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Review Article

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Immune Checkpoint Inhibitors in 10 Years: Contribution of Basic Research and Clinical Application in Cancer Immunotherapy

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ABSTRACT

Targeting immune evasion via immune checkpoint pathways has changed the treatment paradigm in cancer. Since CTLA-4 antibody was first approved in 2011 for treatment of metastatic melanoma, eight immune checkpoint inhibitors (ICIs) centered on PD-1 pathway blockade are approved and currently administered to treat 18 different types of cancers. The first part of the review focuses on the history of CTLA-4 and PD-1 discovery and the preclinical experiments that demonstrated the possibility of anti-CTLA-4 and anti-PD-1 as anti-cancer therapeutics. The approval process of clinical trials and clinical utility of ICIs are described, specifically focusing on non-small cell lung cancer (NSCLC), in which immunotherapies are most actively applied. Additionally, this review covers the combination therapy and novel ICIs currently under investigation in NSCLC. Although ICIs are now key pivotal cancer therapy option in clinical settings, they show inconsistent therapeutic efficacy and limited responsiveness. Thus, newly proposed action mechanism to overcome the limitations of ICIs in a near future are also discussed.

Keywords: CTLA-4; PD-1; Immune checkpoint inhibitors; Cancer immunotherapy; Lung cancer

INTRODUCTION

Over the years, treatment for cancer has evolved, and the survival rate of cancer patients rapidly increased due to the diverse treatment options. Among the various cancer treatment strategies currently available, the most recent and promising treatment option is immunotherapy. While conventional chemotherapy is a method of controlling cancer cell proliferation by targeting cancer cells, immunotherapy differs by targeting immune cells and restoring immune function, thereby killing cancer cells.

Many attempts to treat cancer using the body's immune response have been historically documented, but the first case of using specific immunogenic substances for cancer treatment was in 1891 when William Coley, an American surgeon, injected a bacterium called *Streptococcus* into cancer patients (1). After several tests, Coley finally developed a mixture

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Conflicts of Interest
The authors declare no potential conflicts of interest.

Abbreviations
BMS, Bristol Myers Squibb; CCRT, concurrent chemoradiotherapy; CTx, chemotherapy; DFS, disease-free survival; dMMR, mismatch repair deficient; EC, esophageal cancer; ESCC, esophageal squamous cell carcinoma; Eso, Esophageal; GC, gastric cancer; GEJ, gastroesophageal junction cancer; HCC, hepatocellular carcinoma; ICIs, immune checkpoint inhibitors; I-O, immune-oncologic; mOS, median overall survival; MSI-H, microsatellite instability high; NA, not-applicable; Non-SqCC, non-squamous cell carcinoma; NR, not reached; NSCLC, non-small cell lung cancer; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; R/M, recurrent/metastatic; RCC, renal cell carcinoma; SCCHN, squamous cell carcinoma of the head and neck; SCLC, small cell lung cancer; SqCC, squamous cell carcinoma; TNBC, triple-negative breast cancer; UC, urothelial carcinoma.

Data Availability Statement
Data sharing is not applicable. No datasets were generated or analyzed in this review article.

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called Coley's toxin, which consisted of two bacteria species, *Streptococcus Pyogenes* and *Serratia Marces*. Since then, various immune stimulatory drugs, such as IFN-α and IL-2, have been approved by the United States Food and Drug Administration (U.S. FDA) and applied clinically, but failed to gain clinical significance due to their low efficacy and unpredictable side effects (2). The first immune cell therapy for cancer treatment was Sipuleucel-T (trade name Provenge), an active dendritic cell therapy for prostate cancer developed by Dendreon in 2010. Although it was approved by the FDA and raised expectations as the first immune cell therapy for cancer treatment, Sipuleucel-T failed to show sufficient clinical efficacy (3,4).

Ipilimumab (trade name Yervoy), an CTLA-4 antibody developed by Bristol Myers Squibb (BMS) in 2011, was approved by the FDA as treatment for metastatic melanoma, and opened the era of immuno-cancer treatment. Subsequently, two PD-1 antibodies, pembrolizumab (trade name Keytruda) and nivolumab (trade name Opdivo), developed by Merck and BMS respectively, have obtained FDA approval. Since then, immune-oncologic (I-O) drugs called immune checkpoint inhibitors (ICIs) have begun to be widely applied in various cancers. As of November 2021, 8 ICIs are approved for 18 cancer types, and ICIs targeting diverse immune checkpoints in combination with PD-1 signaling blockers, are under clinical investigation as novel ICIs (5-7). In contrast to the previous immune-related drugs that directly strengthen immune activity, ICIs aimed to overcome immuno-suppressive microenvironment (8). Basic immunological studies showed the importance of overcoming immune suppression, rather than triggering immune activation, thus paving the way for the era of cancer immunotherapy.

2021 is a monumental year since a decade has passed since the approval of FDA of ipilimumab. In this comprehensive review, the discovery and function of CTLA-4 and PD-1, which are the key immune checkpoint molecules most actively targeted are discussed. In addition, a series of studies leading to the drug development of ICIs and their FDA approvals are addressed. Specifically, the focus is on the treatment landscape and future perspectives of ICIs in non-small cell lung cancer (NSCLC), which serve as an example of how ICIs were successfully incorporated into clinical settings. In addition, studies on the existing mechanism of action of ICI such as reactivating the function of exhaustion T cells in tumor microenvironments, as well as recent discoveries on diverse mechanisms of action of ICI are addressed. Lastly, strategies to overcome the limitations of the current ICIs based on the latest research results are discussed.

DISCOVERY OF CTLA-4 AND PD-1 AND EVALUATION OF THEIR BIOLOGICAL FUNCTION

Among immune co-inhibitory molecules, CTLA-4 is the first molecule to be cloned and identified as a new immunoglobulin gene superfamily in activated CD8 T cells by Pierre Golstein in 1987 (9). In 1991, Jeffrey Ledbetter and Peter Linsley, who studied the interaction between CD28 and B7 in BMS, revealed that CTLA-4 can interact with B7 with stronger affinity than CD28 (10). In the following year, Jeffrey Bluestone group and Peter Linsley group independently demonstrated through *in vivo* studies that CTLA4-Ig, a soluble form of CTLA-4, extends the survival of islet graft and inhibits T cell-independent antibody response, presenting a possibility that CTLA-4 is a negative regulator to control T cell activity (11,12). In 1994, Jeffrey Bluestone demonstrated the inhibitory role of CTLA-4 in activating T cell response (13). He showed that CTLA-4 expressed by activated T cells directly bound to B7 on antigen-presenting cells, thereby reducing T cell proliferation and function. James

Allison also independently studied the function of CTLA-4 and reported in 1995 that CD28 and CTLA-4 share the ligand, B7, but both play opposite roles in T cell functionality when B7 interacts with each receptor (14). CTLA-4 gene knockout mice were generated in 1995 by independent two groups, Tak Mak group and Arlene Sharpe group. The mice represented a dramatic lymphoproliferative disorder followed by an early lethality, which clearly defining CTLA-4 as a native molecule of T cell activation and proliferation (15,16).

