

# Programmed Death 1 Immune Checkpoint Inhibitors

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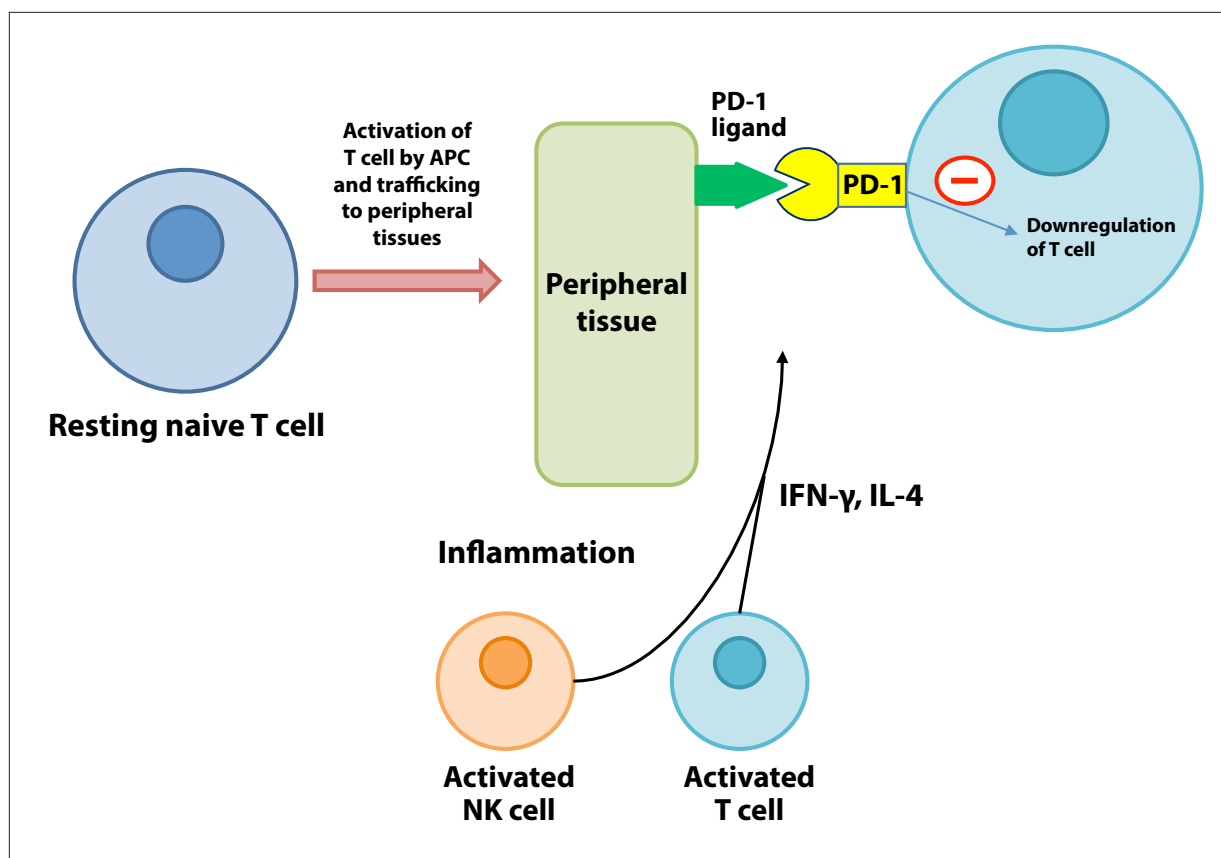
## Keywords

Immune checkpoint inhibitors, nivolumab, PD-1 inhibitors, pembrolizumab

**Abstract:** Programmed death 1 (PD-1) is an immune checkpoint that provides inhibitory signals to the immune system in order to modulate the activity of T cells in peripheral tissues and maintain self-tolerance in the setting of infection and inflammation. In cancer, the immune checkpoints are exploited so that the tumor cells are able to evade the immune system. Immune checkpoint inhibitors are a type of cancer immunotherapy that targets pathways such as PD-1 in order to reinvigorate and enhance the immune response against tumor cells. The US Food and Drug Administration (FDA) has approved 2 PD-1 inhibitors, nivolumab and pembrolizumab, and several others are under investigation. Although PD-1 inhibitors have demonstrated activity in many different types of malignancies, FDA approval has been granted only in melanoma and in non-small cell lung cancer (NSCLC). Identifying biomarkers that can predict response to PD-1 inhibitors is critical to maximizing the benefit of these agents. Future directions for PD-1 inhibitors include investigation of combination therapies, use in malignancies other than melanoma and NSCLC, and refinement of biomarkers.

