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...
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.

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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
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                        | Refractory     | 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   | Nivolumab           | Melanoma                          | 2nd line       | KEYNOTE002         |
| Jan 2016   | Nivolumab + ipilimumab | Melanoma across BRAF status     | 1st line       | CheckMate 069      |
| Mar 2016   | Atezolizumab        | Urothelial carcinoma              | 2nd line       | CheckMate 275      |
| Aug 2016   | Pembrolizumab       | UCSSH                             | 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       | KEYNOTE077         |
| Dec 2018   | Atezolizumab + Bevacizumab + CTx | Non-SqCC NSCLC | 1st line | IMpower150 |
| Feb 2019   | Pembrolizumab       | Melanoma with lymph node          | Adjuvant       | KEYNOTE054         |
| Mar 2019   | Pembrolizumab + nab-paclitaxel | TNBC with PD-L1 | 1st line | Impassion130 |
| Mar 2019   | Atezolizumab + CTx  | SCLC                              | 1st line       | IMpower133         |
| Apr 2019   | Pembrolizumab       | SCLC                              | 1st line       | KEYNOTE426         |
| Apr 2019   | Pembrolizumab + axitinib | RCC                               | 1st line       | KEYNOTE042         |
| May 2019   | Avelumab + axitinib  | RCC                               | 1st line       | JAVELIN Renal 101  |
| Jun 2019   | Pembrolizumab or Pembrolizumab + CTx | SCLC | 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>3%                  | 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 | RCC                               | 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        |
| Jan 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        |
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 mainstay 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-1 inhibitor 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 1. (Continued) FDA approval history of solid tumors

| Date       | Drug name + /− bevacizumab | Indication | Treatment line | Key trial |
|------------|-----------------------------|------------|----------------|-----------|
| Oct 2020   | Pembrolizumab               | Malignant pleural mesothelioma | 1st line | CheckMate 743 |
| Nov 2020   | Pembrolizumab + CTx         | TNBC with PD-L1 | 1st line | KEYNOTE-355 |
| Jan 2021   | Pembrolizumab + caborzinib  | RCC        | 1st line | CheckMate 9ER |
| Mar 2021   | Pembrolizumab + CTx         | Eso or GEJ carcinoma | 1st line | KEYNOTE-590 |
| Apr 2021   | Pembrolizumab + CTx         | GC, GEJ, and Eso adeno carcinoma | 1st line | CheckMate 649 |
| Apr 2021   | Pembrolizumab + trastuzumab + CTx | HER2+ GC or GEJ cancer | 1st line | KEYNOTE-811 |
| May 2021   | Pembrolizumab + trastuzumab + CTx | HER2+ GC or GEJ cancer | 1st line | KEYNOTE-811 |
| Jul 2021   | Pembrolizumab + lenvatinib  | Endometrial carcinoma | 2nd line | KEYNOTE-775 |
| Jul 2021   | Pembrolizumab + CTx, then pembrolizumab | TNBC | Neoadjuvant/Adjuvant | KEYNOTE522 |
| Aug 2021   | Pembrolizumab               | High-risk urothelial carcinoma | Adjuvant | CheckMate 274 |
| Aug 2021   | Pembrolizumab + lenvatinib  | RCC        | 1st line | KEYNOTE-581 |
| Oct 2021   | Pembrolizumab + CTx +/− bevacizumab | Cervical cancer with PD-L1 | 2nd line | KEYNOTE-158 |
| Oct 2021   | Atezolizumab                | NSCLC      | Adj | IMpower010 |
| Nov 2021   | Pembrolizumab               | RCC        | Adj | KEYNOTE-564 |

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.
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 chemotheraphy (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

### 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 |
|-------------------|-----------|---------|------------------------------------|-------------|----------|--------------|-----------------|--------------|--------------|
| **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         | 12.9 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                       | Platinum doublet | 27 vs. 27    | NR         | 5.4 vs. 6.5     | NR           | 16.7 vs. 12.1 | 0.0018       |
| IMPower 110       | All       | ≥50%    | Atezolizumab                        | Platinum doublet | 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       |           |         |                                    |             |          |              |                 |              |              |
| Monotherapy       |           |         |                                    |             |          |              |                 |              |              |
| KEYNOTE 189       | Non-SqCC  | NA      | Pembrolizumab, 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  |             | 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 |             | 63.5 vs. 48 | NR           | 8.3 vs. 6.8     | <0.001       | 19.2 vs. 14.7 | 0.02         |
| Combination       |           |         |                                    |             |          |              |                 |              |              |
| Monotherapy       |           |         |                                    |             |          |              |                 |              |              |
| KEYNOTE 189       | Non-SqCC  | NA      | Pembrolizumab, 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  |             | 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 |             | 63.5 vs. 48 | NR           | 8.3 vs. 6.8     | <0.001       | 19.2 vs. 14.7 | 0.02         |

**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.
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 immunotheapy 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-
mediated phosphorylation rather than TCR-mediated 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-positive Treg cells (133,134). Therefore, detailed studies using the conditional knockout mouse in which PD-1 gene is knocked out 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-1 inhibitors, 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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REFERENCES

1. Coley WB. Contribution to the knowledge of sarcoma. *Ann Surg* 1891;14:199-220.

2. Kirkwood JM, Butterfield LH, Tarhini AA, Zarour H, Kalinski P, Ferrone S. Immunotherapy of cancer in 2012. *CA Cancer J Clin* 2012;62:309-335.

3. Small EJ, Schellhammer PF, Higano CS, Redfern CH, Nemunaitis JJ, Valone FH, Verjee SS, Jones LA, Hershberg RM. Placebo-controlled phase III trial of immunologic therapy with sipuleucel-T (APC8015) in patients with metastatic, asymptomatic hormone refractory prostate cancer. *J Clin Oncol* 2006;24:3089-3094.

4. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, Redfern CH, Ferrari AC, Dreicer R, Sims RB, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *N Engl J Med* 2010;363:411-422.