The cloning of PD-1 gene and the studies on PD-1 function were conducted in a very similar process to those of CTLA-4. The PD-1 gene was cloned as a new immunoglobulin gene superfamily from stimulated mouse T cell hybridoma by Tasuku Honjo in 1992 (17). Subsequently, the Honjo group generated PD-1 gene knockout mouse, but unlike CTLA-4 gene knockout mouse, the phenotype did not appear in a short period of time. About six months later, however, lupus-like arthritis and glomerulonephritis were observed in the aged mice of C57BL/6 background (18). In addition, T cells isolated from the PD-1 gene knockout mouse displayed far more activated phenotype than those from wild-type mouse, demonstrating the role of PD-1 as a co-inhibitory molecule. In 2001, a different type of autoimmune disease such as cardiomyopathy was observed in PD-1 gene knockout mouse with BALB/c background (19). These studies demonstrated that PD-1 also negatively regulates the process of T cell activation, although CTLA-4 and PD-1 might not play the same role possibly due to their different action mechanism. The next task was to find out a real PD-1 ligand showing physical interaction and inhibitory function. Lieping Chen group and the Drew Pardoll group identified PD-L1 and PD-L2 as PD-1 ligands, respectively (20,21). Through further study, Gordon Freeman and colleagues have shown that both PD-1/PD-L1 and PD-1/PD-L2 contribute to the negative regulation in T cell response by the physical interaction between receptor and ligand (22,23). PD-L1 gene knockout mice were generated by two independent teams, Lieping Chen group and Arlene Sharpe group, in 2004 (24,25). PD-L2 gene knockout mice, which were independently generated in 2006 by Chen Dong group and Arlene Sharpe group (26,27). They demonstrated that both ligands can directly bind to PD-1 and promote T cell tolerance.

James Allison and Tasuku Honjo changed the paradigm of cancer immunotherapy with their research on CTLA-4 and PD-1, and received the 2018 Nobel Prize in Physiology and Medicine. The timeline and milestones of the basic studies for immune checkpoints, CTLA-4 and PD-1, are represented in **Fig. 1**.

PRECLINICAL AND CLINICAL STUDY FOR ANTI-CTLA-4 AND ANTI-PD-1 AS CANCER IMMUNOTHERAPY

Basic research on the functional regulation of CTLA-4 molecule paved the way for cancer immunotherapy. In 1996, James Allison demonstrated that tumor growth was significantly delayed when the antibody against CTLA-4 was injected into the syngenic mouse tumor model (28). Later, James Allison, along with biotech company, made a fully humanized antibody against human CTLA-4, named ipilimumab. In 2000, James Allison, Steve Hodi, and Glenn Dranoff noted the tumor antigen-specific T cell activity in patients with metastatic melanoma treated with ipilimumab, who were pre-vaccinated with irradiated, autologous GM-CSF-secreting melanoma cells (29). Ipilimumab monotherapy showed significant objective response rate, but was associated with immune-related toxicity in phase 1/2 clinical trial in patients with advanced melanoma (30). Considering the risks and benefits, BMS performed two consecutive