## Introduction

Immunotherapy in cancer is the use of therapeutic modalities to manipulate the immune system in order to induce a response in tumors. The first use of immunotherapy in cancer is often attributed to William B. Coley, a sarcoma surgeon considered the “father of immunotherapy.”<sup>1</sup> In 1891, after reviewing the literature and finding several cases of the beneficial effects of infections on tumors, he injected streptococcal organisms into a patient with inoperable cancer and achieved an excellent response in the tumor. His treatment of 3 patients with this therapy was published for the first time in 1891 in the *Annals of Surgery*.<sup>1</sup> This finding led to the development of “Coley’s toxins,” bacteria or bacterial products that achieved clinical benefit when injected into patients with various cancers.<sup>1</sup> Decades



**Figure.** In the programmed death 1 (PD-1) pathway, resting naive T cells are activated by antigen-presenting cells and trafficked to peripheral tissues. Following activation, T cells express PD-1. In peripheral tissues where there is inflammation, the activated natural killer cells and T cells release cytokines, such as interferon  $\gamma$  and interleukin 4. This induces the peripheral tissues to express PD-1 ligands (PD-L1 and PD-L2). When PD-1 binds to its ligand, an inhibitory signal is produced and the activity of T cells is downregulated, thus preventing damage to tissue.

APC, antigen-presenting cell; IFN- $\gamma$ , interferon  $\gamma$ ; IL-4, interleukin 4; NK, natural killer; PD-1, programmed death 1.

after these reports, other immunotherapeutic agents and strategies were investigated and developed, including tumor vaccines, adoptive T-cell transfer, cytokine therapy, and monoclonal antibodies. More recently, the effective targeting of immunologic checkpoints has led to dramatic clinical responses in patients with advanced melanoma, lung cancer, and other malignancies.

Immune checkpoints function to maintain self-tolerance and limit collateral tissue damage during the development of immune responses to infections and inflammation.<sup>2,3</sup> These checkpoints, which provide inhibitory signals to the immune system, include the following: cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), B- and T-lymphocyte attenuator (BTLA), lymphocyte activation gene 3 (LAG-3), T-cell immunoglobulin and mucin protein 3 (TIM-3), and programmed death 1 (PD-1).<sup>3</sup> In the development of cancer, dysregulation of the expression of checkpoint proteins allows tumor cells to evade the immune system.<sup>3</sup> Targeting these immune checkpoints to reinvigorate and enhance the

immune response against tumor cells is a valid therapeutic goal. Although many immune checkpoints are under investigation, this review focuses on the PD-1 pathway.

### Programmed Death 1 Pathway

The PD-1 pathway was shown to be an immune checkpoint in 2000.<sup>4</sup> PD-1 functions to modulate the activity of T cells in peripheral tissues and maintain self-tolerance (see the figure).<sup>5</sup> PD-1 is not present on resting naive and memory T cells. It is expressed on the surface of activated T cells, activated B cells, regulatory T (Treg) cells, and natural killer (NK) cells.<sup>2,3</sup> Transcriptional activation is necessary for the expression of PD-1 on the surface of activated T cells, and for this reason, expression is delayed.<sup>2</sup> There are 2 ligands for PD-1: programmed death ligand 1 (PD-L1) and programmed death ligand 2 (PD-L2).<sup>4,6,7</sup> Both ligands interact with other receptors in addition to PD-1. PD-L1, also known as B7-H1 or CD274, is induced by the cytokine

