacted the response to ICI therapy (184). Moreover, a further study analyzed the HLA-I genotype of 1,535 patients with advanced tumors treated with ICIs. The results demonstrated that in patients with maximal heterozygosity at the HLA-I loci ('A', 'B', and 'C'), OS was improved following treatment with ICIs, compared with that of patients who were homozygous for at least one HLA locus. This may improve the ability of providing a wider range of tumor antigens to T cells (185). Therefore, these studies indicated that the recognition of neoantigens by peripheral T lymphocytes is the main mechanism of antitumor immune response. The widespread application of these technologies still requires further validation.

Peripheral blood biomarkers. Considering the advantages of non-invasive surgery, peripheral blood detection technology has remained the focus of research surrounding hematological markers associated with the efficacy of ICIs. In patients with metastatic melanoma treated with ipilimumab, previous studies demonstrated that survival was significantly associated with low serum lactate dehydrogenase, absolute monocyte counts, myeloid-derived suppressor cells (MDSCs), high CD8 effector-memory type 1 T cells, absolute eosinophil counts and absolute lymphocyte counts (186-189). Moreover, baseline absolute neutrophil counts and derived neutrophil-to-lymphocyte ratios have been reported to be significantly associated with the survival of patients with melanoma treated with ipilimumab (187). The neutrophil-to-lymphocyte ratio was also shown to be significantly associated with survival in patients with metastatic RCC (190). In patients with melanoma treated with pembrolizumab, results of a previous study demonstrated that increased relative lymphocyte count at baseline was associated with improved clinical outcomes (191). In patients with NSCLC treated with anti-PD-1/PD-L1 agents as second- or third-line therapies, PD-L1 expression in circulating tumor cells exhibited potential as a prognostic biomarker (192). In addition, numerous features of peripheral blood components are associated with the response to ICIs, including classical monocyte ($CD14^+CD16^-CD33^+HLA\text{-}DR^+$) frequency (193), serum vascular endothelial growth factor level (194,195), soluble CD25 levels (188), and serum cytokine levels of IFN-γ, IL-18, IL-6 and IL-8 (124,196,197). Moreover, circulating exosomes containing PD-L1, PD-1 or CD28 may be associated with responses to ICIs (198-200). These blood-based biomarkers may be obtained in a clinical setting and do not incur any additional costs to the patient. However, there is still much to learn from more retrospective and prospective studies evaluating the value of both approved and developing peripheral blood biomarkers, meanwhile care should be taken to avoiding redundancy between biomarkers.

Intestinal microbiota. Previous studies have demonstrated that intestinal microbiota may affect the occurrence and development of cancer, through modulating immunity and regulating

cell metabolism (201,202). Within antitumor immunotherapy, intestinal microbiota may impact the therapeutic effects of ICIs. Results of a previous study demonstrated that tumors in antibiotic-treated or germ-free mice did not respond to a CTLA inhibitor; however, treatment response occurred following gavage with *Bacteroides fragilis* (203). Moreover, results of a further study demonstrated that treatment with an oral microorganism combined with a PD-L1 antibody reduced tumor outgrowth in mice (204). These studies demonstrated that controlling the microbiota may aid in regulating cancer immunotherapy. Notably, comparable results were observed in the clinic. Results of a clinical retrospective study demonstrated that clinical benefits were reduced in patients who used antibiotics before and after treatment with ICIs, compared with patients who did not use antibiotics. Notably, the antibiotics may have impacted the homeostasis of the intestinal microflora (205). Patients with NSCLC, RCC, metastatic melanoma or UC with a high microbial diversity, including specific species such as *Ruminococcus*, *Bifidobacterium* or *Enterococcus*, exhibited favorable responses to PD-1 inhibitors (205-207). Moreover, results of previous studies demonstrated that germ-free mice that received fecal microbiota transplantation from patients with cancer who responded to ICIs exhibited improved responses to ICIs, compared with those that received fecal microbiota transplantation from non-responders (203,205,206). Collectively, these results demonstrated that intestinal microbiota are influential in antitumor immunity and responses to ICI. At present, the immune regulation mechanism of intestinal microbiota is not fully understood. With the development of the Human Microbiome Program and the advancement of sequencing analysis technology, research on intestinal microbiota is increasing, and metabolomics analysis technology further enhances the exploration of the relationship between intestinal microbiota and host immunity. Two caveats should be mentioned regarding research on intestinal microbiota. First, the differences in the application of microbial sequencing analysis techniques among different studies have limited comparability between research results. Second, the intestinal microbiota is influenced by various factors, such as diet, medication, age and environment, and there is also serious interference between different microorganisms. Regardless, reasonable antibiotic selection provides new possibilities for improving the antitumor efficacy and reducing adverse reactions of ICIs (204).

