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항암 면역 치료제에 관한 최근 임상 정보

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Updates to Clinical Information on Anticancer Immunotherapy

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ABSTRACT

Objective: Over the last several years, immunotherapy has become one of the most promising therapeutic options for cancer. This study aims to summarize the updates on cancer immunotherapy focusing on immune checkpoint inhibitors, such as programmed cell death-1 (PD-1), programmed death-ligand 1 (PD-L1), and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) inhibitors, which have received attention as new anticancer therapeutic agents. **Methods:** A literature survey was carried out on PubMed to identify high-impact papers on cancer immunotherapy from 2010. The most recent data on clinical efficacy and safety have been included highlighting the response characteristics to recently approved immunotherapeutic agents. **Results:** In various cancers, immune checkpoints are a means for cancer cells to evade the immune system. Furthermore, CTLA-4 and PD-L1 can be overexpressed, allowing malignant cells to evade T-cells. Numerous clinical trials have been performed to seek appropriate indication of these products in various cancer types. Among them, the most conspicuous types are melanoma, non-small-cell lung cancer, and head and neck cancer. The approval of ipilimumab by Food and Drug Administration (FDA) commenced a new era of cancer immunotherapy. This was followed by the approval of nivolumab and pembrolizumab. Currently, combination therapies are being investigated for various cancer types. **Conclusion:** In this study, we reviewed recently reported scientific and clinical evidence for currently approved immune checkpoint inhibitors. Although these novel checkpoint inhibitors are ever evolving for cancer therapies, there exist limitations that need to be overcome, indicating the necessity for further studies aiming to improve their efficacy, toxicity, and cost.

KEY WORDS: CTLA-4, PD-1, PD-L1, checkpoint inhibitor

In an aging society the number of patients with cancer has still been increasing although unceasing efforts have been made and have succeeded in developing novel cancer treatment agents. Until now the conventional cytotoxic agents have been considered as the basis of most cancer treatment therapies. Those agents are very effective in eradicating cancer cells, but have many serious adverse effects, which represent the most limiting factors of their clinical use. A series of attempts have been made to develop new cancer treatment drugs, not only effective, but also less cytotoxic on normal cells. The notion that cancer cells thrive, because they can exploit certain ways to hide themselves from one's immune system, has enabled the development of various new immunotherapies. The current types of immunotherapies are monoclonal antibodies, immune checkpoint inhibitors, cancer vaccines, adoptive T cell transfer, and general immunotherapies. Monoclonal antibodies are designed to bind to and destroy the targeted antigen, which is usually located on the membrane of cancer cells. Until now, therapy with monoclonal antibodies, also called as targeted therapy, has been widely applied in multiple cancer treatments,

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gaining reputation for its effectiveness and less adverse effects comparing to cytotoxic agents. With insurance coverage expansion, the use of these agents is rapidly increasing. On the other hand, efforts to restore latent anti-tumor immunity have been made focusing on antibody-based agents, which target CTLA-4, PD-1 on T cells and its principal ligand, and PD-L1 on the surface of tumor cells. The clinical development has been pioneered by the antibody, ipilimumab, which blocks CTLA-4 and has demonstrated durable long-term anti-tumor responses and prolonged survival in patients with advanced melanoma, leading to its FDA approval. Capitalizing on this success, the research on the clinical implication of T cell checkpoint inhibition has been boosted. Consequently, PD-1, PD-L1, and PD-L2 inhibitors have been proven to be effective enough to draw accelerated FDA approvals (Table 1). These checkpoint inhibitors not only have yielded new therapeutic options for patients with cancer, but are regarded as the fourth cornerstone of anticancer treatment attracting many clinicians and pharmacists. This article summarizes the mechanism of action, differences, and similarities of checkpoint inhibitors, therapeutic uses, and predictive biomarkers of response, as well as their limitations in clinical application.

Mechanism of checkpoint inhibitors

CTLA-4 inhibitor

The immune system is characterized by a complex system of control and balance to protect the host from exogenous pathogens by distinguishing "self" from "nonself." This system involves both stimulatory and inhibitory components, and multiple mechanisms of peripheral tolerance.[1)] T lymphocytes function as one of the most significant effectors and play an important regulatory role. T cells develop in the thymus, where immature cells proliferate and create a wide repertoire

Table 1. Clinically approved and applied immune checkpoint inhibitors to treat various cancers