5. Lee JB, Ha SJ, Kim HR. Clinical insights into novel immune checkpoint inhibitors. *Front Pharmacol* 2021;12:681320.

6. Sengupta R, Zaidi SK. Aacr cancer progress report 2021: discovery science driving clinical breakthroughs. *Clin Cancer Res* 2021;27:5757-5759.

7. Jeong S, Park SH. Co-stimulatory receptors in cancers and their implications for cancer immunotherapy. *Immune Netw* 2020;20:e3.

8. Ha SJ, West EE, Araki K, Smith KA, Ahmed R. Manipulating both the inhibitory and stimulatory immune system towards the success of therapeutic vaccination against chronic viral infections. *Immunol Rev* 2008;223:317-333.

9. Brunet JF, Denizot F, Luciani MF, Roux-Dosseto M, Suzan M, Mattei MG, Golstein P. A new member of the immunoglobulin superfamily--CTLA-4. *Nature* 1987;328:267-270.

10. Linsley PS, Brady W, Umes M, Grosmaire LS, Damle NK, Ledbetter JA. CTLA-4 is a second receptor for the B cell activation antigens B7. *J Exp Med* 1991;174:561-569.

11. Lenschow DJ, Zeng Y, Thistlethwaite JR, Montag A, Brady W, Gibson MG, Linsley PS, Bluestone JA. Long-term survival of xenogeneic pancreatic islet grafts induced by CTLA4Ig. *Science* 1992;257:789-792.

12. Linsley PS, Wallace DM, Johnson J, Gibson MG, Greene JL, Ledbetter JA, Singh C, Tepper MA. Immunosuppression in vivo by a soluble form of the CTLA-4 T cell activation molecule. *Science* 1992;257:792-795.

13. Walunas TL, Lenschow DJ, Bakker CY, Linsley PS, Freeman GI, Green JM, Thompson CB, Bluestone JA. CTLA-4 can function as a negative regulator of T cell activation. *Immunity* 1994;1:405-413.

14. Krummel ME, Allison JP. CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. *J Exp Med* 1995;182:459-465.

15. Waterhouse P, Penninger JM, Timms E, Wakeham A, Shahinian A, Lee KP, Thompson CB, Griesser H, Mak TW. Lymphoproliferative disorders with early lethality in mice deficient in Ctlan-4. *Science* 1995;270:985-988.

16. Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, Sharpe AH. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. *Immunity* 1995;3:541-547.

17. Ishida Y, Agata Y, Shibahara K, Honjo T. Induced expression of PD-1, a novel member of the immunoglobulin gene superfamily, upon programmed cell death. *EMBO J* 1992;11:3887-3895.

18. Nishimura H, Nose M, Hiai H, Minato N, Honjo T. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. *Immunity* 1999;11:141-151.
19. Nishimura H, Okazaki T, Tanaka Y, Nakatani K, Hara M, Matsumori A, Sasayama S, Mizoguchi A, Hiai H, Minato N, et al. Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. *Science* 2001;291:319-322.

20. Dong H, Zhu G, Tamada K, Chen L. B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. *Nat Med* 1999;5:1365-1369.

21. Tseng SY, Otsuji M, Gorski K, Huang X, Slansky JE, Pai SI, Shalabi A, Shin T, Pardoll DM, Tsuchiya H. B7-DC, a new dendritic cell molecule with potent costimulatory properties for T cells. *J Exp Med* 2001;193:839-846.

22. Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Fitz LJ, Malenkovich N, Okazaki T, Byrne MC, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J Exp Med* 2000;192:1027-1034.

23. Latchman Y, Wood CR, Chernova T, Chaudhary D, Bosde M, Chernova I, Iwai Y, Long AJ, Brown JA, Nunes R, et al. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. *Immunity* 2004;20:327-336.

24. Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Fitz LJ, Malenkovich N, Okazaki T, Byrne MC, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J Exp Med* 2000;192:1027-1034.

25. Latchman Y, Wood CR, Chernova T, Chaudhary D, Bosde M, Chernova I, Iwai Y, Long AJ, Brown JA, Nunes R, et al. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. *Immunity* 2004;20:327-336.

26. Pfister H, Chung Y, Bishop C, Daugherty B, Chute H, Holst P, Kurohara C, Lotf F, Sun N, Welcher AA, et al. Regulation of T cell activation and tolerance by PD-L2. *Proc Natl Acad Sci U S A* 2006;103:11695-11700.

27. Liang SC, Greenwald RJ, Latchman Y, Ross L, Salojar A, Freeman GJ, Sharpe AH. PD-L1 and PD-L2 have distinct roles in regulating host immunity to cutaneous leishmaniasis. *Eur J Immunol* 2006;36:58-64.

28. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. *Science* 1996;271:1734-1736.

29. Hodi FS, Mihm MC, Soiffer RJ, Haluska FG, Butler M, Seiden MV, Davis T, Henry-Spires R, MacRae S, Willman A, et al. Biologic activity of cytotoxic T lymphocyte-associated antigen 4 antibody blockade in previously vaccinated metastatic melanoma and ovarian carcinoma patients. *Proc Natl Acad Sci U S A* 2003;100:4712-4717.

30. Phan GQ, Yang JC, Sherry RM, Hwu P, Topalian SL, Schwartzztuber DJ, Restifo NP, Haworth LR, Seipp CA, Freezer L, et al. Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. *Proc Natl Acad Sci U S A* 2003;100:8372-8377.

31. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Gonzalez R, Robert C, Schadendorf D, Hassel JC, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* 2010;363:711-723.

32. Robert C, Thomas L, Bondarenko I, O'Day S, Weber J, Garbe C, Lebbe C, Baurain JF, Testori A, Grob JJ, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N Engl J Med* 2011;364:2517-2526.