**Table 1.** Anti-PD-1/PD-L1 Antibodies That Have Been Approved by the FDA or Are Under Investigation

Target	Agent	Sponsor	Class	Clinical Testing Phase
PD-1	Nivolumab	Bristol-Myers Squibb	Human IgG4	FDA-approved for treatment of refractory unresectable melanoma and for metastatic NSCLC
	Pembrolizumab	Merck	Humanized IgG4	FDA-approved for treatment of refractory unresectable melanoma and for metastatic NSCLC that expresses PD-1
	CT-011	CureTech	Humanized IgG1k	Phase 1-2
	AMP-224	Amplimmune	PD-L2 IgG2a fusion protein	Phase 1
	MEDI0680 (AMP-514)	Amplimmune	PD-L2 fusion protein	Phase 1-2
	REGN2810	Regeneron	Human IgG4	Phase 1
	PDR001	Novartis	Information not available	Phase 1-2
PD-L1	BMS-936559	Bristol-Myers Squibb	Human IgG4	Phase 1-2
	MEDI4736	MedImmune/AstraZeneca	Humanized IgG1k	Phase 1-3
	MPDL3280A	Roche	Human IgG1k	Phase 1-3
	MSB0010718C	Merck Serono	Human IgG1	Phase 1-3

FDA, US Food and Drug Administration; IgG, immunoglobulin; NSCLC, non-small cell lung cancer; PD-1, programmed death 1; PD-L1, programmed death ligand 1.

interferon  $\gamma$  (IFN- $\gamma$ ), which is produced by some activated T cells and NK cells. After exposure to IFN- $\gamma$ , PD-L1 can be expressed broadly on many cell types, including activated hematopoietic cells and epithelial cells.<sup>2,8</sup> PD-L2, also known as B7-DC or CD273, is primarily induced by interleukin 4 (IL-4) and is expressed on a more limited number of cell types, which include activated dendritic cells and some macrophages.<sup>2</sup> When PD-L1 or PD-L2 engages with PD-1, downregulation of T-cell activity occurs.<sup>4,6</sup> This limits damage to tissues in the setting of immune stimulation, such as in infection.<sup>3</sup> In settings such as chronic infection, in which antigen exposure is ongoing, there can be excessive induction of PD-1 on T cells, resulting in an anergic or exhausted state.<sup>3,9</sup> Another function of the PD-1 pathway is shifting the balance from T-cell activation to tolerance within lymphoid tissues.<sup>3</sup>

Cancer uses the checkpoints to evade immune destruction.<sup>10</sup> In many tumor types, PD-1 is overexpressed on both CD4+ and CD8+ tumor-infiltrating lymphocytes (TILs).<sup>3</sup> Additionally, upregulation of checkpoint ligands commonly occurs in the tumor microenvironment.<sup>10,11</sup> In many solid tumors, such as melanoma, ovarian cancer, non-small cell lung cancer (NSCLC), renal cell carcinoma (RCC), and gastric cancer, PD-L1 expression is upregulated.<sup>12</sup> In fact, PD-L1 expression has been associated with poor prognosis in several of these malignancies, including RCC,<sup>13,14</sup> melanoma,<sup>15,16</sup> esophageal cancer,<sup>17</sup> gastric cancer,<sup>18</sup> and pancreatic cancer.<sup>19</sup> PD-L2 upregulation can be seen in B-cell lymphomas.<sup>3</sup>

There are 2 hypothesized mechanisms of immunologic evasion by the tumor related to the upregulation of PD-1 ligands: innate immune resistance and adaptive resistance. With innate immune resistance, PD-1 ligand overexpression occurs via constitutive oncogenic signaling in the tumor cell. This has been described in glioblastomas through phosphatase and tensin homolog (PTEN) silencing and in lymphomas through constitutive anaplastic lymphoma kinase (ALK) signaling.<sup>20,21</sup> The other mechanism for PD-1 ligand upregulation is through adaptive response, in which PD-1 ligand expression is induced on tumor cells by the secretion of interferons, particularly IFN- $\gamma$ , by TILs. This represents the recognition by the tumor cells of an inflammatory immune microenvironment and their adaptation to evade immune destruction.<sup>2,3,22</sup>

Given that the upregulation of PD-1 on T cells and PD-1 ligands in the tumor microenvironment allows tumor cells to escape immune destruction, it was hypothesized that targeting this interaction could enhance antitumor immune responses. This approach to cancer therapy is novel in 2 ways: (1) it does not directly target tumor cells, but rather T cells, which play a major role in immune response, and (2) it does not activate the immune system to target the tumor cells, but instead eliminates the inhibitory pathways that prevent effective antitumor T-cell responses.<sup>8</sup> Currently, 2 PD-1 inhibitors are approved by the US Food and Drug Administration (FDA), and several other agents targeting PD-1 and PD-L1 are under investigation (Table 1).