Target	Name	Cancer type	Year
CTLA-4	**Ipilimumab**	Late stage melanoma	Mar. 2011
	(Yervoy)	Reduce the risk of melanoma returning after surgery	Oct. 2015
PD-1	**Pembrolizumab**	Advanced melanoma	Sep. 2014
	(Keytruda)	Advanced NSCLC	Oct. 2015
		Extended indication for advanced melanoma	Dec. 2015
		Recurrent or metastatic head and neck	
		Squamous cell carcinoma	Aug. 2016
		1st line treatment of certain patients with metastatic NSCLC	Oct. 2016
		Metastatic NSCLC Classical Hodgkin Lymphoma	Mar. 2017
		1st line combination therapy for patients with metastatic	
		Nonsquamous NSCLC irrespective of PD-L1 expression	May. 2017
	Nivolumab	Advanced melanoma	Dec. 2014
	(Opdivo)	Lung cancer	Mar. 2015
		Nivolumab+Ipilimumab for BRAF V600wt melanoma	Oct. 2015
		Metastatic Renal cell carcinoma	Nov. 2015
		Nivolumab+Yervoy for unresectable or	
		Metastatic melanoma across BRAF status	Jan. 2016
		Hodgkin Lymphoma	May 2016
		Head and neck cancer	Nov. 2016
		Previously treated locally advanced or metastatic	
		Urothelial carcinoma	Feb. 2017
PD-L1	**Atezolizumab**	Advanced urothelian carcinoma	May 2016
	(Tecentriq)	Specific type of metastatic lung cancer	Oct. 2016
		Advanced bladder cancer	Apr. 2017
	Avelumab	Metastatic Markel cell carcinoma	Mar. 2017
	(Bavencio)	Urothelial carcinoma	May 2017
	Durvalumab (Imfinzi)	Urothelial carcinoma	May. 2017

of T-cell receptors (TCRs) through recombination of the TCR gene segments. A selection process then begins, through which T cells with strong reactivity to self-peptides are eliminated in the thymus to prevent auto-reactivity in a process called central tolerance.[2] However, a fraction of self-reactive lymphocytes still escapes to the periphery and poses a threat to cause autoimmunity.[3] The immune system evolved various mechanisms to constrain such autoreactive T cells and maintain peripheral tolerance, including T cell anergy, deletion, and suppression by regulatory T cells (Tregs).[3] T cell anergy is a tolerance mechanism in which the lymphocyte is intrinsically functionally inactivated following an antigen encounter, but remains alive for an extended period of time in a hypo-responsive state.[3] The CTLA-4 and PD-1 immune checkpoints also work as negative regulators of T cell immune function.[2] Inhibition of these targets, resulting in increased activation of immune system, has facilitated the attenuation of disease progression in some cancer types.

T cell activation is modulated by stimulatory and inhibitory signals that work collaboratively to coordinate the immune system's response to a threat. The cell surface molecules CD28 and CTLA-4 provide positive (CD28) and negative (CTLA-4) modulatory signals in the early stage of an immune response (Fig 1).[1] CTLA-4 is a CD28 homolog with much higher binding affinity for its receptor compared to CD28; however, unlike CD28, binding of CTLA-4 does not produce a stimulatory signal.[2] Therefore, the rationale for T cells' proliferation is that CD28 is constitutively expressed on the surface of both naïve and activated T cells, and is present in 90% of CD4+ and in 50% of CD8+ T cells. CTLA-4 expression is only induced by the activation of T cells and its upregulation reaches a maximum of 2~3 days after initiation of response.[1] The relative amount of CD28 binding versus CTLA-4 binding determines whether a T cell will undergo activation or anergy.[2]

Other aspects of immune controls by CTLA-4 involve Tregs which control functions of the effector T cells, and thus are key players in maintaining peripheral tolerance. Unlike effector T cells, Tregs constitutively express CTLA-4, which is thought to be important for their suppressive functions.[2] The identification of key players in the stimulatory and inhibitory mechanisms of the immune systems is very significant for cancer treatment. Anti-CTLA-4 therapy is one of the first therapeutic methods that demonstrates definite clinical benefit through direct T cell activation.

PD-1/PD-L1 pathway

Molecules of B7-CD28 family are involved in T cell activation and tolerance. These molecules are not only responsible for providing positive co-stimulatory signals to

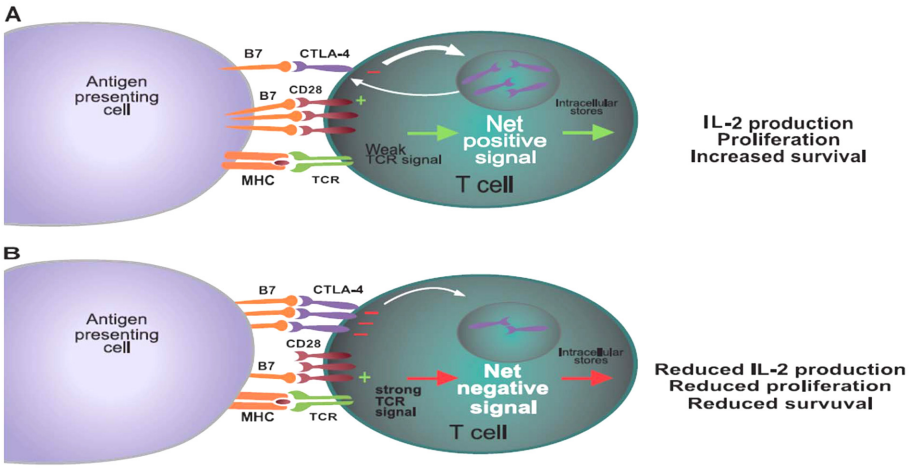

Fig. 1. CTLA-4-mediated inhibition of T cells. T cells are activated when TCRs bind an antigen displayed in the MHC on antigen presenting cells in concert with CD28:B7-mediated co-stimulation.
In the case of a weak TCR stimulus **(case A)**, CD28:B7 binding predominates resulting in a net positive activating signal and increased IL-2 production, proliferation, and increased survival.
In the case of a strong TCR stimulus **(case B)**, CTLA-4 expression is upregulated by increased transport to the cell surface from intracellular stores and decreased internalization.CTLA-4 competes with CD28 for binding of B7 molecules. Increased CTLA-4:B7 binding can result in a net negative signal, which limits IL-2 production and proliferation, as well as survival of the T cell. CTLA-4, cytotoxic T-lymphocyte–associated antigen 4; IL-2, interleukin-2; MHC, major histocompatibility complex; TCR, T-cell receptor. (cited with permission from Buchbinder E.I. *et al*., Am J Clin Oncol 2016;39:99-106)