33. Iwai Y, Ishida M, Tanaka Y, Okazaki T, Honjo T, Minato N. Involvement of PD-L1 on tumor cells in the escape from host immune system and tumor immunotherapy by PD-L1 blockade. *Proc Natl Acad Sci U S A* 2002;99:12293-12297.

34. Dong H, Strome SE, Salamaa DR, Tamura H, Hirano F, Flies DB, Roche PC, Lu J, Zhu G, Tamada K, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat Med* 2002;8:793-800.
35. Hirano F, Kaneko K, Tamura H, Dong H, Wang S, Ichikawa M, Rietz C, Flies DB, Lau JS, Zhu G, et al. Blockade of B7-H1 and PD-1 by monoclonal antibodies potentiates cancer therapeutic immunity. **Cancer Res** 2005;65:1089-1096. 
**PUBMED** | **CROSSREF**

36. Barber DL, Wherry EJ, Masopust D, Zhu B, Allison JP, Sharpe AH, Freeman GJ, Ahmed R. Restoring function in exhausted CD8 T cells during chronic viral infection. **Nature** 2006;439:682-687. 
**PUBMED** | **CROSSREF**

37. Brahmer JR, Drake CG, Wollner I, Powderly JD, Picus J, Sharfman WH, Stankevich E, Pons A, Salay TM, McMiller TL, et al. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. **J Clin Oncol** 2010;28:3167-3175. 
**PUBMED** | **CROSSREF**

38. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Powderly JD, Carvajal RD, Somon JA, Atkins MB, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. **N Engl J Med** 2012;366:2443-2454. 
**PUBMED** | **CROSSREF**

39. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P, Drake CG, Camacho LH, Kauh J, Odunsi K, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. **N Engl J Med** 2012;366:2455-2465. 
**PUBMED** | **CROSSREF**

40. Hamid O, Robert C, Daud A, Hodi FS, Hwu WJ, Kefford R, Hersey P, Joseph RW, Weber JS, et al. Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. **N Engl J Med** 2013;369:134-144. 
**PUBMED** | **CROSSREF**

41. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Cowey CL, Lao CD, Schadendorf D, Dummer R, Smylie M, Rutkowski P, et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. **N Engl J Med** 2015;373:23-34. 
**PUBMED** | **CROSSREF**

42. Balar AV, Galsky MD, Rosenberg JE, Powles T, Petrylak DP, Bellmunt J, Loriot Y, Necchi A, Hoffman-Censits J, Perez-Gracia JL, et al. Atezolizumab as first-line treatment in cisplatin-ineligible patients with locally advanced and metastatic urothelial carcinoma: a single-arm, multicentre, phase 2 trial. **Lancet** 2017;389:67-76. 
**PUBMED** | **CROSSREF**

43. Burtness B, Harrington KJ, Greil R, Soulères D, Tahara M, de Castro G Jr, Payri A, Basté N, Neupane P, Bratland Å, et al. Pembrolizumab alone or with chemotherapy versus cetuximab with chemotherapy for recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-048): a randomised, open-label, phase 3 study. **Lancet** 2019;394:1915-1928. 
**PUBMED** | **CROSSREF**

44. Gandhi L, Rodriguez-Abreu D, Gadgeel S, Esteban E, Felip E, De Angelis F, Domine M, Clingan P, Hochmair PJ, Powell SF, et al. Pembrolizumab plus chemotherapy in metastatic non-small-cell lung cancer. **N Engl J Med** 2018;378:2078-2092. 
**PUBMED** | **CROSSREF**

45. Hellmann MD, Paz-Ares L, Bernabe Caro R, Zurawski B, Kim SW, Carceny Costa E, Park K, Alexandru A, Lupinacci L, de la Mora Jimenez E, et al. Nivolumab plus ipilimumab in advanced non-small-cell lung cancer. **N Engl J Med** 2019;381:2020-2031. 
**PUBMED** | **CROSSREF**

46. Hodi FS, Chesney J, Pavlick AC, Robert C, Grossmann KF, McDermott DF, Linette GP, Meyer N, Giguere JK, Agarwala SS, et al. Combined nivolumab and ipilimumab versus ipilimumab alone in patients with advanced melanoma: 2-year overall survival outcomes in a multicentre, randomised, controlled, phase 2 trial. **Lancet Oncol** 2016;17:1558-1568. 
**PUBMED** | **CROSSREF**

47. Horn L, Mansfield AS, Szczesna A, Havel L, Kruzakowski M, Hochmair MJ, Huerer F, Losonczy G, Johnson ML, Nishio M, et al. First-line atezolizumab plus chemotherapy in extensive-stage small-cell lung cancer. **N Engl J Med** 2018;379:2220-2229. 
**PUBMED** | **CROSSREF**

48. Langer CJ, Gadgeel SM, Borghaei H, Papadimitrakopoulou VA, Patnaik A, Powell SF, Gentzler RD, Martins RG, Stevenson JP, Jalal SI, et al. Carboplatin and pemetrexed with or without pembrolizumab for advanced, non-squamous non-small-cell lung cancer: a randomised, phase 2 cohort of the open-label KEYNOTE-021 study. **Lancet Oncol** 2016;17:1497-1508. 
**PUBMED** | **CROSSREF**
49. Mok TS, Wu YL, Kudaba I, Kowalski DM, Cho BC, Tuma HZ, Castro G Jr, Srimuninnimit V, Laktionov KK, Bondarenko I, et al. Pembrolizumab versus chemotherapy for previously untreated, PD-L1-expressing, locally advanced or metastatic non-small-cell lung cancer (KEYNOTE-042): a randomised, open-label, controlled, phase 3 trial. *Lancet* 2019;393:1819-1830.

50. Motzer RJ, Penkov K, Haanen J, Rini B, Albiges L, Campbell MT, Venugopal B, Kollmannsberger C, Negrier S, Uemura M, et al. Avelumab plus axitinib versus sunitinib for advanced renal-cell carcinoma. *N Engl J Med* 2019;380:1103-1115.