In addition, epigenetic signatures, liquid biomarkers and the tumor metabolism microenvironment may be associated with responses to ICIs (206,208). In conclusion, there are numerous potential biomarkers for predicting the effectiveness of ICIs; however, these efficacy prediction biomarkers often do not work alone, and different biomarkers interact in tumor specimens or blood specimens. The combined application of multiple predictive biomarkers can better screen out the population that will respond best to ICIs, and maximize the clinical benefits of patients. Thus, further large-scale prospective studies for comparison and validation are required prior to use in clinical practice. Further detection of multiple biomarkers, establishing standard biomarker test procedures, and maintaining high repeatability and low costs are all considerations that must be addressed.

5. Resistance mechanisms to ICIs

ICIs exhibit potential in the treatment of some advanced tumors; however, the majority of patients do not benefit from ICIs as a single therapy due to the complexity of drug resistance mechanisms. Improving the current understanding of mechanisms limiting cancer immunotherapy may aid in the discovery of novel therapeutic targets and provide potential combination treatment strategies. Multiple tumor-intrinsic and -extrinsic factors may contribute to both primary resistance, in which tumors do not respond to initial therapy, and acquired resistance, such as relapse after an initial response.

Tumor-intrinsic factors for primary resistance. Tumor cells may escape from immune surveillance through abnormal antigen processing and presentation. Genetic mutations in β2M, a component of the MHC-I molecule required for antigen presentation, are present in various cancer types and associated with evading the T-cell immune response (166,183,209-212). Therefore, alterations in antigen-presenting machinery must be taken into consideration prior to ICI treatment.

Gene alterations in specific signaling pathways also serve significant and complex roles in mediating immunotherapy resistance. Alterations in oncogenic signaling pathways include: i) Loss of IFN-γ signaling pathways; ii) upregulation of the Wnt-β-catenin signaling pathway; and iii) loss of PTEN expression, which enhances PI3K signaling. Loss-of-function mutations in JAK1 and/or JAK2 involved in the IFN-γ signaling pathway have been observed in patients who exhibited resistance to PD-1 inhibitors (167). Other genetic mutations in the IFN-γ pathway, including IFN-γ receptor 1 and 2, and interferon regulatory factor 1, have also been observed in patients who exhibited resistance to CTLA-4 inhibitors (165). These mutations may inhibit IFN-γ signal transduction and allow tumor cells to escape from T cells. In addition, mutations in this pathway may lead to the downregulation of PD-L1 expression following IFN-γ exposure, thus reducing the therapeutic effect of PD-1/PD-L1 antibodies (213). Upregulation of the Wnt-β-catenin signaling pathway has been detected in a subset of patients with melanoma, and this was revealed to be associated with resistance to ICIs (214). Notably, β-catenin suppresses CCL4 secretion, a chemokine that attracts DCs, subsequently leading to failure of T-cell activation and function (215). Loss of PTEN expression has also been associated with ICI resistance, resulting from enhanced PI3K signaling (169,170,216). Moreover, mutations in the MAPK signaling pathway may lead to cancer immune evasion, through enhancing the expression of the immunoregulatory cytokines IL-6 and IL-10 (217).

An additional tumor intrinsic mechanism is the compensatory upregulation of other immune checkpoints, such as VISTA, Tim-3 and TIGIT (218-220). Moreover, the transition of epithelial cells to mesenchymal cells, characterized by increased migration and invasion, and resistance to apoptosis, may also have a role in ICI resistance (221-223). Numerous genes have been reported to be associated with a lack of response to PD-1 blockade, known as innate anti-PD-1 resistance signatures (80). Further understanding of these resistance mechanisms is required for informing clinical management and developing personalized therapies.