sustain T cell activity, but also contribute inhibitory signals that modulate the magnitude of T cell responses. PD-1 with its ligands PD-L1and PD-L2 constitutes one such inhibitory pathway.[29] PD-1 interacts with its two ligands PD-L1 and PD-L2 and plays a very important role in lowering the immune system through suppression of T cells function and upregulation of Tregs, which in turn reduces autoimmunity and promotes self-tolerance.[32] After binding of PD-L1 or PD-L2, recruitment of tyrosine phosphatases is stimulated, which generates an inhibitory signal, leading to cell cycle arrest and suppressed T cell activation.[32] The main ligand for PD-1, PD-L1 induces a co-inhibitory signal in activated T cells and promotes T cell apoptosis, anergy and functional exhaustion.[4] Since T cell activation requires various TCR -mediated signals in addition to TCR signaling, the strength and duration of T cell activation are mainly determined by the net effect of positive and negative co-stimulation, as well as by cytokines from antigen presenting cells (APCs).[4]

T cell activation induces the expression of PD-1, whereas cytokines, such as interferon-γ and interleukin-4, are produced after T cell activation. PD-1 ligands are also upregulated, establishing a feedback loop that attenuates immune responses and limits the extent of immune-mediated tissue damage, unless the activation is overridden by strong co-stimulatory signals (Fig. 2).[4] Therefore, PD-1 expression is a hallmark of "exhausted" T cells that have experienced high levels of stimulation or reduced CD4+ T cell help. This state of exhaustion, which occurs during chronic infections and cancer, is characterized by T cell dysfunction, resulting in suboptimal control of infections and tumors.[2] PD-1/PD-L1 interaction ensures that the immune system is activated only at the appropriate time in order to minimize the possibility of chronic autoimmune inflammation.

Studies in various types of human cancers have confirmed that tumors exploit PD-1 mediated immune suppression to escape immune surveillance.[3] A wide variety of solid tumors, including urothelial, ovarian, breast, cervical, colon, pancreatic, gastric, melanoma, glioblastoma, NSCLC, and hematologic malignancies, have been found to express PD-L1, and to a lesser extent PD-L2, which correlates with a better clinical response to PD-1/PD-L1 checkpoint blockade therapy.[2]

Clinically used checkpoints inhibitors

Ipilimumab (Yervoy)

Ipilimumab, a fully human monoclonal antibody that blocks

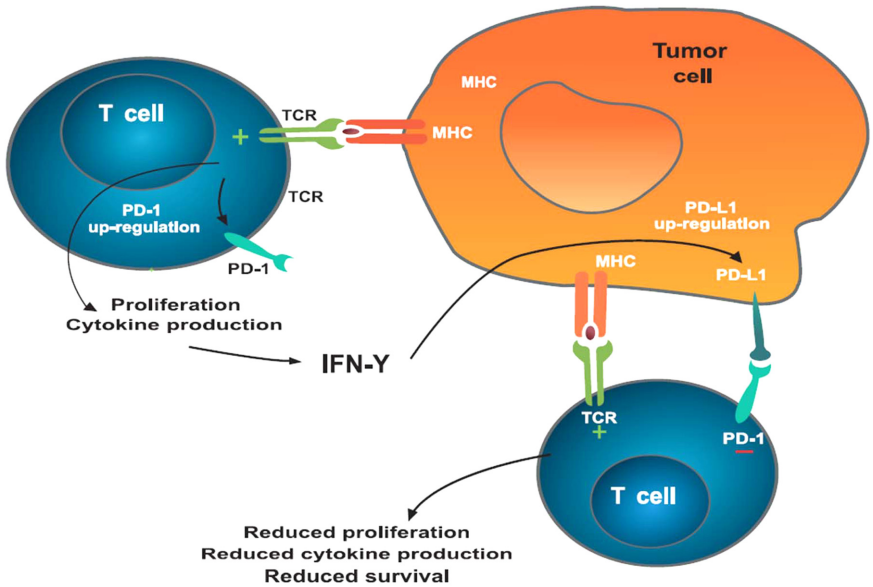

Fig. 2. PD-1-mediated inhibition of T cells. T cells recognizing tumor antigens can be activated to proliferate, secrete inflammatory cytokines, and resist cell death. Prolonged TCR stimulation during an ongoing immune response can cause upregulated PD-1 expression. Tumor cells can express PD-L1 (and PD-L2, not shown) as a consequence of inflammatory cytokines and/or oncogenic signaling pathways. PD-1:PD-L1 binding inhibits TCR-mediated positive signaling, leading to reduced proliferation, cytokine secretion, and survival. IFN-γ, interferon-γ; MHC, major histocompatibility complex; PD-1, programmed death protein 1; PD-L1, programmed death ligand 1; PD-L2, programmed death ligand 2; TCR, T-cell receptor. (cited with permission from Buchbinder E.I. et al., Am J Clin Oncol 2016;39:99-106)

CTLA-4 to promote antitumor immunity, has shown efficacy in patients with metastatic melanoma when it has been used as a monotherapy.