51. Paz-Ares L, Luft A, Vicente D, Tafreshi A, Gümrü M, Mazières J, Hermes B, Çay Şenler F, Csösz T, Fülöp A, et al. Pembrolizumab plus chemotherapy for squamous non-small-cell lung cancer. *N Engl J Med* 2018;379:2040-2051.

52. Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csösz T, Fülöp A, Gottfried M, Peled N, Tafreshi A, Cuffe S, et al. Pembrolizumab versus chemotherapy for PD-L1-positive non-small-cell lung cancer. *N Engl J Med* 2016;375:1823-1833.

53. Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csösz T, Fülöp A, Gottfried M, Peled N, Tafreshi A, Cuffe S, et al. Updated analysis of keynotes-024: pembrolizumab versus platinum-based chemotherapy for advanced non-small-cell lung cancer with PD-L1 tumor proportion score of 50% or greater. *J Clin Oncol* 2019;37:537-546.

54. Rini BI, Plimack ER, Stus V, Gafanov R, Hawkins R, Nosov D, Pouliot F, Alekseev B, Soulères D, Melichar B, et al. Pembrolizumab plus axitinib versus sunitinib for advanced renal-cell carcinoma. *N Engl J Med* 2019;380:1116-1127.

55. Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, Diéras V, Hegg R, Im SA, Shaw Wright G, et al. Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. *N Engl J Med* 2018;379:2108-2121.

56. Bajorin DF, Witjes JA, Gschwend JE, Schenker M, Valderrama BP, Tomita Y, Bamias A, Lebret T, Shariat SF, Park SH, et al. Adjuvant nivolumab versus placebo in muscle-invasive urothelial carcinoma. *N Engl J Med* 2021;384:2102-2114.

57. Eggermont AM, Blank CU, Mandala M, Long GV, Atkinson V, Dalle S, Haydon A, Lichinitser M, Khattak A, Carlini MS, et al. Adjuvant pembrolizumab versus placebo in resected stage iii melanoma. *N Engl J Med* 2018;378:1789-1801.

58. Kelly RJ, Ajani JA, Kuzdzal J, Zander T, Van Cutsem E, Piessen G, Mendez G, Feliciano J, Motoyama S, Lévrarme A, et al. Adjuvant nivolumab in resected esophageal or gastroesophageal junction cancer. *N Engl J Med* 2021;384:1191-1203.

59. Schmid P, Cortes J, Pusztai L, McArthur H, Kümmel S, Bergh J, Denkert C, Park YH, Hui R, Harbeck N, et al. Pembrolizumab for early triple-negative breast cancer. *N Engl J Med* 2020;382:810-821.

60. Wakelee HA, Altorki NK, Zhou C, Csösz T, Vynnychenko IO, Goloborodko O, Luft A, Akopov A, Martinez-Marti A, Kinnotts H, et al. Impower010: Primary results of a phase iii global study of atezolizumab versus best supportive care after adjuvant chemotherapy in resected stage ib-iiiia non-small cell lung cancer (nsclc). *J Clin Oncol* 2021;39 15_suppl:8500-8500.

61. Cetin K, Ettinger DS, Hei YJ, O’Malley CD. Survival by histologic subtype in stage IV nonsmall cell lung cancer based on data from the Surveillance, Epidemiology and End Results Program. *Clin Epidemiol* 2011;3:139-148.

62. Paz-Ares LG, de Marinis F, Dediu M, Thomas M, Pujol JL, Bidoli P, Molinier O, Sahoo TP, Laack E, Reck M, et al. PARAMOUNT: Final overall survival results of the phase III study of maintenance pemetrexed versus placebo immediately after induction treatment with pemetrexed plus cisplatin for advanced nonsquamous non-small-cell lung cancer. *J Clin Oncol* 2013;31:2895-2902.
63. Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, Zhu J, Johnson DH; Eastern Cooperative Oncology Group. Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. *N Engl J Med* 2002;346:92-98.  
[PubMed] [Crossref]

64. Etinger DS, Wood DE, Aisner DL, Akerley W, Balmanoukian AS, Bharat A, Bruno DS, Chang JY, Chiriac LR, D’Amico TA, et al. NCCN guidelines insights: non-small cell lung cancer, version 2.2021. *J Natl Compr Canc Netw* 2021;19:254-266.  
[PubMed] [Crossref]

65. Vaddepatly RK, Kharel P, Pandey R, Garje R, Chandra AB. Review of indications of fda-approved immune checkpoint inhibitors per NCCN guidelines with the level of evidence. *Cancers (Basel)* 2020;12:738.  
[PubMed] [Crossref]

66. Steuer CE, Ramalingam SS. Advances in immunotherapy and implications for current practice in non-small-cell lung cancer. *JCO Oncol Pract* 2021;17:662-668.  
[PubMed] [Crossref]

67. Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP, Patnaik A, Aggarwal C, Gubens M, Horn L, et al. Pembrolizumab for the treatment of non-small-cell lung cancer. *N Engl J Med* 2015;372:2018-2028.  
[PubMed] [Crossref]

68. Brahmer J, Reckamp KL, Baas P, Crinó L, Eberhardt WE, Poddubskaya E, Antonia S, Polumanski A, Vokes EE, Holgado E, et al. Nivolumab versus docetaxel in advanced squamous-cell non-small-cell lung cancer. *N Engl J Med* 2015;373:123-135.  
[PubMed] [Crossref]

69. Herbst RS, Baas P, Kim DW, Felip E, Pérez-Gracia JL, Han JY, Molina J, Kim JH, Arvis CD, Ahn MJ, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. *Lancet* 2016;387:1540-1550.  
[PubMed] [Crossref]

70. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, Chow LQ, Vokes EE, Felip E, Holgado E, et al. Nivolumab versus docetaxel in advanced nonsquamous non-small-cell lung cancer. *N Engl J Med* 2015;373:1627-1639.  
[PubMed] [Crossref]