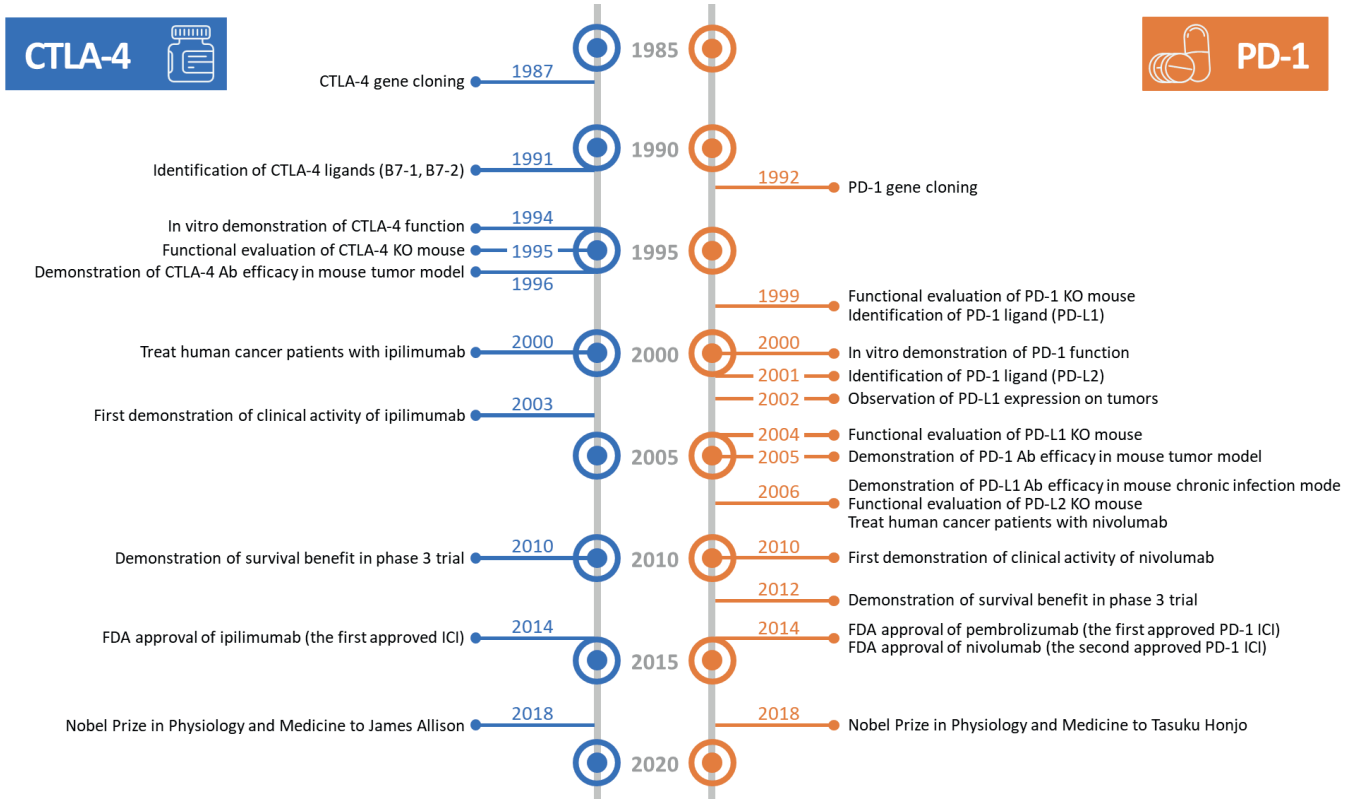

Figure 1. Timelines and milestones of immune checkpoints, CTLA-4 and PD-1, from basic to clinic.
Major events leading up to the development of anti-CTLA-4 (left) and anti-PD-1 (right) are indicated in chronological order.

phase 3 clinical trials in 2010 and 2011 (31,32). Based on these results, the FDA approved ipilimumab as the first ICI, thus opening the era of cancer immunotherapy.

In 2002, targeting the PD-1/PD-L1 axis began when Honjo group and the Lieping Chen group revealed that PD-L1 expressed by tumor cells could inhibit tumor-reactive T cells as one of the immune evolution mechanisms (33,34). In 2005, Lieping Chen demonstrated that antibodies to PD-1 or PD-L1 could delay tumor growth in syngenic mouse tumor model (35). In addition, Rafi Ahmed, who first proposed the concept of T cell exhaustion occurring during the chronic virus infection, reported in 2006 that antibody against PD-L1, which was produced by Gordon Freeman, could rejuvenate the proliferation and function of exhausted CD8 T cells *in vivo* (36). These series of animal experimental results triggered the development of PD-1 antibodies. In 2006, Ono and Medarex developed antibodies targeting human PD-1 and human PD-L1, and started phase 1/2 clinical study. Although the study was conducted in patients with various metastatic cancers who were refractory to standard treatments, improved clinical response with manageable side effect were observed in a subset of patients (37). Based on the results of this clinical trial, BMS, which developed ipilimumab, purchased Medarex and conducted two large-scale phase 3 clinical trials (38,39). Notably, nivolumab, an anti-PD-1, showed significant and durable clinical responses in patients with refractory melanoma, renal cancer, and NSCLC (38). Since the immune-related adverse events of nivolumab were significantly lower than those of ipilimumab, antibodies targeting PD-1 emerged as the better candidate of I-O drug. In the meantime, Merck, began to speed up the clinical development of pembrolizumab, anti-PD-1, by running the largest phase 1 clinical trial in oncology (40). This resulted in the FDA's breakthrough therapy designation for pembrolizumab. On September 2014, under the

fast-track development program, the FDA approved pembrolizumab in advanced melanoma patients who were refractory to treatments including ipilimumab and BRAF inhibitor. On December 2014, the FDA approved also nivolumab as a new option for the treatment of patients with unresectable or metastatic melanoma. In 2016, BMS received the FDA approval of ipilimumab and nivolumab combination therapy in metastatic melanoma patients (41). The timeline and milestones for the development of ICIs involving anti-CTLA-4 and anti-PD-1 in preclinical and clinical studies are represented in **Fig. 1**.

APPROVED ICIs IN SOLID CANCERS

Currently, 8 different ICIs approved by FDA in solid tumors are 1) ipilimumab, an anti-CTLA-4 inhibitor, 2) PD-1 inhibitors such as pembrolizumab, nivolumab, cemiplimab (trade name Libtayo), and dostarlimab (trade name Jemperli), 3) anti-PD-L1 inhibitors including atezolizumab (trade name Tecentriq), durvalumab (trade name Imfinzi), and Avelumab (trade name Bavencio). The related information is found in the website (https://www.fda.gov/drugs/resources-information-approved-drugs/oncology-cancer-hematologic-malignancies-approval-notifications). Here, we will take a look at the indications for ipilimumab as a CTLA-4 inhibitor, and nivolumab and pembrolizumab as PD-1 inhibitors.

Ipilimumab is a human CTLA-4-blocking antibody indicated for the treatment of melanoma. Ipilimumab is also used in combination with nivolumab for the treatment in melanoma, renal cell carcinoma (RCC), microsatellite instability high (MSI-H) or mismatch repair deficient (dMMR) metastatic colorectal cancer, hepatocellular carcinoma (HCC), NSCLC, and malignant pleural mesothelioma. Nivolumab is approved for the treatment of melanoma, NSCLC, malignant pleural mesothelioma, RCC, squamous cell carcinoma of the head and neck (SCCHN), urothelial carcinoma (UC), MSI-H or dMMR metastatic colorectal cancer, HCC, esophageal cancer (EC), gastric cancer (GC), and gastroesophageal junction cancer. Pembrolizumab is indicated for the treatment of melanoma, NSCLC, SCCHN, UC, MSI-H or dMMR cancer, MSI-H or dMMR colorectal cancer, GC, EC, cervical cancer, HCC, merkel cell carcinoma, RCC, endometrial carcinoma, tumor mutational burden-high cancer, cutaneous squamous cell carcinoma, and triple-negative breast cancer (TNBC).

Based on early phase studies, nivolumab and pembrolizumab were approved in patients with heavily treated solid cancers who failed to standard treatment. Clinical trials then focused on the efficacy of these drug as a front-line setting and determined whether they prolonged survival in comparison with the control group. Currently, the anti-PD-1 inhibitor alone or combined chemotherapy are treated in front-line setting in metastatic GC, advanced RCC, metastatic TNBC, MSI-H or dMMR colorectal cancer, metastatic SCCHN, metastatic NSCLC (42-55). More recently, ICIs are expanding their indications to early settings, and have been approved as adjuvant treatment after surgery in RCC, UC, melanoma, NSCLC and as neoadjuvant treatment in TNBC (56-60). Details of the approved drugs, cancer type and settings are summarized in the **Table 1**.