**Table 2.** Phase 3 Trials of Programmed Death 1 Inhibitors

Trial	Population	PD-1 Inhibitor	Comparator	N	OS, Median, mo	OS at 1 y, %	PFS, Median, mo	ORR, %	CR Rate, %
CheckMate 066 <sup>32</sup>	Untreated metastatic melanoma without <i>BRAF</i> mutations	Nivolumab 3 mg/kg every 2 wk	Dacarbazine 1000 mg/m <sup>2</sup> every 3 wk	418	Not reached vs 10.8 <sup>a</sup>	72.9 vs 42.1	5.1 vs 2.2	40.0 vs 13.9	7.6 vs 1.0
KEYNOTE-006 <sup>44</sup>	Advanced melanoma with ≤1 prior therapy	Pembrolizumab 10 mg/kg every 2 or 3 wk	Ipilimumab 3 mg/kg every 3 wk	834	Not reached in any groups	74.1 vs 68.4 vs 58.2 <sup>a</sup>	5.5 vs 4.1 vs 2.8 <sup>a</sup>	33.7 vs 32.9 vs 11.9	5.0 vs 6.1 vs 1.4
CheckMate 037 <sup>33</sup>	Advanced melanoma after progression on ipilimumab	Nivolumab 3 mg/kg every 2 wk	Investigator's choice of chemotherapy	405	NR	NR	4.7 vs 4.2	31.7 vs 10.6 <sup>a</sup>	3.3 vs 0
CheckMate 067 <sup>57</sup>	Untreated advanced melanoma	Nivolumab 3 mg/kg every 2 wk OR nivolumab 1 mg/kg every 3 wk plus ipilimumab 3 mg/kg for 4 doses followed by nivolumab 3 mg/g every 2 wk	Ipilimumab 3 mg/kg every 3 wk	945	NR	NR	6.9 vs 11.5 vs 2.9 <sup>a</sup>	43.7 vs 57.6 vs 19.0	8.9 vs 11.5 vs 2.2
CheckMate 017 <sup>37</sup>	Advanced squamous cell NSCLC after progression on first-line chemotherapy	Nivolumab 3 mg/kg every 2 wk	Docetaxel 75 mg/m <sup>2</sup> every 3 wk	272	9.2 vs 6.0 <sup>a</sup>	42.0 vs 24.0	3.5 vs 2.8	20.0 vs 9.0	1.0 vs 0

CR, complete response; NR, not reported; NSCLC, non-small cell lung cancer; ORR, objective response rate; OS, overall survival; PD-1, programmed death 1; PFS, progression-free survival; wk, weeks; y, year.

<sup>a</sup>Primary endpoint(s) of study.

## Programmed Death 1 Inhibitors

The 2 PD-1 inhibitors that have FDA approval are nivolumab (Opdivo, Bristol-Myers Squibb) and pembrolizumab (Keytruda, Merck). The results of the completed phase 3 trials for these agents are summarized in Table 2. It should be noted that with immunotherapy, there are unique considerations with regard to adverse events and response.<sup>23,24</sup> Immune-related adverse events (irAEs) associated with immune checkpoint inhibition consist of autoimmune-like syndromes due to T cell activation. These syndromes include skin-related toxicities, colitis, hepatitis, endocrinopathies, pneumonitis, neuropathies, and others. Symptoms typically can be reversed with

corticosteroids, and guidelines for the management of irAEs have been developed.<sup>25</sup> Symptoms of fevers, chills, and lethargy also can be observed with checkpoint inhibitors.<sup>26</sup> In general, the irAEs caused by PD-1 inhibitors seem to be less severe than those observed with the CTLA-4 inhibitor ipilimumab (Yervoy, Bristol-Myers Squibb; Table 3).