71. Rittmeyer A, Barlesi F, Waterkamp D, Park K, Ciardiello F, von Pawel J, Gadgeel SM, Hida T, Kowalski DM, Dols MC, et al. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. *Lancet* 2017;389:255-265.  
[PubMed] [Crossref]

72. Herbst RS, Giaccone G, de Marinis F, Reinmuth N, Vergnenegre A, Barrios CH, Morise M, Felip E, Andric Z, Geater S, et al. Atezolizumab for first-line treatment of pd-li-selected patients with NSCLC. *N Engl J Med* 2020;383:1328-1339.  
[PubMed] [Crossref]

73. Sozinski MA, Jotte RM, Cappuzzo F, Orlandi F, Stroyakovskiy D, Nogami N, Rodriguez-Abrue D, Morosiblot D, Thomas CA, Barlesi F, et al.; IMpower150 Study Group. Atezolizumab for first-line treatment of metastatic nonsquamous nsclc. *N Engl J Med* 2018;378:2288-2301.  
[PubMed] [Crossref]

74. Rodriguez-Abrue D, Powell SE, Hochmair MJ, Gadgeel S, Esteban E, Felip E, Speranza G, De Angelis F, Démine M, Cheng SY, et al. Pemetrexed plus platinum with or without pembrolizumab in patients with previously untreated metastatic nonsquamous NSCLC: protocol-specified final analysis from KEYNOTE-189. *Ann Oncol* 2021;32:881-895.  
[PubMed] [Crossref]
79. Paz-Ares L, Vicente D, Tafreshi A, Robinson A, Soto Parra H, Mazières J, Hermes B, Cicin I, Medgyasszay B, Rodríguez-Cid J, et al. A randomized, placebo-controlled trial of pembrolizumab plus chemotherapy in patients with metastatic squamous NSCLC: Protocol-specified final analysis of keynote-407. J Thorac Oncol 2020;15:1657-1669.

80. Sheth S. Current and emerging therapies for patients with advanced non-small-cell lung cancer. Am J Health Syst Pharm 2010;67 Suppl 1:S9-S14.

81. Soldéra SV, Leighl NB. Update on the treatment of metastatic squamous non-small cell lung cancer in new era of personalized medicine. Front Oncol 2017;7:50.

82. Paz-Ares L, Ciuleanu TE, Cobo M, Schenker M, Zurawski B, Menezes J, Richardet E, Bennouna J, Felip E, Juan-Vidal O, et al. First-line nivolumab plus ipilimumab combined with two cycles of chemotherapy in patients with non-small-cell lung cancer (CheckMate 9LA): an international, randomised, open-label, phase 3 trial. Lancet Oncol 2021;22:198-211.

83. Reck M, Ciuleanu TE, Cobo M, Schenker M, Zurawski B, Janoski de Menezes J, Richardet E, Bennouna J, Felip E, Juan-Vidal O, et al. First-line nivolumab (NIVO) plus ipilimumab (IPi) plus two cycles of chemotheraphy (chemo) versus chemo alone (4 cycles) in patients with advanced non-small cell lung cancer (NSCLC): Two-year update from checkmate 9la. J Clin Oncol 2021;39 Suppl 9:0000.

84. Antonia SJ, Villegas A, Daniel D, Vicente D, Murakami S, Hui R, Yokoi T, Chiappori A, Lee KH, de Wit M, et al. Durvalumab after chemoradiotherapy in stage iii non–small-cell lung cancer. N Engl J Med 2017;377:1919-1929.

85. Spigel DR, Faitre-Finn C, Gray JE, Vicente D, Planchard D, Paz-Ares LG, Vansteenkiste JF, Garassino MC, Hui R, Quantin X, et al. Five-year survival outcomes with durvalumab after chemoradiotherapy in unresectable stage III NSCLC: an update from the pacific trial. J Clin Oncol 2021;39 15 Suppl:8511.

86. Jabbour SK, Lee KH, Frost N, Breder VW, Kowalski DM, Alawin IA, Levchenko E, Reguart N, Martinez-Marti A, Houghton B, et al. Keynote-799: Phase 2 trial of pembrolizumab plus platinum chemotherapy and radiotherapy for unresectable, locally advanced, stage 3 nsclc. J Clin Oncol 2021;39 15 Suppl:8512.

87. Peters S, Felip E, Dafni U, Tufman A, Guckenberger M, Álvarez R, Nadal E, Becker A, Vees H, Pless M, et al. Progression-free and overall survival for concurrent nivolumab with standard concurrent chemoradiotherapy in locally advanced stage IIIA-b NSCLC: Results from the European thoracic oncology platform NICOLAS phase ii trial (European thoracic oncology platform 6-44). J Thorac Oncol 2021;16:278-288.

88. Lin SH, Lin Y, Yao L, Kalhor N, Carter BW, Ahtan M, Blumenschein G, Byers LA, Fossella F, Gibbons DL, et al. Phase ii trial of concurrent atezolizumab with chemoradiation for unresectable NSCLC. J Thorac Oncol 2020;15:248-257.

89. Bai R, Li L, Chen X, Chen N, Song W, Cui J. Neoadjuvant and adjuvant immunotherapy: opening new horizons for patients with early-stage non-small cell lung cancer. Front Oncol 2020;10:575472.

90. Lee YJ, Lee JB, Ha SJ, Kim HR. Clinical perspectives to overcome acquired resistance to anti-programmed death-1 and anti-programmed death ligand-1 therapy in non-small cell lung cancer. Mol Cells 2021;44:363-373.

91. Rodriguez-Abreu D, Johnson ML, Hussein MA, Cobo M, Patel AJ, Szczych S, Massuti B, Hirat S, Yang JC, et al. Primary analysis of a randomized, double-blind, phase II study of the anti-TIGIT antibody tiragolumab (tira) plus atezolizumab (atezo) versus placebo plus atezo as first-line (1L) treatment in patients with PD-L1-selected NSCLC (CITYSCAPE). J Clin Oncol 2020;38 15 Suppl:9503.