CLINICAL USE OF ICIs FOCUSING ON LUNG CANCER

Among various cancers, NSCLC is one of the representative solid tumors which immunotherapy shows dramatic response. The bench-to bedside research of immunotherapy

Table 1. FDA approval history of solid tumor

Date	Drug name	Indication	Treatment line	Key trial
Mar 2011	Ipilimumab	Melanoma	Refractory	MDX010-020
Sep 2014	Pembrolizumab	Melanoma	2nd line	KEYNOTE001
Dec 2014	Nivolumab	Melanoma	Refractory	CheckMate 037
Mar 2015	Nivolumab	SqCC NSCLC	2nd line	CheckMate 063, CheckMate 017
Oct 2015	Nivolumab + ipilimumab	BRAF V600 wild-type melanoma	1st line	CheckMate 069
Oct 2015	Pembrolizumab	NSCLC	2nd line	KEYNOTE001, KEYNOTE010
Oct 2015	Nivolumab	Non-SqCC NSCLC	2nd line	CheckMate 057
Nov 2015	Nivolumab	RCC	2nd line	CheckMate 025
Dec 2015	Pembrolizumab	Melanoma	2nd line	KEYNOTE002
Jan 2016	Nivolumab + ipilimumab	Melanoma across BRAF status	1st line	CheckMate 069
Mar 2016	Atezolizumab	Urothelial carcinoma	2nd line	Imvigor210 (cohort2)
Aug 2016	Pembrolizumab	SCCHN	2nd line	KEYNOTE012
Oct 2016	Pembrolizumab	NSCLC	1st line	KEYNOTE024
Oct 2016	Atezolizumab	NSCLC	2nd line	BIRCH, POPLAR, FIR, OAK
Nov 2016	Nivolumab	SCCHN	2nd line	CheckMate 141
Feb 2017	Nivolumab	Urothelial carcinoma	2nd line	CheckMate 275
Mar 2017	Avelumab	Merkel cell carcinoma	2nd line	JAVELIN Merkel 200
May 2017	Pembrolizumab + CTx	Non-SqCC NSCLC	1st line	KEYNOTE021
Apr 2017	Atezolizumab	Urothelial carcinoma	1st line	Imvigor210 (cohort2)
May 2017	Pembrolizumab	Urothelial carcinoma	2nd line	KEYNOTE045
May 2017	Pembrolizumab	Solid tumor with a MSI-H or dMMR	2nd line	NCT01876511
May 2017	Avelumab	Urothelial carcinoma	2nd line	JAVELIN solid tumor
Aug 2017	Nivolumab	MSI-H or dMMR colorectal cancer	2nd line	CheckMate 142
Sep 2017	Nivolumab	HCC failed to sorafenib	2nd line	CheckMate 040
Sep 2017	Pembrolizumab	GC or GEJ with PD-L1	3rd line	KEYNOTE059
Dec 2017	Nivolumab	Melanoma	Adjuvant	CheckMate 238
Feb 2018	Durvalumab	Unresectable Stage III NSCLC	Consolidation	PACIFIC
Apr 2018	Nivolumab + ipilimumab	Intermediate-/poor-risk RCC	1st line	CheckMate 214
Jun 2018	Pembrolizumab	Cervical cancer with PD-L1	2nd line	KEYNOTE158
Jul 2018	Nivolumab + ipilimumab	MSI-H/dMMR colorectal cancer	3rd line	CheckMate 142
Aug 2018	Nivolumab	SCLC	3rd line	CheckMate 032
Aug 2018	Pembrolizumab + CTx	Non-SqCC NSCLC	1st line	KEYNOTE189
Oct 2018	Pembrolizumab + CTx	SqCC NSCLC	1st line	KEYNOTE407
Nov 2018	Pembrolizumab	HCC failed to sorafenib	2nd line	KEYNOTE224
Dec 2018	Pembrolizumab	Merkel cell carcinoma	1st line	KEYNOTE017
Dec 2018	Atezolizumab + Bevacizumab + CTx	Non-SqCC NSCLC	1st line	IMpower150
Feb 2019	Pembrolizumab	Melanoma with lymph node	Adjuvant	KEYNOTE054
Mar 2019	Atezolizumab + nab-paclitaxel	TNBC with PD-L1	1st line	Impassion130
Mar 2019	Atezolizumab + CTx	SCLC	1st line	IMpower133
Apr 2019	Pembrolizumab	NSCLC	1st line	KEYNOTE042
Apr 2019	Pembrolizumab + axitinib	RCC	1st line	KEYNOTE426
May 2019	Avelumab + axitinib	RCC	1st line	JAVELIN Renal 101
Jun 2019	Pembrolizumab or Pembrolizumab + CTx	SCCHN	1st line	KEYNOTE048
Dec 2019	Atezolizumab + CTx	Non-SqCC NSCLC	1st line	IMpower130
Mar 2020	Nivolumab + ipilimumab	HCC failed to sorafenib	2nd line	CheckMate 040
Mar 2020	Durvalumab + CTx	SCLC	1st line	CASPIAN
May 2020	Nivolumab + ipilimumab	NSCLC PD-L1≥1%	1st line	CheckMate 227
May 2020	Nivolumab + ipilimumab + 2 cycles CTx	NSCLC	1st line	CheckMate 9LA
May 2020	Atezolizumab	NSCLC with high PD-L1	1st line	IMpower110
May 2020	Atezolizumab + bevacizumab	HCC	1st line	IMbrave150
Jun 2020	Nivolumab	ESCC	2nd line	ATTRACTION-3
Jun 2020	Avelumab	Urothelial carcinoma	1st line	JAVELIN Bladder 100
Jun 2019	Pembrolizumab	SCLC	3rd line	KEYNOTE158, KEYNOTE028
Jul 2019	Pembrolizumab	ESCC	2nd line	KEYNOTE180, KEYNOTE181
Jan 2020	Pembrolizumab	Invasive bladder cancer	2nd line	KEYNOTE-057
Jun 2020	Pembrolizumab	Cutaneous squamous cell carcinoma	1st line	KEYNOTE-629
Jun 2020	Pembrolizumab	MSI-H or dMMR Colorectal Cancer	1st line	KEYNOTE-177
Jul 2020	Atezolizumab + cobimetinib + vemurafenib	BRAF V600E melanoma	1st line	IMspire150

(continued to the next page)