The specific trial involved 676 patients with stage III or IV metastatic melanoma and reported a median overall survival (OS) of 10.1 months in patients receiving ipilimumab monotherapy compared with 6.4 months in those receiving the experimental glycoprotein 100 (gp100) peptide vaccine (HR, 0.68; $p < 0.0001$). The 1 and 2 year survival rates following ipilimumab monotherapy (46% and 24%, respectively), were nearly double of those following gp100 vaccine (25% and 14%, respectively). Immune-related adverse events (irAEs) occurred in approximately 60% of patients receiving ipilimumab compared with about 32% of those receiving only gp100.[5] The most common irAEs include rash and pruritus, colitis and diarrhea, vitiligo, endocrinopathies involving pituitary, thyroid or adrenal gland, as well as hepatitis and uveitis.[6] Management guidelines (algorithms) for irAEs involve close patient follow-up and the administration of high-dose systemic corticosteroids — which, in the trial, were considered necessary for grade 3 or 4 events.[5]

Ipilimumab was approved by FDA in March 2011 as monotherapy (3 mg/kg every 3 weeks for 4 doses) for the treatment of advanced (unresectable or metastatic) melanoma both in pre-treated or chemotherapy naive patients. Ipilimumab is the first agent that has demonstrated to improve OS in patients with metastatic melanoma, which has a very poor prognosis, in randomized phase 3 clinical trials.[5] The patterns of tumor response to ipilimumab differ from those observed with cytotoxic chemotherapeutic agents, since patients may have a delayed yet durable response and obtain long-term survival benefit despite an initial tumor growth.[36] Further development of ipilimumab includes its use in the neoadjuvant or adjuvant high-risk melanoma setting and for the treatment of other refractory and advanced solid tumors, either as a single agent or in combination with additional immune-stimulating agents or molecularly targeted therapies.[6]

Both nivolumab (PD-1 checkpoint inhibitor) and ipilimumab enhance T-cell antitumor activity in different sites; specifically have been shown to have complementary activity in metastatic melanoma. This combination therapy has also been approved by FDA for the treatment of melanoma. In a randomized, double-blind, phase 3 study, 945 previously untreated patients with unresectable stage III or IV melanoma were assigned to nivolumab alone, nivolumab plus ipilimumab, or ipilimumab alone. The median progression-free survival was 11.5 months (95% confidence interval CI, 8.9 to 16.7) with nivolumab plus ipilimumab, compared with 2.9 months (95% CI, 2.8 to 3.4) with ipilimumab (HR for death or disease progression, 0.42; 99.5% CI, 0.31 to 0.57; $P<0.001$), and 6.9 months (95% CI, 4.3 to 9.5) with nivolumab (HR, 0.57; 99.5% CI, 0.43 to 0.76; $P < 0.001$). In patients with tumors positive for PD-L1, the median progression-free survival was 14.0 months in both the nivolumab-plus-ipilimumab group and the nivolumab group. However, in patients with PD-L1 negative tumors, progression-free survival was longer in patients treated with the combination therapy than those treated with nivolumab alone (11.2 months [95% CI, 8.0 to not reached] vs. 5.3 months [95% CI,2.8 to 7.1]). Treatment-related adverse events of grade 3 or 4 occurred in 16.3% of patients in the nivolumab group, 55.0% of those in the nivolumab-plus-ipilimumab group, and 27.3% of those in the ipilimumab group.[7] The combination of nivolumab plus ipilimumab is tolerable with promising clinical activity, including high response rates in patients with PD-L1-positive tumors and the potential for deep and durable responses. These findings represent the first evidence, to our knowledge, of improved benefit of immunotherapy combinations as a first-line treatment of NSCLC. Several ongoing phase 3 studies are assessing dual checkpoint inhibitor blockade or immunotherapy plus chemotherapy, such as NCT02453282, NCT02367781, NCT02578680, and NCT02477826 (CheckMate 227—a prospective phase 3 study of nivolumab, nivolumab plus ipilimumab, or nivolumab plus chemotherapy versus chemotherapy for the first-line treatment of patients with advanced NSCLC). These efforts collectively aim for an improved first-line strategy (or strategies) for patients with advanced NSCLC.[8]

Nivolumab (Opdivo)

Nivolumab is a PD-1 blocking antibody indicated for the treatment of unresectable or metastatic melanoma, metastatic NSCLC, advanced renal cell carcinoma, classical Hodgkin lymphoma, recurrent metastatic squamous cell carcinoma of head and neck, and locally advanced or metastatic urothelial carcinoma.