Tumor-extrinsic factors for primary resistance. The highly immunosuppressive TME is considered the tumor extrinsic mechanism of ICI resistance. The TME includes immunosuppressive cells, cytokines, chemokines and metabolites that restrain antitumor immunity (121). The immunosuppressive cells include Tregs, MDSCs, tumor-associated macrophages (TAMs) and MSCs, and these suppress immune responses through numerous mechanisms (224-228). The immunosuppressive cytokines, chemokines and metabolites, such as transforming growth factor-β (TGF-β), CCL5, CXCL8 and IDO are secreted by cancer cells and immunosuppressive cells in the TME (229-232). Collectively, these factors create a functionally inhibitory microenvironment that causes resistance to immunotherapy.

Acquired resistance to immunotherapy. Acquired resistance refers to cancer that progresses and relapses following initial responses to immunotherapy. The potential mechanisms underlying acquired resistance include the inability of T cells to recognize tumor cells, due to lack of antigen expression or antigen presentation function defects, upregulation of alternative immune checkpoints, T-cell depletion, and immune escape. The mechanisms of abnormal antigen recognition or upregulation of other immune checkpoints have been aforementioned. The factors that lead to T-cell depletion are multifaceted. For example, epigenetic dysfunction makes T cells resistant to remodeling and activation (233). In addition, elevated IDO or lactate dehydrogenase in the TME may diminish T-cell responses. Furthermore, impaired production of memory T cells may lead to the weakening of the effects of ICIs over time, leading to acquired drug resistance (233).

Although resistance to immunotherapy may manifest at different times, from initial therapy to relapse after an initial response, similar or overlapping mechanisms enable tumor cells to evade antitumor immune responses. For example, IFN-γ signaling pathways are key factors for both primary and acquired drug resistance (165,167,213,233). Moreover, resistance mechanisms are dynamic (233). Therefore, targeting a single drug resistance mechanism is unlikely to be sufficient to eradicate immunotherapeutically refractory tumors. Thus, the precise selection of sensitive populations, dynamic monitoring of drug resistance, an increased search for synergistic combination therapies, and the development of novel targets and drugs are required to overcome resistance to immunotherapy.

6. Combination of ICIs with other therapeutic strategies

The effectiveness of ICI therapy is limited by various factors, and further investigations into reducing resistance are required. To determine an optimal antitumor immune response, combination treatments that combine ICIs with other therapeutic strategies, such as surgery, radiotherapy, chemotherapy and other forms of immunotherapy are required.

Immunotherapy may be combined with surgery in a neoadjuvant setting (before surgery) or an adjuvant setting (after surgery). Neoadjuvant treatment using chemotherapy or radiotherapy before surgery exhibits specific advantages over adjuvant treatment; however, current literature surrounding neoadjuvant immunotherapy is lacking. From a biological standpoint, neoadjuvant immunotherapy may reinvigorate exhausted cytotoxic T cells when antigens are encountered, and the exposure to antigens during the presence of major tumor mass may increase the breadth and persistence of tumor-specific T-cell responses. Compared with adjuvant immunotherapy, neoadjuvant immunotherapy may effectively reduce tumor mass and improve the probability of complete surgical resection (234). Besides, neoadjuvant immunotherapy has been reported to be superior to adjuvant immunotherapy in eradicating micrometastases, thereby reducing the probability of recurrence (234). Moreover, fewer infusions of neoadjuvant immunotherapy provides reduced exposure to immunotherapy, limiting the development of resistant clones in relapsed patients. The results of previous preclinical and clinical studies have demonstrated that neoadjuvant immunotherapy can improve response and survival rates, compared with the same therapy administered in the adjuvant setting (234-236). In the first clinical trial that performed a head-to-head comparison of neoadjuvant and adjuvant ICIs for the treatment of stage III resectable melanoma, the patients were treated either post-surgery for 12 weeks with a combination of ipilimumab + nivolumab, or in a split design for 6 weeks before surgery and for 6 weeks post-surgery (NCT02437279). The result showed that OS was 90% for patients treated with neoadjuvant ICI therapy and 67% for patients treated with adjuvant ICI therapy at a median follow-up time of 32 months (234). At the European Society of Internal Oncology Immunooncology Conference in 2022, a phase II CA209-8D8 study of neoadjuvant therapy for NSCLC based on nivolumab, led by Professor Wu Yilong (Guangdong Provincial People's Hospital, Guangzhou, China), also announced the advantages of neoadjuvant immunotherapy (237). Specifically, patients can benefit from nivolumab + chemotherapy regardless of PD-L1 expression, and the neoadjuvant therapy does not affect the timing and feasibility of surgery, nor does it increase the difficulty of surgery (237). However, further investigations into the optimal duration of neoadjuvant immunotherapy and surgery, the optimal type of immunotherapy, and the efficacy and safety of neoadjuvant immunotherapy are required.