Table 1. (Continued) FDA approval history of solid tumor

Date	Drug name	Indication	Treatment line	Key trial
Oct 2020	Nivolumab + ipilimumab	Malignant pleural mesothelioma	1st line	CheckMate 743
Nov 2020	Pembrolizumab + CTx	TNBC with PD-L1	1st line	KEYNOTE-355
Jan 2021	Nivolumab + cabozantinib	RCC	1st line	CheckMate 9ER
Mar 2021	Pembrolizumab + CTx	Eso or GEJ carcinoma	1st line	KEYNOTE-590
Apr 2021	Nivolumab + CTx	GC, GEJ, and Eso adenocarcinoma	1st line	CheckMate 649
Apr 2021	Dostarlimab	dMMR endometrial cancer	2nd line	GARNET
May 2021	Pembrolizumab + trastuzumab + CTx	HER2+ GC or GEJ cancer	1st line	KEYNOTE-811
May 2021	Nivolumab	Eso or GEJ cancer	Adjuvant	CheckMate 577
Jul 2021	Pembrolizumab + lenvatinib	Endometrial carcinoma	2nd line	KEYNOTE-775
Jul 2021	Pembrolizumab + CTx, then pembrolizumab	TNBC	Neoadjuvant/Adjuvant	KEYNOTE522
Aug 2021	Nivolumab	High-risk urothelial carcinoma	Adjuvant	CheckMate 274
Aug 2021	Dostarlimab	dMMR solid tumors	2nd line	GARNET
Aug 2021	Pembrolizumab + lenvatinib	RCC	1st line	KEYNOTE581
Oct 2021	Pembrolizumab + CTx +/– bevacizumab	Cervical cancer with PD-L1	2nd line	KEYNOTE158
Oct 2021	Atezolizumab	NSCLC	Adj	IMpower010
Nov 2021	Pembrolizumab	RCC	Adj	KEYNOTE564

Abbreviations: NSCLC, non-small cell lung cancer; PD-L1, programmed death-ligand-1; SqCC, squamous cell carcinoma; Non-SqCC, non-squamous cell carcinoma; RCC, renal cell carcinoma; TNBC, triple negative breast cancer; HCC, hepatocellular carcinoma; Eso, Esophageal, GC, gastric cancer; GEJ, gastroesophageal junction, ESCC, esophageal squamous cell carcinoma; CTx, chemotherapy; SCLC, small cell lung cancer; SCCHN, squamous cell carcinoma head and neck; MSI-H, microsatellite instability high; dMMR, mismatch repair deficient.

in the treatment landscape for patients with NSCLC without molecular alterations is exemplary, and the clinical trials that led to the application of ICIs focusing on lung cancer are discussed. Prior to the advent of immunotherapy and targeted agents, platinum doublet chemotherapy was the only treatment option for metastatic NSCLC with dismal overall survival (OS) of 8–12 months (61-63). In NSCLC patients who do not have oncogenic driver-mutations, ICIs are the main stay for treatment, and the addition of chemotherapy is based on PD-L1 expressions, as well as the patient's individual characteristics (64). In metastatic NSCLC, the ICIs was first approved by the FDA in 2015 (65). So far, the 6 approved ICIs include 1) PD-1 inhibitors such as pembrolizumab, nivolumab, and cemiplimab, 2) anti-PD-L1 inhibitors including atezolizumab and durvalumab, and 3) ipilimumab, an anti-CTLA-4 inhibitor (66). ICIs are the standard of treatment for metastatic and unresectable, stage 3 NSCLC. Recently, ICIs are rapidly expanding its indications to recurrent/metastatic (R/M) NSCLC. The pivotal trials which led to the approval of ICIs in metastatic, unresectable stage 3, and resected NSCLC, as well as the future perspectives of ICIs and ongoing clinical trials are discussed.

The success of phase 1 clinical studies of PD-1inhibitor in heavily treated R/M NSCLC patients resulted in various clinical studies (38,67). Four pivotal phase 3 trials, CheckMate 017, CheckMate057, KEYNOTE 010, and OAK trial initialized the paradigm shift in the treatment options for metastatic NSCLC in second line setting (68-71). Although there are some differences in inclusion criteria for each study, these phase 3 studies compared PD-1/PD-L1 inhibitors with docetaxel as secondary treatment in NSCLC patients who failed to prior platinum-based chemotherapy. PD-1/PD-L1 inhibitor clearly showed OS benefit and durable response compared to docetaxel as conventional standard treatment (**Table 1**). Based on these pivotal studies, PD-1 pathway inhibitors, including nivolumab, pembrolizumab, and atezolizumab were firstly approved in NSCLC patients in more than second line setting in 2015.

Similar to the chemotherapy development process in other cancer types, multiple clinical trials were conducted to test the effectiveness of PD-1 inhibitors as a front-line treatment in

Table 2. Summary of key trials on immune checkpoint inhibitors in metastatic NSCLC