92. Pauken KE, Sammons MA, Odorizzi PM, Manne S, Godec J, Khan O, Drake AM, Chen Z, Sen DR, Kurachi M, et al. Epigenetic stability of exhausted T cells limits durability of reinvigoration by PD-1 blockade. Science 2016;354:1160-1165.

93. Sen DR, Kaminiski J, Barnitz RA, Kurachi M, Gerdemann U, Yates KB, Tsao HW, Godec J, LaFleur MW, Brown FD, et al. The epigenetic landscape of T cell exhaustion. Science 2016;354:1165-1169.
94. Turner SJ, Russ BE. Can T cells be too exhausted to fight back? *Science* 2016;354:1104-1105.

95. Ghoneim HE, Fan Y, Moustaki A, Abdelsamed HA, Dash P, Dogra P, Carter R, Awad W, Nesle G, Thomas PG, et al. De novo epigenetic programs inhibit PD-1 blockade-mediated T cell rejuvenation. *Cell* 2017;170:142-157.e119.

96. Im SJ, Hashimoto M, Gerner MY, Lee J, Kissick HT, Burger MC, Shan Q, Hale JS, Lee I, Nasti TH, et al. Defining CD8+ T cells that provide the proliferative burst after PD-1 therapy. *Nature* 2016;537:417-421.

97. He R, Hou S, Liu C, Zhang A, Bai Q, Han M, Yang Y, Wei G, Shen T, Yang X, et al. Follicular CXCR5-expressing CD8(+) T cells curtail chronic viral infection. *Nature* 2016;537:412-428.

98. Utzschneider DT, Charmoy M, Chennupati V, Pousse L, Ferreira DP, Calderon-Copete S, Danilo M, Alfei F, Hofmann M, Wieland D, et al. T cell factor 1-expressing memory-like cd8(+) T cells sustain the immune response to chronic viral infections. *Immunity* 2016;45:415-427.

99. Wu T, Ji Y, Moseman EA, Xu HC, Manglani M, Kirby M, Anderson SM, Handon R, Kenyon E, Elkahloun A, et al. The TCF1-Bcl6 axis counteracts type I interferon to repress exhaustion and maintain T cell stemness. *Sci Immunol* 2016;1:1.

100. Leong YA, Chen Y, Ong HS, Wu D, Man K, Deleage C, Minnich M, Meckiff BJ, Wei Y, Hou Z, et al. CXCR5(+) follicular cytotoxic T cells control viral infection in B cell follicles. *Nat Immunol* 2016;17:1187-1196.

101. Miller BC, Sen DR, Al Abosy R, Bi K, Virkud YV, LaFleur MW, Yates KB, Lako A, Felt K, Naik GS, et al. Subsets of exhausted CD8+ T cells differentially mediate tumor control and respond to checkpoint blockade. *Nat Immunol* 2019;20:326-336.

102. Kurtulus S, Madi A, Escobar G, Klaphozl M, Nyman J, Christian E, Pawlak M, Xiao J, Rozenblatt-Rosen O, et al. Checkpoint blockade immunotherapy induces dynamic changes in PD-1(-)CD8(+) tumor-infiltrating T cells. *Immunity* 2019;50:181-194.e186.

103. Chu F, Li HS, Liu X, Cao J, Ma W, Ma Y, Weng J, Zhu Z, Cheng X, Wang Z, et al. CXCR5(+)CD8(+) T cells are a distinct functional subset with an antitumor activity. *Leukemia* 2019;33:2640-2653.

104. Jansen CS, Prokhnevska N, Master VA, Sandra MG, Carlisle JW, Bilen MA, Cardenas M, Wilkinson S, Lake R, Sowalsky AG, et al. An intra-tumoral niche maintains and differentiates stem-like CD8 T cells. *Nature* 2019;576:465-470.

105. Eberhardt CS, Kissick HT, Patel MR, Cardenas MA, Prokhnevska N, Obeng RC, Nasti TH, Griffith CC, Im SJ, Wang X, et al. Functional HPV-specific PD-1(+)-stem-like CD8(+) T cells in head and neck cancer. *Nature* 2021;597:279-284.

106. Hudson WH, Gensheimer J, Hashimoto M, Wieland A, Valanparambil RM, Li P, Lin IX, Konieczny BT, Im SJ, Freeman GJ, et al. Proliferating transitory T cells with an effector-like transcriptional signature emerge from PD-1(+) stem-like CD8(+) T cells during chronic infection. *Immunity* 2019;51:1043-1058.e1044.

107. Beltra JC, Manne S, Abdel-Hakeem MS, Kurachi M, Giles JR, Chen Z, Casella V, Ngioe SF, Khan O, Huang YJ, et al. Developmental relationships of four exhausted CD8(+) T cell subsets reveals underlying transcriptional and epigenetic landscape control mechanisms. *Immunity* 2020;52:825-841.e828.

108. Im SJ, Ha SJ. Re-defining t-cell exhaustion: subset, function, and regulation. *Immune Netw* 2020;20:e2.

109. Kamphorst AO, Wieland A, Nasti T, Yang S, Zhang R, Barber DL, Konieczny BT, Daugherty CZ, Koenig L, Yu K, et al. Rescue of exhausted CD8 T cells by PD-1-targeted therapies is CD28-dependent. *Science* 2017;355:1423-1427.

110. Hui E, Cheung I, Zhu J, Su X, Taylor MJ, Wallweber HA, Sasmal DK, Huang J, Kim JM, Mellman I, et al. T cell costimulatory receptor CD28 is a primary target for PD-1-mediated inhibition. *Science* 2017;355:1428-1433.
111. Clouthier DL, Ohashi PS. Costimulation, a surprising connection for immunotherapy. *Science* 2017;355:1373-1374.