The response to immunotherapies must be assessed differently from the response to cytotoxic therapies. With cytotoxic therapy, increase in tumor size or the appearance of new tumors typically indicates failure of treatment.<sup>27</sup> With immunotherapy, an increase in tumor burden or the development of new lesions is possible, but this can be followed later by tumor regression and eventual disease

**Table 3.** Comparison of the Rates of Immune-Related Adverse Events in Phase 3 Studies of Ipilimumab, Nivolumab, and Pembrolizumab

	<b>Ipilimumab 3 mg/kg every 3 wk for 4 doses, N=131, n (%)<sup>54</sup></b>	<b>Nivolumab 3 mg/kg every 2 wk, N=206, n (%)<sup>32</sup></b>	<b>Pembrolizumab 10 mg/kg every 3 wk, N=277, n (%)<sup>44</sup></b>
Any irAE	80 (61.1)	141 (68.5)	NR
Grade 3/4 irAE	19 (14.5)	12 (5.8)	NR
Any dermatologic irAE	57 (43.5)	77 (37.4)	NR
Rash	25 (19.1)	31 (15.0)	37 (13.4)
Pruritus	32 (24.4)	35 (17.0)	39 (14.1)
Any gastrointestinal irAE	38 (29.0)	35 (17.0)	NR
Diarrhea	36 (27.5)	33 (16.0)	40 (14.4)
Colitis	10 (7.6)	2 (1.0)	10 (3.6)
Any pulmonary irAE	0 (0)	3 (1.5)	NR
Pneumonitis	0 (0)	3 (1.5)	5 (1.8)
Any endocrine irAE	10 (7.6)	15 (7.3)	NR
Hypothyroidism	2 (1.5)	9 (4.4)	24 (8.7)
Hyperthyroidism	0 (0)	7 (3.4)	9 (3.2)
Hypophysitis	2 (1.5)	1 (0.5)	2 (0.7)

irAE, immune-related adverse event; NR, not reported; wk, weeks.

stability or response.<sup>24</sup> For this reason, commonly used response criteria, such as the Response Evaluation Criteria in Solid Tumors (RECIST), may not appropriately characterize the response to immunotherapy. Wolchok and colleagues developed the immune-related response criteria (irRC) in an attempt to capture these delayed immunologic responses and better categorize the response to immunotherapies.<sup>24</sup> The irRC allow the tumor burden to be assessed as a continuous variable over multiple points. Additionally, the criteria may be more clinically meaningful with immunotherapies.<sup>23</sup> At the 2015 annual meeting of the American Society of Clinical Oncology, Wolchok and colleagues presented data on atypical patterns of response in patients with metastatic melanoma treated with pembrolizumab in KEYNOTE-001 (Study of Pembrolizumab [MK-3475] in Participants With Progressive Locally Advanced or Metastatic Carcinoma, Melanoma, or Non–Small Cell Lung Carcinoma). Of 594 patients included in the analysis, 15 (3%) had progression of disease per RECIST but a complete response or partial response per irRC. The overall survival (OS) for these patients was similar to that of the 205 patients who had a complete response or partial response per RECIST.<sup>28</sup>

#### **Data for Single-Agent Nivolumab**

The first in-human trial of nivolumab was published in 2010 by Brahmer and colleagues.<sup>29</sup> This phase 1 trial investigated the use of nivolumab in 39 patients with advanced, treatment-refractory solid tumors, including melanoma (26%), prostate cancer (21%), NSCLC (15%),

RCC (3%), and colorectal cancer (36%). A durable complete response was observed in 1 patient with colorectal cancer, and partial responses were achieved in 2 patients, the first in a patient with melanoma and the other in a patient with RCC. In addition, 1 patient with NSCLC and 1 patient with melanoma achieved significant tumor regression that did not meet partial response criteria. The drug was well tolerated, with only 1 serious adverse event of inflammatory colitis observed.<sup>29</sup>