The clinical study CheckMate-066 has been conducted with previously untreated patients with BRAF wild-type unresectable stage III and IV melanoma. The trial has enrolled 418 patients who were randomized to receive either nivolumab 3 mg/kg every 2 weeks or dacarbazine 1000 mg/m^2 every 3 weeks. A primary

endpoint of overall survival and secondary endpoints of progression-free survival and objective response rate have been assessed. It has been found that 1 year OS was 72.9% (95% CI, 65.5 to 78.9) in the nivolumab group, and 42.1% (95% CI, 33.0 to 50.9) in the dacarbazine group (HR for death, 0.42; 99.79% CI, 0.25 to 0.73; $p < 0.001$). A median progression-free survival of 5.1 months in the nivolumab group versus 2.2 months in the dacarbazine group (HR for death or progression of disease, 0.43; 95% CI, 0.34 to 0.56; $P < 0.001$) has been observed. The objective response rate has been found to be 40.0% (95% CI, 33.3 to 47.0) in the nivolumab group versus 13.9% in the dacarbazine group (OR, 4.06; 95% CI, 9.5 to 19.4; $p < 0.001$). A survival benefit with nivolumab versus dacarbazine has been observed across pre-specified subgroups, including subgroups defined by status regarding PD-L1. Common adverse events associated with nivolumab have included fatigue, pruritus, and nausea. Drug related adverse events of grade 3 or 4 have occurred in 11.7% of patients treated with nivolumab and 17.6% of those treated with dacarbazine.[9] The outcome of CheckMate-066 has become an important milestone in the field of immune-oncology as it represents the first well-controlled, randomized phase 3 trial of an investigational PD-1 checkpoint inhibitor that has demonstrated an OS benefit of the inhibitor and has been terminated early due to the superior OS of nivolumab compared to dacarbazine. Since then continuous studies have been established, which have facilitated the sequential approval of nivolumab by FDA in multiple cancer treatments.

Pembrolizumab (Keytruda)

Pembrolizumab is a monoclonal antibody that targets the PD-1 receptor, and was approved by the FDA in September 2014 for the treatment of advanced melanoma. In October 2015 and October 2016, it was approved by the FDA as a therapeutic strategy in NSCLC metastatic and first-line settings, respectively ; In August 2016, it received accelerated approval for head and neck cancer, and in February 2017, it was granted a priority review for urothelial carcinoma as a first-line treatment for patients who are ineligible for cisplatin-containing therapy and as a second-line treatment for patients whose disease progressed on or after platinum-containing chemotherapy. The first positive report of pembrolizumab activity, published in 2013, included 135 patients with advanced melanoma who demonstrated durable tumor responses after a median follow-up of 11 months.[5] Keynote-002 is a randomized phase 2 trial of 540 patients with melanoma that progressed on ipilimumab, and if $BRAF^{V600}$ mutant- positive, previously treated with a BRAF or MEK inhibitor or both; those patients have few treatment options. Based on 410 total progression-free survival events, the study has met the pre-specified criteria to show significant improvement in progression-free survival, with hazard ratios of 1.57 (95% CI, 0.45 to 1.73) for pembrolizumab 2 mg/kg and 1.5 (95%CI, 1.39 to1.64) for pembrolizumab10 mg/kg compared with chemotherapy. The most common treatment-related grade 3–4 adverse event that has been observed in the pembrolizumab groups was fatigue (2 [1%] out of 178 patients in the 2 mg/kg group and 1 [<1%] out of 179 patients in the 10 mg/kg group, compared with 8 [5%] out of 171 in the chemotherapy group). Other treatment-related grade 3–4 adverse events included generalized edema and myalgia (2 [1%] patients each) after administration of pembrolizumab 2 mg/kg; hypopituitarism, colitis, diarrhea, decreased appetite, hyponatremia, and pneumonitis (each in two [1%]) in those given pembrolizumab 10 mg/kg; and anemia (9 [5%] patients), fatigue (8 [5%] patients), neutropenia (6 [4%] patinets), and leucopenia (6 [4%] patients) following chemotherapy. These findings indicate that pembrolizumab can serve as a new standard care for the treatment of ipilimumab refractory melanoma.[10] In another randomized, controlled, phase 3 study comparing pembrolizumab with ipilimumab in advanced melanoma, the estimated 6-month progression-free-survival rates of 47.3% after pembrolizumab treatment every 2 weeks, 46.4% after pembrolizumab treatment every 3 weeks, and 26.5% after ipilimumab treatment have been observed (HR for disease progression following treatment with pembrolizumab regimens versus ipilimumab, 0.58; $p < 0.001$; 95% CI, 0.46 to 0.72 and 0.47 to 0.72, respectively). Ongoing responses have been observed in 89.4%, 96.7%, and 87.9% of patients, respectively, after a median follow-up of 7.9 months. Similar efficacy in the 2 pembrolizumab groups has been observed with lower rates of treatment-related adverse events of grade 3 to 5 severity (13.3% and 10.1%) than in the ipilimumab group (19.9%).[11] The former US president Jimmy Carter appears to be among one of the rare patients who have shown highly durable efficacy after treatment with pembrolizumab.