112. Khan O, Giles JR, McDonald S, Manne S, Ngiov SF, Patel KP, Werner MT, Huang AC, Alexander KA, Wu JE, et al. TOX transcriptionally and epigenetically programs CD8+ T cell exhaustion. *Nature* 2019;571:211-218.

113. Alfei F, Kanoe K, Hofmann M, Wu M, Ghoneim HE, Roelli P, Utschneider DT, von Hoeslum M, Cullen JG, Fan Y, et al. TOX reinforces the phenotype and longevity of exhausted T cells in chronic viral infection. *Nature* 2019;571:265-269.

114. Scott AC, Dündar F, Zumbo P, Chandran SS, Klebanoff CA, Shakiba M, Trivedi P, Menocal L, Appleby H, Camara S, et al. TOX is a critical regulator of tumour-specific T cell differentiation. *Nature* 2019;571:270-274.

115. Yao C, Sun HW, Lacey NE, Ji Y, Moesman EA, Shih HY, Heuston EF, Kirby M, Anderson S, Cheng J, et al. Single-cell RNA-seq reveals TOX as a key regulator of CD8+ T cell persistence in chronic infection. *Nat Immunol* 2019;20:890-901.

116. Seo H, Chen J, Gonzalez-Avalos E, Samaniego-Castruita D, Das A, Wang YH, Lopez-Moyado IF, Georges RO, Zhang W, Onodera A, et al. TOX and TOX2 transcription factors cooperate with NR4A transcription factors to impose CD8+ T cell exhaustion. *Proc Natl Acad Sci U S A* 2019;116:12410-12415.

117. Kim CG, Jang M, Kim Y, Leem G, Kim KH, Lee H, Kim TS, Choi SJ, Kim HD, Han JW, et al. TOX drives CD8+ T cell exhaustion in anti-PD-1-resistant microsatellite stable colorectal cancers. *Sci Immunol* 2019;4:4.

118. Wang X, He Q, Shen H, Xia A, Tian W, Yu W, Sun B. TOX promotes the exhaustion of antitumor CD8+ T cells by preventing PD1 degradation in hepatocellular carcinoma. *J Hepatol* 2019;71:731-741.

119. Blank CU, Haining WN, Held W, Hogan PG, Kallies A, Lugli E, Lynn RC, Philip M, Rao A, Restifo NP, et al. Defining T cell exhaustion. *Nat Rev Immunol* 2019;19:665-674.

120. Kim MJ, Ha SJ. Differential role of PD-1 expressed by various immune and tumor cells in the tumor immune microenvironment: expression, function, therapeutic efficacy, and resistance to cancer immunotherapy. *Front Cell Dev Biol* 2021;9:767466.

121. van der Leun AM, Thommen DS, Schumacher TN. CD8+ T cell states in human cancer: insights from single-cell analysis. *Nat Rev Cancer* 2020;20:218-232.

122. Gordon SR, Maute RL, Duklen BW, Hutter G, George BM, McCracken MN, Gupta R, Tsai JM, Sinha R, Corey D, et al. PD-1 expression by tumour-associated macrophages inhibits phagocytosis and tumour immunity. *Nature* 2017;545:495-499.

123. Park HJ, Kusnadi A, Lee EJ, Kim WW, Cho BC, Lee JI, Seong J, Ha SJ. Tumor-infiltrating regulatory T cells delineated by upregulation of PD-1 and inhibitory receptors. *Cell Immunol* 2012;278:76-83.

124. Kim HR, Park HJ, Son J, Lee JG, Chung KY, Cho NH, Shin HS, Park S, Kim G, In Yoon H, et al. Tumor microenvironment dictates regulatory T cell phenotype: upregulated immune checkpoints reinforce suppressive function. *J Immunother Cancer* 2019;7:339.

125. Son J, Cho JW, Park HJ, Moon J, Park S, Lee H, Lee J, Kim G, Park SM, Lira SA, et al. Tumor-infiltrating regulatory T-cell accumulation in the tumor microenvironment is mediated by IL33/ST2 signaling. *Cancer Immunol Res* 2020;8:1393-1406.
128. Park HJ, Park JS, Jeong YH, Son J, Ban YH, Lee BH, Chen L, Chang J, Chung DH, Choi I, et al. PD-1 upregulated on regulatory T cells during chronic virus infection enhances the suppression of CD8+ T cell immune response via the interaction with PD-L1 expressed on CD8+ T cells. *J Immunol* 2015;194:5801-5811. [PUBMED](https://doi.org/10.4110/in.2022.22.e2) [CROSSREF](https://doi.org/10.4110/in.2022.22.e2)

129. Yoshida K, Okamoto M, Sasaki I, Kuroda C, Ishida H, Ueda K, Ideta H, Kamanaka T, Sobajima A, Takizawa T, et al. Anti-PD-1 antibody decreases tumour-infiltrating regulatory T cells. *BMC Cancer* 2020;20:25. [PUBMED](https://doi.org/10.4110/in.2022.22.e2) [CROSSREF](https://doi.org/10.4110/in.2022.22.e2)

130. Stathopoulou C, Gangaplasra A, Mallett G, Flomerfelt FA, Liniany LP, Knight D, Samsel LA, Berlinguer-Palmini R, Yim JJ, Felizardo TC, et al. PD-1 inhibitory receptor downregulates asparaginyl endopeptidase and maintains foxp3 transcription factor stability in induced regulatory T cells. *Immunity* 2018;49:247-263.e247. [PUBMED](https://doi.org/10.4110/in.2022.22.e2) [CROSSREF](https://doi.org/10.4110/in.2022.22.e2)

131. Leung CS, Yang KY, Li X, Chan VW, Ku M, Waldmann H, Hori S, Tsang JC, Lo YM, Lui KO. Single-cell transcriptomics reveal that PD-1 mediates immune tolerance by regulating proliferation of regulatory T cells. *Genome Med* 2018;10:71. [PUBMED](https://doi.org/10.4110/in.2022.22.e2) [CROSSREF](https://doi.org/10.4110/in.2022.22.e2)