Following this small pilot study, a larger, multiple-dose phase 1 trial of nivolumab in 296 patients was published by Topalian and colleagues in 2012.<sup>30</sup> The patients had advanced melanoma, NSCLC, RCC, prostate cancer, or colorectal cancer, and the majority were heavily pretreated. The study enrolled 236 patients with evaluable disease. Among these, objective responses were seen in patients with NSCLC (objective response rate [ORR], 18%), melanoma (ORR, 28%), and RCC (ORR, 27%). In this study, no objective responses were observed in patients with colorectal or prostate cancer. Some irAEs were observed, including 3 deaths from pulmonary toxicity.<sup>30</sup> These data were encouraging for further investigation of nivolumab in melanoma, NSCLC, and RCC. Long-term follow-up of a phase 1 expansion cohort of 107 patients with advanced melanoma receiving nivolumab revealed an objective response in 31% of patients.<sup>30</sup> The median duration of response was 2 years. The median OS was 16.8 months, and the 1-year OS rate was 62%, which compared favorably with the results of studies of similar patient populations treated with ipilimumab.<sup>31</sup>

Two phase 3 randomized, controlled trials investigating nivolumab were published in 2015. Robert and colleagues randomly assigned 418 patients with previously untreated advanced melanoma to either nivolumab or dacarbazine in a double-blind trial (CheckMate 066). The 1-year OS rate in the nivolumab group was 72.9%, compared with 42.1% in the dacarbazine group (hazard ratio [HR], 0.42;  $P < .001$ ). Overall, the patients on nivolumab had a high response rate, rapid time to median response, and durable response.<sup>32</sup> In a randomized, controlled, open-label phase 3 trial of nivolumab vs chemotherapy in 405 patients with advanced melanoma who progressed after ipilimumab (CheckMate 037), the interim analysis reported a greater proportion of objective responses in the nivolumab arm than in the chemotherapy arm (31.7% vs 10.6%). Additionally, grade 3 or 4 drug-related adverse events occurred in 9% of patients in the nivolumab arm and in 31% of patients in the chemotherapy arm; however, the rates of serious grade 3 or 4 drug-related adverse events were comparable in the 2 arms (5% in the nivolumab arm vs 9% in the chemotherapy arm).<sup>33</sup> Based upon these data, the FDA approved nivolumab administered at a dose of 3 mg/kg every 2 weeks for use in patients who had unresectable or metastatic melanoma with disease progression on ipilimumab therapy and on a BRAF inhibitor if the tumor was positive for the V600 mutation of the *BRAF* gene. The National Comprehensive Cancer Network (NCCN) panel recommends that given the higher response rate and lower toxicity of nivolumab compared with ipilimumab, nivolumab should be included as an option for first-line treatment in unresectable or metastatic melanoma.<sup>34</sup>

Long-term follow up of an NSCLC expansion cohort of the phase 1 trial of nivolumab<sup>30</sup> in 129 heavily pretreated patients showed a median OS of 9.9 months and 1-year OS rate of 42%. There was a 17% ORR, and the median duration of response was 17 months. Grade 3 or 4 treatment-related adverse events occurred in 14% of patients, and there were 3 treatment-related deaths secondary to pneumonitis.<sup>35</sup> In a phase 2, single-arm trial of nivolumab in 117 patients with advanced, refractory squamous cell NSCLC (CheckMate 063), 14.5% of patients had an objective response, and the median duration of response was not reached. Median progression-free survival (PFS) was 1.9 months, and median OS was 8.2 months. Grade 3 or 4 treatment-related adverse events developed in 17% of the patients.<sup>36</sup>

Ongoing phase 3 trials are investigating the use of nivolumab in patients with previously treated and untreated NSCLC. The results of the CheckMate 017 phase 3 randomized, open-label trial of nivolumab vs docetaxel in 272 patients with previously treated advanced squamous cell NSCLC were recently published

by Brahmer and colleagues.<sup>37</sup> The median OS was 9.2 months in the nivolumab group vs 6.0 months in the docetaxel group (HR, 0.59;  $P < .001$ ), and 1-year OS was 42% in the nivolumab group vs 24% in the docetaxel group. The ORR was higher in the nivolumab group than in the docetaxel group (20% vs 9%,  $P = .008$ ), and the median duration of response was not reached. Treatment-related adverse events with a grade above 3 occurred less frequently in nivolumab-treated patients than in docetaxel-treated patients (7% vs 57%), a finding that was mainly attributable to the hematologic toxicity of docetaxel.<sup>37</sup> In 2015, the FDA approved nivolumab for the treatment of metastatic NSCLC after progression on or following platinum-based chemotherapy. The initial approval was for metastatic squamous cell NSCLC only, but now nivolumab is approved for use in metastatic squamous cell and nonsquamous cell NSCLC).