Atezolizumab (Tecentriq)

Atezolizumab was the first PD-L1 inhibitor that has been found to be active in bladder cancer, and is currently the only PD-L1 inhibitor specifically approved for the treatment of

patients with locally advanced or metastatic urothelial carcinoma, who progressed on or after platinum-based chemotherapy. This monoclonal antibody was granted accelerated approval by the FDA in May 2016. The initial studies on atezolizumab from 2014, have been established in NSCLC, with approval for this indication granted in October 2016.[5)]

IMvigor 210 is an open-label, multicenter, two-cohort phase 2 study that has evaluated the safety and efficacy of atezolizumab in patients with locally advanced or metastatic urothelial carcinoma, regardless of PD-L1 expression. Between May 13, 2014, and November 19, 2014, 486 patients were screened, with 315 of them being enrolled into the study. Of these patients, 310 have received atezolizumab treatment (the remaining 5 patients have not met the eligibility criteria and have not been treated with the examind drug).[12)] The primary analysis (data cutoff May 5, 2015) showed that compared with a historical control overall response rate (ORR) of 10%, treatment with atezolizumab resulted in a significantly improved objective response rate according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1; the objective response rate in each pre-specified immune cell (IC) group was follows: IC2/3: 27% (95% CI, 19 to 37), $p < 0{\cdot}0001$; IC1/2/3, 18% (95% CI,13 to 24), $p=0{\cdot}0004$, and in all patients was 15% (95% CI, 11to 20), $p=0{\cdot}0058$. Re-evaluation with longer follow-up (data cutoff Sept 14, 2015), by independent review, the objective response rate was 26% (95% CI,18 to 36) in the IC 2/3 group, 18% (95% CI, 13 to 24) in the IC1/2/3 group, and 15% (95% CI,11 to19) in all 310 patients. With a median follow-up of 11.7 months (95% CI 11·4-12·2), ongoing responses have been recorded in 38 (84%) out of 45 responders. Grade 3-4 treatment-related adverse events, with fatigue being the most common (5 [2%] patients), have occurred in 50 (16%) out of 310 treated patients.[12)] Grade 3-4 immune-mediated adverse events have occurred in 15 (5%) out of 310 treated patients, with pneumonitis, increased aspartate aminotransferase, increased alanine aminotransferase, rash, and dyspnea being the most common. No treatment-related deaths have occurred during the study.[12)] Atezolizumab has shown durable activity and good tolerability in this patient population.[12)] Increased levels of PD-L1 expression in immune cells have been associated with increased response.[15)] Metastatic urothelial cancer is associated with a poor prognosis and limited treatment options. Until atezolizumab's approval in the US in May 2016, there had been no major advances for more than 30 years in treating urothelial carcinoma. Extensive clinical trials are ongoing, investigating atezolizumab's efficacy on several types of cancer such as lung, kidney, skin, breast, colorectal, prostate, ovarian, bladder and blood cancers.

Avelumab (Bavencio)

Avelumab, a PD-L1 blocking human IgG1 lambda monoclonal antibody, is the first FDA approved treatment drug for metastatic Merkel cell carcinoma (MCC). JAVELIN Merkel 200 trial, an open-label, single-arm, multi-center clinical trial has demonstrated a clinically meaningful and durable responses. The primary endpoint of confirmed objective response (complete response or partial response) has been assessed according to RECIST version 1.1, by an independent review committee. Safety and clinical activity have been assessed in all patients who received at least 1 dose of study drug (the modified intention-to-treat population). Between July 25, 2014, and September 3, 2015, 88 patients were enrolled and received at least 1 dose of avelumab. Patients have been followed up for a median of 10 - 4 months (IQR 8.6 to 13.1). The proportion of patients who have achieved an objective response was 28 (31.8% [95% CI, 21.9 to 43.1]) out of 88 patients, including 8 complete responses and 20 partial responses. Ongoing responses have been found in 23 (82%) out of 28 patients at the time of analysis.[13)] Five grade 3 treatment-related adverse events have occurred in 4 (5%) patients: lymphopenia in 2 patients, blood creatine phosphokinase increase in 1 patient, aminotransferase increase in 1 patient, and blood cholesterol increase in 1 patient; no treatment-related grade 4 adverse events or treatment-related deaths have been observed. Serious treatment-related adverse events have been reported in 5 patients (6%): enterocolitis, infusion-related reaction, aminotransferases increase, chondrocalcinosis, synovitis, and interstitial nephritis (n=1 each).[13)] Objective responses to avelumab in all subgroups have been analyzed. It has been noted that the proportion of patients with response was higher in those who received fewer lines of previous therapy compared with those who received more lines of previous therapy. One possible explanation for this observation is that patients who received fewer lines of cytotoxic therapy might be more likely to have fully functioning immune systems than those who received more lines of therapy; thus those patients might respond in a more robust way to immunotherapy with a checkpoint inhibitor. These results indicate that anti-PD-L1/PD-1 therapies could become the standard of care in treatment-naive and advanced

Merkel cell carcinoma.[13] Additionally, these studies not only support the clinical activity and safety of anti-PD-L1/PD-1 monotherapy in the treatment framework but also provide possible efficacy of combination approaches with anti-PD-L1/PD-1 antibodies and other immunotherapies. Avelumab also has its accelerated approval for the treatment of locally advanced or metastatic urothelial carcinoma with disease progression during or following platinum containing chemotherapy or platinum ineligible.[37] Several active clinical trials investigating avelumab in stomach cancer, head and neck cancer, and NSCLC are ongoing.