Study	Histology	PD-L1	Treatment	Control CTx	ORR		PFS		OS	
					ICI vs. CTx	p	ICI vs. CTx	p	ICI vs. CTx	p
SECOND LINE										
KEYNOTE 010	All	≥1%	Pembrolizumab	Docetaxel	18 vs. 9	0.002	4 vs. 4	0.004	12.7 vs. 8.5	<0.0001
CheckMate 017	SqCC	NA	Nivolumab		20 vs. 9	0.008	3.5 vs. 2.8	<0.001	9.2 vs. 6	<0.001
CheckMate 057	Non-SqCC	NA	Nivolumab		19 vs. 12	0.02	2.3 vs. 4.2	0.39	12.2 vs. 9.4	<0.002
OAK	All	NA	Atezolizumab		14 vs. 13	NR	2.8 vs. 4	0.49	13.8 vs. 9.6	0.0003
FIRST LINE										
Monotherapy										
KEYNOTE 024	All	≥50%	Pembrolizumab	Platinum doublet	44.8 vs. 27.8	NR	10.3 vs. 6	<0.001	30 vs. 14.2	0.002
KEYNOTE 042	All	≥1%	Pembrolizumab		27 vs. 27	NR	5.4 vs. 6.5	NR	16.7 vs. 12.1	0.0018
IMpower 110	All	≥50%	Atezolizumab		38.3 vs. 28.6	NR	8.1 vs. 5	NR	20.2 vs. 13.1	0.01
EMPOWER-lung 1	All	≥50%	Cemiplimab		39 vs. 20	<0.001	8.2 vs. 5.7	<0.0001	NR vs. 14.2	0.0002
CheckMate 026	All	≥5%	Nivolumab		25 vs. 33	NR	4.2 vs. 5.9	0.25	14.4 vs. 13.2	NR
MYSTIC	All	≥25%	Durvalumab		35.6 vs. 37.7	-	4.7 vs. 5.4	<0.05	16.3 vs. 12.9	0.04
Combination										
IO-chemotherapy										
KEYNOTE 189	Non-SqCC	NA	Pembrolizumab, platinum, pemetrexed	Platinum, pemetrexed	47.6 vs. 18.9	<0.001	8.8 vs. 4.9	<0.001	NR vs. 11.3	<0.001
KEYNOTE 407	SqCC	NA	Pembrolizumab, carboplatin, taxane	Carboplatin, taxane	57.9 vs. 38.4	NR	6.4 vs. 4.8	<0.001	15.9 vs. 11.3	<0.001
IMpower 150	Non-SqCC	NA	Atezolizumab, bevacizumab, carboplatin, paclitaxel	Bevacizumab, carboplatin, paclitaxel	63.5 vs. 48	NR	8.3 vs. 6.8	<0.001	19.2 vs. 14.7	0.02
IO-IO										
CheckMate 227	All	≥1%	Nivolumab, ipilimumab	Platinum doublet	35.9 vs. 30	NR	5.1 vs. 5.6	NR	17.1 vs. 14.9	0.007
	All	0%	Nivolumab, ipilimumab		27.3 vs. 23.1	NR	5.1 vs. 4.7	NR	17.2 vs. 12.2	NR
CheckMate 9LA	All	NA	Nivolumab, ipilimumab, platinum doublet		37.7 vs. 25.1	0.0003	6.8 vs. 5	0.00012	15.6 vs. 10.9	0.00065
MYSTIC	All	≥25%	Durvalumab, tremelimumab		34.4 vs. 37.7	-	3.9 vs. 2.4	0.71	11.9 vs. 12.9	0.20

Abbreviations: ICIs, immune checkpoint inhibitors; NSCLC, non-small cell lung cancer; ORR, overall response rate; PFS, progression-free survival; OS, overall survival; PD-L1, programmed death-ligand-1; CTx, chemotherapy; SqCC, squamous cell carcinoma; Non-SqCC, non-squamous cell carcinoma; NR, not reached; NA, not-applicable.

metastatic NSCLC patients (**Table 2**). The role of PD-1 and PD-L1 agents were evaluated in front-line monotherapy (KEYNOTE 024, KEYNOTE 042, IMpower 110, EMPOWER-lung-1, CheckMate 026) and combination with chemotherapy (KEYNOTE 189, KEYNOTE 407, IMpower 150) or I-O combinations (CheckMate 227, CheckMate 9LA) in the pivotal phase 3 trials for the treatment naïve, metastatic NSCLC (**Table 2**).

KEYNOTE 024 investigated the role of pembrolizumab in front-line settings in patients with highly expressed PD-L1 (≥50%) (52). Updated analysis continued to support the OS benefit in pembrolizumab monotherapy compared to standard platinum-based chemotherapy with median overall survival (mOS) of 30.0 vs. 14.2 months (53). IMpower110 study assessed the atezolizumab monotherapy in PD-L1 selected population (≥1% of tumor cell or immune cell) (72). The primary endpoint of OS favored the atezolizumab group over the chemotherapy group. Recently, the EMPOWER-lung-1 study showed the efficacy of cemiplimab in the first-line treatment of metastatic NSCLC with PD-L1 ≥50% (73). Considering the magnitude of survival benefit, PD-1 inhibitor monotherapy is used as a standard therapy in patients with PD-L1 ≥50% in front-line setting.

Second, the success of anti-PD-1/PD-L1 as monotherapy in front-line setting proved possibility of combination of chemotherapy with ICIs. Pre-clinical studies showed that chemotherapeutic agents augment PD-L1 expression on tumor cells (74). Thus, treatment with immunotherapy plus chemotherapy is a plausible combination since both agents can synergistically induce

anti-tumor activity via reducing T-regulatory cell activities and increasing presentation of tumor antigens (75,76). The KEYNOTE 189 and IMpower150, and KEYNOTE 407 support the addition of immunotherapy with chemotherapy in front-line R/M NSCLC for non-squamous and squamous histology, respectively (44,51,77-79) (**Table 1**). The phase 3 KEYNOTE 189 was conducted, and the co-primary endpoints were progression-free survival (PFS) and OS, and both results were superior in the pembrolizumab combination group, despite the permission of placebo group to crossover to second line pembrolizumab monotherapy (44,78). The KEYNOTE 407 was designed to evaluate the efficacy of the combination in squamous metastatic NSCLC (51). The addition of pembrolizumab to chemotherapy succeeded in prolonging both PFS and OS, regardless of PD-L1 expression (79). This trial resulted in the significant paradigm shift for the treatment of squamous R/M NSCLC, since the only available treatment was cytotoxic chemotherapy for squamous histology due to the lack of targetable aberrations (80,81). IMpower150 explored the role of atezolizumab in combination with bevacizumab, a monoclonal antibody which targets VEGF-A in front-line, non-squamous metastatic NSCLC (77). In addition, ABCP regimen, in which atezolizumab, bevacizumab, carboplatin, and paclitaxel are combined, was well tolerated, and is one of the treatment options for treatment naïve, non-squamous metastatic NSCLC.

The combination of nivolumab plus ipilimumab in previously untreated, metastatic NSCLC was assessed in the CheckMate 227 (45) (**Table 1**). Results showed the superior mOS of nivolumab plus ipilimumab in comparison with chemotherapy. To overcome the early progression in some cases, CheckMate 9LA was conducted with two cycles of chemotherapy added to the combination regardless of PD-L1 expressions (82). The addition of 2 cycles of chemotherapy with nivolumab plus ipilimumab improved mOS compared with the chemotherapy alone (83).

Following the success of ICIs in metastatic NSCLC, ICIs were subsequently explored in surgically unresectable, locally advanced NSCLC (84). Updated 5-year analysis of the PACIFIC trial showed that the addition of durvalumab, an anti-PD-L1 agent as consolidation treatment in patients whose disease had not progressed after concurrent chemoradiotherapy (CCRT), continually improve both the median progression-free survival (mPFS) and mOS (85). Currently, consolidation with durvalumab after CCRT is the gold standard of practice (64). Other immunotherapy agents such as pembrolizumab, nivolumab and atezolizumab have also been explored in the phase 2 studies of KEYNOTE-799 (NCT0363178), NICOLAS (NCT02434081), and DETERRED study (NCT02525757), respectively (86-88).