RCC was another malignancy in which nivolumab showed promise in the initial phase 1 trial. In a dose escalation, cohort expansion phase 1 study, 34 patients who had previously treated advanced RCC were treated with nivolumab. The overall ORR was 29.4%, with a median duration of response of 12.9 months. The median PFS was 7.3 months, and the median OS was 22.4 months. Grade 3 or 4 treatment-related adverse events were seen in 9% of patients.<sup>38</sup> The results of a blinded, randomized, multicenter phase 2 trial of nivolumab in 168 patients with pretreated metastatic RCC were recently published. This trial evaluated nivolumab at 3 doses (0.3, 2, and 10 mg/kg) in order to identify any dose-response relationship and to assess efficacy and safety. The median PFS times were 2.7, 4.0, and 4.2 months for the 0.3-, 2-, and 10-mg/kg arms, respectively, and no dose-response relationship was found. Similarly, there was no dose-response relationship for ORR, with ORRs of 20%, 22%, and 20% in the 0.3-, 2-, and 10-mg/kg arms, respectively. The median OS times were 18.2, 25.5, and 24.7 months for the 0.3-, 2-, and 10-mg/kg arms, respectively. Grade 3 or 4 treatment-related adverse events developed in 11% of patients.<sup>39</sup> A phase 3 trial comparing nivolumab with everolimus in patients with pretreated metastatic RCC (NCT01668784) is ongoing.

#### **Data for Single-Agent Pembrolizumab**

The first in-human phase 1 dose escalation study for pembrolizumab in patients with solid tumors was presented in abstract form in 2012 and published in 2015 (KEYNOTE-001). Patnaik and colleagues treated 30 patients with escalating doses of pembrolizumab. The patients had various advanced cancers, including melanoma ( $n=7$ ) and NSCLC ( $n=6$ ). A complete response with treatment was achieved in 2 patients (the first with Merkel cell carcinoma and the other with melanoma).

Partial responses were observed in 3 patients (all with melanoma), and 15 patients achieved stable disease. There were no grade 3 or 4 treatment-related adverse events.<sup>40</sup>

Given the encouraging response in melanoma, 2 phase 1 expansion cohort studies of the KEYNOTE-001 trial investigating pembrolizumab in advanced melanoma were subsequently published. Hamid and colleagues investigated the safety and antitumor activity of pembrolizumab in 135 patients with advanced or metastatic melanoma, of whom 48 had previously been treated with ipilimumab. The response rate was 38% and did not vary significantly between those patients who had and had not received prior ipilimumab treatment. The responses were durable in a majority of the patients. Grade 3 or 4 treatment-related adverse events occurred in 13% of patients.<sup>41</sup> Another expansion cohort of KEYNOTE-001 was studied by Robert and colleagues to investigate the benefit of pembrolizumab in patients with advanced melanoma who progressed after ipilimumab. The study randomly assigned 173 patients to receive a dose of pembrolizumab of either 2 mg/kg (n=89) or 10 mg/kg (n=84). At both doses, the ORR was 26%. The only grade 3 treatment-related adverse event was fatigue (3%), and there were no grade 4 adverse events.<sup>42</sup>