Durvalumab (Imfinzi)

Durvalumab, a monoclonal antibody against PD-L1, was granted a breakthrough therapy designation by the FDA in February 2016 for patients with PD-L1 inoperable or metastatic urothelial bladder cancer, whose tumor has progressed during or after a standard platinum-based regimen.[5] It is also currently under investigation for the treatment of NSCLC, head and neck cancer, gastric cancer, pancreatic cancer, hepatocellular carcinoma, mesothelioma, and hematologic cancers. Breakthrough therapy designation was based on the phase 1/2 study (NCT01693562) on durvalumab (10 mg/kg IV Q2W) in patients (n = 61) with inoperable or metastatic urothelial bladder cancer.[14] An ORR of 31% has been observed in the overall population and 46% in the PD-L1 high (defined as TC or IC = 25%) subgroup versus 0 in the PD-L1 low/negative subgroup (defined as TC and IC <25%). The median duration of response has not yet been reached (range: 4–49 weeks), and responses have been ongoing in 12 out of 13 patients at the time of publication. The most common adverse events have been found to be fatigue (13%), diarrhea (10%), and decreased appetite (8%), and grade 3 adverse events have occurred in 5% of patients; no grade 4 or 5 events have been oberved.[14] The combination of durvalumab plus the CTLA-4 inhibitor, tremelimumab, which is currently being examined (DANUBE; NCT02516241) versus a standard-of-care chemotherapy in patients with stage IV urothelial bladder cancer, is expected to be completed in 2019.[15]

Predictive biomarkers for checkpoint inhibitor-based immunotherapy

Despite the recent approvals for multiple therapeutic antibodies that block CTLA-4 and PD-1 in melanoma, NSCLC, and kidney cancer, and additional immune checkpoints being targeted clinically, many questions still remain regarding the optimal use of drugs that block these checkpoint pathways. Defining biomarkers that predict therapeutic effects and adverse events is a crucial mandate, highlighted by recent approvals for 2 PD-L1 diagnostic tests. The biomarkers for anti-PD-1 therapy are based on immunological, genetic and virological criteria. The unique biology of the CTLA-4 immune checkpoint, compared with PD1, requires a different approach to biomarker development.[16]

The CTLA-4 immune checkpoint unlike PD1/PD-L1, predominantly functions early in the life cycle of the immune response, during T cell priming and activation, through down-modulation of CD4+ T effector (Teff) cells and enhancement of Treg cell activity.[2] This indicates its global impact on the immune system and therefore, biomarkers of response and resistance to anti-CTLA-4 therapy may differ from other immune checkpoint inhibitors that have different mechanisms of action.

Therefore, many biomarker studies of anti-CTLA-4 therapies have focused on the diversity, phenotype, and function of peripheral blood lymphocytes (PBLs) before and after therapy, instead of on tumor biopsies. Increased diversity and expression of activation markers on PBLs have been reported following anti-CTLA-4 therapy. At least 2 independent studies have noted that a rise in the absolute lymphocyte count in peripheral blood is correlated with a high rate of response to ipilimumab. CD8+ T cells may be the most relevant subset in this analysis, as CTLA-4 blockade can enhance CD8+ T cell-mediated immune responses indirectly by enhancing the activity of CD4+ T helper cells. Furthermore, patients with melanoma who developed CD4+ and CD8+ PBLs with specificity against the NY-ESO-1 cancer testis antigen have also demonstrated significant tumor shrinkage or stabilization. In contrast, other factors in peripheral blood, such as high levels of soluble CD25 (also known as IL2Rα), have been correlated with resistance to anti-CTLA-4 therapy. Local factors in the pretreatment tumor microenvironment (TME), such as PDL1 expression, are generally not associated with clinical response to anti-CTLA-4 therapy, although 1 study has raised the possibility that patients with an inflamed TME before treatment are more likely to respond to anti-CTLA-4 therapy. Increased expression of the co-stimulatory molecule inducible T cell co-stimulator (ICOS) on PBLs and tumor-infiltrating lymphocytes (TILs) has also been observed following CTLA-4 blockade in patients with various tumor types. Furthermore, an increased Teff cell:Treg cell ratio in tumor tissues has been observed. Despite these correlations, no predictive biomarker for ipilimumab treatment selection, nor any

on-treatment pharmacodynamic marker, have been yet proved sufficiently robust to be used clinically.[16)]

PD-L1 expression has being investigated as a predictive biomarker of response for PD-1/PD-L1 directed therapy.[18)] PD-L1 is expressed in several tumor types, including melanoma, lung, renal, kidney, head and neck and bladder cancer. Preliminary molecular marker studies on melanoma have shown a correlation of PD-L1 expression in pretreatment tumor specimens and objective response to anti-PD-1 therapy.[17)] However, PD-L1 expression in some studies appears to be associated with better prognosis only in metastatic melanoma lesions, suggesting that its predictive value may not be as clear-cut as initially thought. Other issues also add complexity when evaluating different analyses of PD-L1 expression as a predictive factor of response.[18)] PD-L1 expression is IFN-γ–inducible and can be present on either the tumor or infiltrating immune cells. Furthermore, there is currently no standardized methodology to measure PD-L1 expression and its evaluation differs between assays.[18)]