The ICIs were also incorporated in early-stage NSCLC, including neoadjuvant and adjuvant settings (89). In adjuvant settings, the addition of atezolizumab after standard chemotherapy was explored in patients with stage IB-IIIA NSCLC in the IMpower 010 (60). The primary and secondary endpoints were disease-free survival (DFS) and OS, respectively. Patients with stage IB-IIIA NSCLC and PD-L1≥1% treated with atezolizumab had significantly longer median DFS, compared to the control group, and the improvement in DFS was also seen in the atezolizumab treated patients regardless of PD-L1 expressions in stage II-IIIA. IMpower 010 study is the first to show significant benefit of adjuvant PD-L1 inhibitor for DFS in early-stage resected NSCLC, and approved by the FDA. Neoadjuvant immunotherapy has not yet been approved, their role was also explored in several studies (**Table 2**).

Despite the clinical utility of immunotherapy in metastatic NSCLC, there remain several hurdles, including intrinsic and acquired resistance during treatment with ICIs (90). Treatment

strategies to prolong survival and overcome acquired resistance to immunotherapy include addition of other immune checkpoints, such as TIGIT, LAG-3, TIM-3, which enable immune evasion (5). Agents targeting these immune checkpoints are under clinical investigation in various solid tumors, including metastatic NSCLC. One of the promising agent in tiragolumab, an anti-TIGIT antibody (91). The phase 2, CITYSCAPE trial evaluated the combination of tiragolumab with atezolizumab versus placebo with atezolizumab as front-line treatment in PD-L1 ≥1% NSCLC. Tiragolumab plus atezolizumab group had both improved overall response rate and mPFS compared to the placebo plus atezolizumab group. As a result, the FDA grant the use of tiragolumab breakthrough therapy designation for PD-L1 high NSCLC. The phase 3 SKYSCAPER-01 trial (NCT04513925) is currently under investigation.

In summary, the combination treatments of chemotherapy with immunotherapy or immunotherapy combination resulted in significant benefit in OS. Currently, the standard clinical practice for front-line metastatic NSCLC without molecular alterations includes pembrolizumab, atezolizumab, cemiplimab, nivolumab plus ipilimumab for PD-L1 ≥50%, pembrolizumab plus chemotherapy, ABCP regimen, and nivolumab plus ipilimumab for PD-L1 ≥1%–49% (64). For patients with PD-L1 ≥1%–49%, but with high volume tumors, the combination of chemoimmunotherapy is also a feasible option (66).

PREVIOUS AND NOVEL ASPECTS FOR MODE OF ACTION OF PD-1 ICIs

While ICIs targeting PD-1 expanded the scope of treatment to various types of cancers in clinical setting, basic immunological studies support the reasons for the limitations of PD-1 inhibitors have continued. The Rafi Ahmed group reported that inhibition of PD-1 signaling induces functional restoration of exhausted T cells (36). However, Jonh Wherry group and Nicholas Haining group using comparative epigenetic analysis, noted the difficulty of exhausted T cells to be reprogrammed by PD-1 inhibitors into effector T cells, because the genes associated with T cell effector function have been epigenetically silent during exhaustion process (92-94), supporting the rationale for limited efficacy of the current PD-1 ICIs. In this regard, the combination of epigenetic modulatory drug with PD-1 inhibitor, can induce a de novo epigenetic program and enhance the efficacy of PD-1 antibody monotherapy (95), but the drug's ability to specificity modulate epigenetics is questionable.

Recent studies reveal that the exhausted cells are more heterogeneous than expected. Accordingly, studies on which subset could respond to PD-1 inhibitor are under investigation. In 2016, many scientists, including Rafi Ahmed, using chronic virus infection model, proved that stem-like exhausted T cells, maintaining their self-renewal property by Tcf1 expression, may differentiate into fully exhausted T cells, by losing Tcf1 expression and expressing multiple immune checkpoints other than PD-1, under certain condition (96-100) (**Fig. 2**). Another interesting point is that stem-like exhausted T cell subset, but not fully exhausted T cells, proliferate in response to PD-1 inhibitor (96). The presence of stem-like exhausted T cells are seen in tumor mouse model (101-103) and human tumor samples (104,105). In addition to the two aforementioned subsets, further research also revealed the exhausted T cell subsets are more diverse according to their differentiation stage (106-108).

In 2017, *in vitro* biochemical experiment and *in vivo* mouse experiment showed that SHP-2, in response to the trigger of PD-1 signal by PD-L1, inhibits CD28 co-stimulation-