Radiotherapy and chemotherapy may induce apoptosis of tumor cells, also known as immunogenic cell death, resulting in greater antigen presentation and enhanced antitumor immune responses (238). The results of previous studies demonstrated improved efficacy when radiotherapy or chemotherapy was used in combination with ICIs (239-243). Combined targeting of multiple immune checkpoints, including CTLA-4, PD-1, LAG-3, Tim-3, OX40 and glucocorticoid-induced tumor necrosis factor receptor exerts significant survival benefits, compared with single targeting (164,244-246). However, some immune checkpoints are expressed only after initial T-cell priming; thus, ICIs may be limited to tumors that require reverse exhaustion and restoration of T-cell function (247). In addition to antibody-based immunotherapy, the combination of ICIs with other forms of immunotherapy, such as cancer vaccines, oncolytic viruses or T-cell adoptive therapies are being explored in clinical trials at present (248-252). Moreover, the combination of ICIs with small molecule inhibitors targeting i) immunosuppressive cells, such as MDSCs and TAMs; ii) cytokines, such as TGF-β; or iii) metabolites, such as IDO, are being developed to enhance responses to ICIs (229,253). Notably, these studies provide guidance on

delivering combination therapies. There are still a number of issues that need to be addressed before these combination therapies become clinical standards, including indications, applicable population, combination medication sequence, medication time, dosage, efficacy evaluation standards and adverse reaction prediction. Therefore, further preclinical investigations and clinical trial designs are required.

7. Adverse events associated with ICIs

The application of ICIs, either alone or in combination with other therapeutic strategies, has increased in patients with refractory metastatic cancer, and also as adjuvant or neoadjuvant therapy in the early stages of cancer. Although these treatments are often well tolerated, immune-related adverse events (irAEs) may also occur, resulting from activation of the immune system and off-target immune attack on healthy tissues of the host, which may affect almost any organ system with varying severities (254). Notably, irAEs are often graded using the National Cancer Institute Common Terminology Criteria for Adverse events (254).