Taube and colleagues have found a significant correlation between the presence of tumor infiltrating lymphocytes (TILs) and PD-L1 expression in the TME.[18)] The number, type and location of TILs in primary tumors seem to have prognostic value and their presence may be more important for predicting response than PD-L1 expression alone.[18)] However, there is evidence that TILs are necessary but not sufficient for PD-L1 expression in melanoma.[19)] Patients with better response to these therapies express high levels of PD-L1 and have infiltration of T cells within the tumor.[18)] Therefore, evaluation of PD-L1 expression by immunohistochemistry (IHC) together with measurement of immune infiltration might be a good predictor of tumor response to anti PD-L1 agents. There are yet caveats regarding measuring levels of PD-L1 because its expression is constitutive and its overexpression in response to stimuli can vary according to the cell type.[18)] In addition, tumors are heterogeneous and the sample used for the assay may not be representative of the whole tumor. For instance, various levels of PD-L1 expression have been found in different metastases and their primary clear cell renal cell carcinomas.[18)] Moreover, it has been observed that patients with PD-L1 negative tumors can also respond to PD-1 and PD-L1 blockade.[9)] For all these reasons, a standardized definition of PD-L1 positivity that links these different assays is needed to evaluate PD-L1 expression as a predictive factor for PD-1 and/or PD-L1 pathway blockade.[18)]

Other immune biomarkers have also been assessed. Messina and colleagues have found a direct correlation between a 12-chemokine gene expression signature and the presence of lymph nodal structures (immune cells that infiltrate and are organized into intratumoral structures that resemble lymph nodes) associated with increased OS in patients with melanoma, which may be useful in selecting those patients as most suitable for immunotherapy.[20)]

Other studies have demonstrated that tumors with a high somatic mutational frequency, such as melanomas, respond better to PD-1 immune checkpoint inhibitors. The mutational load in melanoma has been found to be associated with clinical benefit, but not predictive of response to treatment.[22)] It has been observed that the characterization of immune infiltration (intratumoral infiltration), chemokine signature, tumor mutational load, and PD-L1 expression of the tumor, may provide the information on which patients may benefit from which type of immune checkpoint inhibitor, either in monotherapy or in combination, and on mechanism of an individual's tumorigenesis.[18)] Optimal agents for combination treatment with immune checkpoint monoclonal antibodies (mAbs) might be those capable of inducing immune infiltration into the TME. Furthermore, PD-L1 expression by IHC is currently the strongest predictive marker of clinical benefit for immune checkpoint therapy, however, data presented so far, do not demonstrate PD-L1 to be a reliable single predictive marker, as the epidermal growth factor receptor is for lung cancer or human epidermal growth factor receptor 2 for breast cancer.[18)] A standardization of PD-L1 IHC is required to explore the relationship between its expression and its impact on prognosis of patients with melanoma treated with PD-1 and/or PD-L1 mAbs. Given that infiltration of TILs is important to obtain an effective antitumor immune response, some categorization of immune infiltration together with PD-L1 expression by IHC or other immunologic assays might help to better predict of tumor response, although the fact that PD-L1 negative patients can also response means clinical application should be approached with caution.[18)] Finally, the identification and application of such possible predictive markers for each patient are crucial for the rational development, research and advance of immunotherapy to guide the decision of the optimal choice of immunotherapy treatment.[18)]

Discussion

A better understanding of the role of the immune system in

tumor immune-surveillance has made it possible to develop a new generation of immunotherapeutic agents. Results from early phase studies on immune checkpoint agents, such as CTLA-4, PD-1, and PD-L1 inhibitors, in a range of solid tumors, including melanoma and NSCLC, are highly promising and provide new therapeutic options for the treatment of those cancers. Although checkpoint inhibitors have proved their prominent efficacy in cancer treatment, their response rate is low and not effective in some cancer types. To overcome this limitation, researchers have investigated a number of combination therapies. Other challenges can be listed including their administration in earlier and more curative settings, identification of predictive biomarkers for each agent, and focus on providing the best supportive care to reduce their adverse effects, especially focusing on irAEs according to the long-term use of those agents.

In addition there are more puzzles to be solved, such as prohibitive cost, low production rate of the agents, and low stability. Nevertheless, the significance of these new immunotherapies in the treatment of intractable types of cancers, such as melanoma, NSCLC, and bladder cancer, cannot be emphasized enough. In the future study we may compare the innovative development of various checkpoint inhibitors to the 4th industrial revolution that utilizes super-intelligence. There is no doubt that immunotherapy will change the standard of care of cancer not long into the future and we aim to facilitate these agents to the maximum efficacy.

Conclusion

It is definite that checkpoint inhibitors usher a new era of cancer treatment therapies. These seemingly perfect drugs of choice in cancer treatment have their own ambivalence that has to be solved. The development of biomarkers for identification of the appropriate treatment strategy is a critical factor because of the high price and fastidious insurance guarantee of these agents. Dealing with irAEs is another aspect that many researchers have to focus on. It is probable that long-time use of these agents causes unexpected autoimmune disease. Lastly, determining the manner in which checkpoint inhibitors can be combined with not only their own category of drugs, but also with different types of anticancer drugs to improve efficacy and response is a critical goal we should strive for.

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