132. Asano T, Meguri Y, Yoshioka T, Kishi Y, Iwamoto M, Nakamura M, Sando Y, Yagita H, Koreth J, Kim HT, et al. PD-1 modulates regulatory T-cell homeostasis during low-dose interleukin-2 therapy. *Blood* 2017;129:2186-2197. [PUBMED](https://doi.org/10.4110/in.2022.22.e2) [CROSSREF](https://doi.org/10.4110/in.2022.22.e2)

133. Kamada T, Togashi Y, Tay C, Ha D, Sasaki A, Nakamura Y, Sato E, Fukuoka S, Tada Y, Tanaka A, et al. PD-1+ regulatory T cells amplified by PD-1 blockade promote hyperprogression of cancer. *Proc Natl Acad Sci U S A* 2019;116:9999-10008. [PUBMED](https://doi.org/10.4110/in.2022.22.e2) [CROSSREF](https://doi.org/10.4110/in.2022.22.e2)

134. Kumagai S, Togashi Y, Kamada T, Sugiyama E, Nishinakamura H, Takeuchi Y, Vitaly K, Itahashi K, Maeda Y, Matsui S, et al. The PD-1 expression balance between effector and regulatory T cells predicts the clinical efficacy of PD-1 blockade therapies. *Nat Immunol* 2020;21:1346-1358. [PUBMED](https://doi.org/10.4110/in.2022.22.e2) [CROSSREF](https://doi.org/10.4110/in.2022.22.e2)

135. Topalian SL, Taube JM, Pardoll DM. Neoadjuvant checkpoint blockade for cancer immunotherapy. *Science* 2020;367:367. [PUBMED](https://doi.org/10.4110/in.2022.22.e2) [CROSSREF](https://doi.org/10.4110/in.2022.22.e2)

136. Philip M, Schietinger A. CD8+ T cell differentiation and dysfunction in cancer. *Nat Rev Immunol* 2021. doi: 10.1038/s41577-021-00574-3. [PUBMED](https://doi.org/10.4110/in.2022.22.e2) [CROSSREF](https://doi.org/10.4110/in.2022.22.e2)

137. Sautès-Fridman C, Petitprez F, Calderaro J, Fridman WH. Tertiary lymphoid structures in the era of cancer immunotherapy. *Nat Rev Cancer* 2019;19:307-325. [PUBMED](https://doi.org/10.4110/in.2022.22.e2) [CROSSREF](https://doi.org/10.4110/in.2022.22.e2)

138. Schumacher TN, Thommen DS. Tertiary lymphoid structures in cancer. *Science* 2022;375:eabf9419. [PUBMED](https://doi.org/10.4110/in.2022.22.e2) [CROSSREF](https://doi.org/10.4110/in.2022.22.e2)

139. Fairfax BP, Taylor CA, Watson RA, Nassiri I, Danielli S, Fang H, Mahé EA, Cooper R, Woodcock V, Traill Z, et al. Peripheral CD8+ T cell characteristics associated with durable responses to immune checkpoint blockade in patients with metastatic melanoma. *Nat Med* 2020;26:193-199. [PUBMED](https://doi.org/10.4110/in.2022.22.e2) [CROSSREF](https://doi.org/10.4110/in.2022.22.e2)

140. Valpione S, Galvani E, Tweedy J, Mundra PA, Banyard A, Middlehurst P, Barry J, Mills S, Salih Z, Weightman J, et al. Immune-awakening revealed by peripheral T cell dynamics after one cycle of immunotherapy. *Nat Can* 2020;1:210-221. [PUBMED](https://doi.org/10.4110/in.2022.22.e2) [CROSSREF](https://doi.org/10.4110/in.2022.22.e2)

141. Wu TD, Madireddi S, de Almeida PE, Banchereau R, Chen YJ, Chitre AS, Chiang EY, Iftikhar H, O’Gorman WE, Au-Yeung A, et al. Peripheral T cell expansion predicts tumour infiltration and clinical response. *Nature* 2020;579:274-278. [PUBMED](https://doi.org/10.4110/in.2022.22.e2) [CROSSREF](https://doi.org/10.4110/in.2022.22.e2)

142. Burger ML, Cruz AM, Crossland GE, Gaglia G, Ritch CC, Blatt SE, Bhukar A, Canner D, Kienka T, Taverna SZ, et al. Antigen dominance hierarchies shape TCF1(+) progenitor CD8+ T cell phenotypes in tumors. *Cell* 2021;184:4996-5014.e4926. [PUBMED](https://doi.org/10.4110/in.2022.22.e2) [CROSSREF](https://doi.org/10.4110/in.2022.22.e2)

143. Connolly KA, Kuchroo V, Venkat A, Khatun A, Wang J, William I, Hornick NI, Fitzgerald BL, Damo M, Kasmani MY, et al. A reservoir of stem-like CD8+ T cells in the tumor-draining lymph node preserves the ongoing antitumor immune response. *Sci Immunol* 2021;6:eabg7836. [PUBMED](https://doi.org/10.4110/in.2022.22.e2) [CROSSREF](https://doi.org/10.4110/in.2022.22.e2)
144. Lucca LE, Axisa PP, Lu B, Harnett B, Jessel S, Zhang L, Raddassi K, Zhang L, Olimo K, Clune J, et al. Circulating clonally expanded T cells reflect functions of tumor-infiltrating T cells. *J Exp Med* 2021;218:218. PUBMED | CROSSREF

145. Schenkel JM, Herbst RH, Canner D, Li A, Hillman M, Shanahan SL, Gibbons G, Smith OC, Kim JY, Westcott P, et al. Conventional type I dendritic cells maintain a reservoir of proliferative tumor-antigen specific TCF-1(+) CD8(+) T cells in tumor-draining lymph nodes. *Immunity* 2021;54:2338-2353.e2336. PUBMED | CROSSREF