As a systemic adverse reaction, fatigue is the most commonly reported, followed by infusion reactions (255). Moreover, adverse reactions of the skin and gastrointestinal tract are the most common following treatment with any approved ICI. Skin rash and pruritus are the most widely reported symptoms of skin toxicity. Notably, anti-CTLA-4 treatment causes the highest rate of adverse reactions, occurring in 40-50% of cases, followed by anti-PD-1 treatment (30-40%). In addition, anti-PD-L1 treatment causes the lowest rate of adverse reactions, occurring in 1-7% of cases (255,256). Other skin toxicities include vitiligo, photosensitive reaction and xerosis. Rare cases of Stevens-Johnson syndrome and toxic epidermal necrolysis have been reported, and these may be fatal (255). Often, the majority of skin toxicities are low-grade and easily managed with emollients, oral antihistamines and topical corticosteroids, while high-grade adverse events require permanent cessation of ICIs. Gastrointestinal toxicities often present as diarrhea and/or colitis. In total, in a previous study, ~30% of patients who received anti-CTLA-4 treatment, 20% of patients who received anti-PD-1 treatment and 45% of patients who received combination treatment developed diarrhea (257,258). Prompt recognition and intervention are crucial in preventing additional complications, such as colonic perforation. It is generally recommended that all patients receiving ICIs who present with diarrhea should undergo stool analyses for enteric pathogens and *Clostridium difficile* toxins. Patients with grade ≥2 diarrhea may require steroid treatment, whereas patients with grade 4 diarrhea/colitis or recurrent diarrhea should stop ICI treatment permanently (259). Endocrine toxicity associated with ICI therapy may involve the thyroid, pituitary or adrenal gland. The most common adverse effect is hypophysitis; however, others include hypothyroidism, hyperthyroidism, thyroiditis, primary adrenal insufficiency, type 1 diabetes mellitus and hypoparathyroidism. Therefore, examination of thyroid function pre-treatment and monitoring during treatment are essential (260). Hepatic adverse events that occur following ICI therapy often present as increases in asymptomatic transaminase, with or without increases in bilirubin; however, autoimmune-like hepatitis with increased severity and acute liver failure may also occur. Patients with grade ≥2 toxicities should be treated with systemic steroid treatment (253). Pulmonary irAEs, such as pneumonitis, are uncommon; however, these may be fatal. The incidence rate of pulmonary irAEs is higher following treatment with anti-PD-1 and/or combined treatment, compared with anti-CTLA-4 treatment. Timing of systemic steroid treatment is crucial and potential infection should be excluded (261). Other irAEs, such as neurologic, ocular, renal, hematological, rheumatologic and cardiovascular toxicities are rare. Following single drug treatment, the incidence rate of these events is <2%; however, following the development of grade 3-4 adverse reactions, patients should stop ICI treatment permanently (259).

Although treatment options are available for irAEs, these can progress, and in some cases, be life threatening. Management of irAEs is often complex and requires close collaboration with clinical experts. Further identification of predictive biomarkers of irAEs, such as T-cell or B-cell biomarkers, microbiome biomarkers and genomic biomarkers, will aid in guiding treatment decisions (262). Furthermore, it is necessary to encourage the establishment of a large-scale pharmacovigilance registration system and collect the records of irAEs in real-world patients following treatment with ICIs. This can not only verify the existing conclusions obtained through real-world large sample data, but also use these records for new research, such as determining the clinical characteristics of various irAEs, exploring their important risk factors, and providing an important basis for the diagnosis and treatment of irAEs.

8. Conclusions

Immunotherapy has emerged as a novel cancer treatment. The present review summarized the history and novel developments of ICIs. However, the number of patients benefiting from ICIs remains low, and further studies should focus on understanding the specific interactions between tumors and the immune system, and resistance mechanisms relevant to immunotherapy. Notably, the immune system of each patient is dynamic and constantly evolving, highlighting that personalized treatment options are required. Future research should focus on developing ICIs for use in an increased number of patients with cancer. In addition, ICIs should also be developed for use in all fields of oncology, to expand the options available for combination strategies. ICIs may be combined with surgery, chemotherapy, radiotherapy, targeted therapy and other forms of immunotherapy; however, efficient toxicity management strategies are required.

Moreover, identification of novel biomarkers that predict response or resistance is essential for accurately selecting specific ICIs. Existing biomarkers, such as PD-L1, dMMR, MSI-H and TMB, are widely used in clinical practice; however, factors such as tumor type, tumor heterogeneity, tumor dynamics and testing procedures may impact the accuracy of these biomarkers. Therefore, optimizing existing biomarkers, and developing new biomarkers or new biomarker systems that integrate immune profiling, tumor biology and treatment history are key in future investigations.

The field of immunotherapy is challenging, but also exhibits potential. In the future, immunotherapy will require developments at a multi-directional level. Further investigations

should explore novel inhibitory checkpoints and pathways, and also integrate other fields, such as cancer biology, genetics and epigenetics. Moreover, further high-quality clinical trials of ICIs are required, to advance evidence-based medicine and develop new cancer treatment options.

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Authors' contributions

HC and JHW contributed to the conception and design of the review. YJW and SY wrote the first draft of the manuscript. LW and WL wrote sections of the manuscript. Data authentication is not applicable. All authors contributed to manuscript revision, and read and approved the final manuscript.

Ethics approval and consent to participate

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Competing interests

The authors declare that they have no competing interests.

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