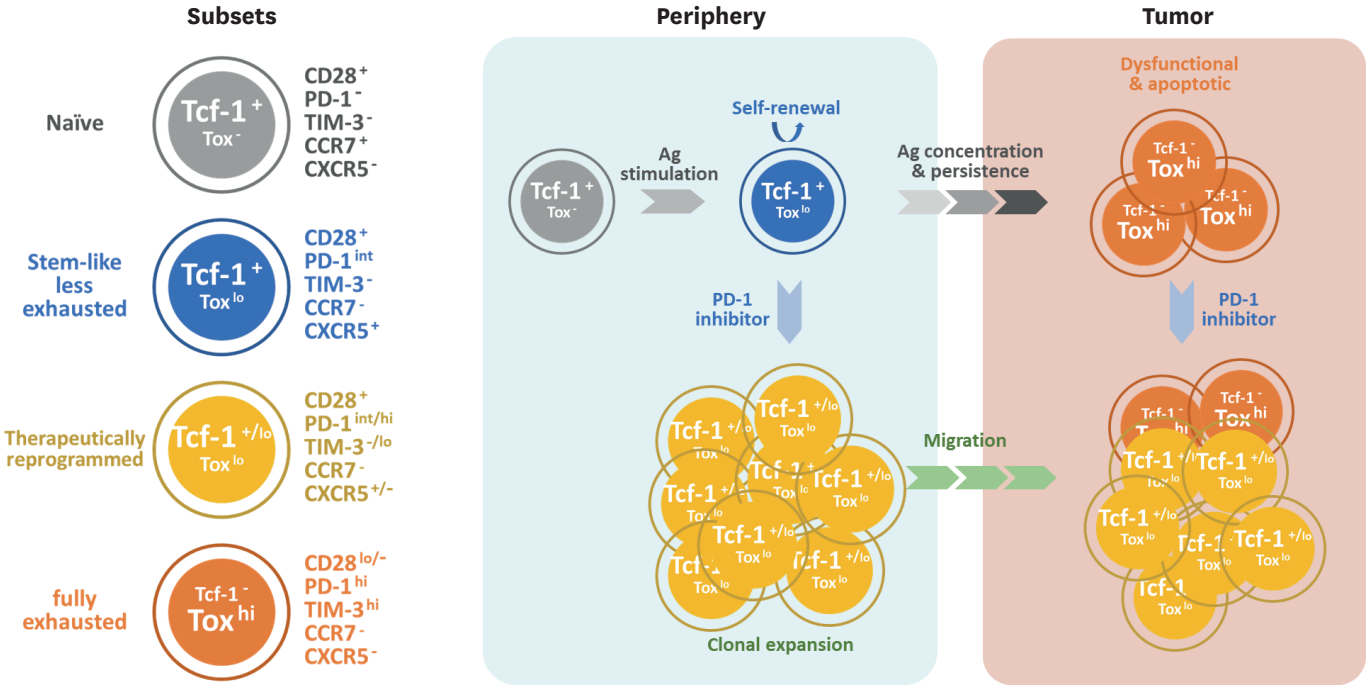

Figure 2. Subsets of tumor-specific CD8 T cells and mode of action of PD-1 inhibitor. Tumor antigen specific CD8 T cells are heterogenous dependent on environment. Representative transcription factor and surface molecules are indicated on each subset (left). Naïve tumor-specific CD8 T cells are differentiated linearly from peripheral tissues including tumor-draining lymph node upon stimulation with tumor antigen (right). Persistent antigen stimulation programs progressive exhaustion in tumor-specific CD8 T cells, thereby differentiating them from stem-like less exhausted state in peripheral tissues into fully exhausted state in tumor tissue in tumor antigen concentration-dependent manner. PD-1 inhibitor treatment partially might reprogram stem-like less exhausted T cells, which clonally expand, migrate into tumor, and display their effector function. In contrast, fully exhausted T cells, which have already entered tumor and become dysfunctional and apoptotic within tumor, do not respond to PD-1 inhibitor.

mediated phosphorylation rather than TCR-medicated phosphorylation (109,110). CD28 is downregulated through a continuous exhaustion program after tumor-infiltrating. Thus, the later stage of exhausted T cells, in which the B7-CD28 signal was inhibited, do not respond to PD-1 blockade (111).

In 2019, TOX, a master transcription factor that transcriptionally and independently programs T cell exhaustion, was identified (112-117). Subsequent studies using human cancer samples showed that TOX can control PD-1 expression and T cell function (118,119) (**Fig. 2**). Given the previous reports that Tcf1 expression in stem-like exhausted T cells increases the reactivity to PD-1 inhibitors, there is a need for a strategy that can increase the response to PD-1 inhibitors through controlling the balance of Tcf1 and Tox expressions (108,120,121).

Meanwhile, studies showing that PD-1 expression is observed in a variety of immune cells in addition to exhausted T cells have opened up the possibility for another mode of action for PD-1 inhibitors (122,123). In fact, low level of PD-1 expressed macrophages present in tumor microenvironment promoted tumor growth by inhibiting the macrophage function, including phagocytosis (124). Since PD-1 expression in tumor-infiltrating Treg cells was reported to be very high than exhausted CD8 T cells (125-127), the following extensive studies were conducted to identify the function of PD-1 in the tumor-infiltrating Treg cells. Studies of chronic virus infection and tumor models have reported that PD-1 expression in Treg cells is involved in inhibiting the function of CD8 T cells by strengthening suppressive function and proliferation of Treg cells (128-132). On the contrary, other studies argued that PD-1 inhibitor treatment

may instead promote cancer hyperprogression in some patients with high frequency of PD-1-expressing Treg cells, because PD-1 inhibitors can induce the proliferation of PD-1-posivie Treg cells (133,134). Therefore, detailed studies using the conditional knockout mouse in which PD-1 gene is knockout specifically in Treg cells, should be conducted for more accurate conclusion. Since strong expression of immune checkpoints other than PD-1 are also observed in tumor-infiltrating Treg cells, studies focusing on the function of Treg cells expressing multiple immune checkpoints warrant further exploration (122,126).

FUTURE PERSPECTIVES TO ENHANCE EFFICACY IN ICIs

The biggest limitation of the currently available ICIs, including PD-1inhibitors, is that only an average of 10%–20% patients treated obtain clinical benefit. Thus, combination with other ICIs that have non-overlapping mechanisms may potentially increase clinical efficacy. Deeper understanding of T cell exhaustion mechanism, further clarification of the tumor immune microenvironment, and understanding the exact mechanism of actions of ICIs will help overcome the limitations of cancer immunotherapy.

Recently, studies have shown the importance of the priming and activation of tumor-specific T cells in the draining lymph node, the migration of tumor-specific T cells into the tumor site, and the formation of a tertiary lymphoid structure within the tumor (135-138). T cells primed in peripheral tissues including tumor-draining lymph node, rather than T cells that have entered and resided within the tumor, could respond efficiently to ICIs, thereby preserving the ongoing anti-tumor immune response (139-145).

T cells differ by its stages in T cell exhaustion, and stem-like exhausted T cells occupy the majority in the tumor-draining lymph node and actively proliferate responding to ICIs, whereas fully exhausted T cells within the tumor site are little responsive to ICIs. Thus, strategies to generate and migrate stem-like exhausted T cells to tumor site, as well as combining these methods with the current ICIs may increase the efficacy of cancer immunotherapy. One method is to combine ICIs and therapeutic cancer vaccine expressing tumor antigens (8). To this end, ways to quickly discover neoantigen must be developed, and the ideal vaccine should be able to continuously express tumor neoantigens. The vaccine containing tumor neoantigens should be delivered to the site in which antigen-presenting cells such as dendritic cells efficiently process and present antigens. The optimization of therapeutic cancer vaccines, in combination with emerging ICIs, may result in increased response in various cancers, and result in effective cancer immunotherapy.

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