A phase 2 study randomly assigned 540 patients with ipilimumab-refractory melanoma in a 1:1:1 manner to pembrolizumab at 2 or 10 mg/kg or to investigator choice chemotherapy (ICC). Patients in the chemotherapy group were allowed to cross over to pembrolizumab if their disease had progressed at the 3-month assessment (KEYNOTE-002). The 6-month PFS rate was 34% for pembrolizumab at 2 mg/kg, 38% for pembrolizumab at 10 mg/kg, and 16% for ICC. The HR was 0.57 for the 2-mg/kg dose and 0.50 for the 10-mg/kg dose compared with the ICC group ( $P<.00001$ ). Additionally, the rate of grade 3 or 4 drug-related adverse events was higher in the ICC arm (26%) than in either of the pembrolizumab arms (11% in the 2-mg/kg group and 14% in the 10-mg/kg group).<sup>43</sup>

A phase 3 randomized, controlled trial of pembrolizumab vs ipilimumab in advanced melanoma called KEYNOTE-006 was published by Robert and colleagues. In this study, 834 patients were randomly assigned in a 1:1:1 manner to pembrolizumab 10 mg/kg every 2 or 3 weeks or to ipilimumab. The 6-month PFS rates were 47.3%, 46.4%, and 26.5% for pembrolizumab 10 mg/kg every 2 weeks, pembrolizumab 10 mg/kg every 3 weeks, and ipilimumab, respectively (HR, 0.58;  $P<.001$ ). The 12-month survival rates were 73.1%, 68.4%, and 58.2% for pembrolizumab 10 mg/kg every 2 weeks, pembrolizumab 10 mg/kg every 3 weeks, and ipilimumab, respectively. The majority of responses were durable. There was less toxicity in the pembrolizumab groups than in the ipilimumab group.<sup>44</sup> Based upon these

data, pembrolizumab 2 mg/kg every 3 weeks was given accelerated FDA approval in 2014 for the treatment of patients who had unresectable or metastatic melanoma with disease progression following ipilimumab therapy and a BRAF inhibitor if the tumor was positive for the V600 mutation of the *BRAF* gene. As for nivolumab, the NCCN panel recommends that given the higher response rate and lower toxicity of pembrolizumab compared with ipilimumab, pembrolizumab should be included as an option for the first-line treatment of patients with unresectable or metastatic melanoma.<sup>34</sup>

An expansion cohort of patients with NSCLC from the phase 1 KEYNOTE-001 trial was also studied to investigate the efficacy and safety of pembrolizumab in this disease. Among 495 patients with locally advanced or metastatic NSCLC, the ORR was 19.4%, and the median duration of response was 12.5 months. Median PFS was 3.7 months, and median OS was 12 months. Grade 3 or higher treatment-related adverse events were reported in 9.5% of patients.<sup>45</sup> The phase 2/3 KEYNOTE-010 study comparing pembrolizumab with docetaxel in patients with previously treated NSCLC is ongoing. Pembrolizumab was approved by the FDA for the treatment of advanced NSCLC expressing PD-L1 as tested using the pharmDx companion diagnostic on October 2, 2015.

## Biomarkers of Programmed Death 1 Pathway

Because only a fraction of patients respond to PD-1 inhibitor therapy, the identification of predictors of response is critical. In many of the trials described above, biomarker studies were performed. The biomarkers studied to date include PD-L1 expression, the presence of TILs, mutational load, and neo-epitope antigen burden.

Several studies have investigated the relationship between PD-L1 expression by immunohistochemistry (IHC) and response to PD-1 inhibitors, but the results have been mixed. Brahmer and colleagues analyzed tumor biopsy specimens from 9 of 39 patients and found that tumor cell surface B7-H1 (PD-L1) expression of 5% or higher on IHC correlated with the likelihood of response to nivolumab treatment.<sup>29</sup> The follow-up study by Topalian and colleagues confirmed this finding by staining for PD-L1 on tumor cells in the tumor biopsy specimens of 42 of 296 patients ( $\geq 5\%$  defined as positive).<sup>30</sup> Taube and colleagues<sup>46</sup> used 68 biopsy samples from 41 patients enrolled in the phase 1 nivolumab trial<sup>30</sup> and performed IHC for PD-L1 in both tumor cells and infiltrating immune cells, with expression of 5% or greater considered positive. As in prior studies, expression of PD-L1 in the pretreatment samples of patients with advanced cancer was associated with an objective response to and clinical benefit from nivolumab use. Although there was

**Table 4.** PD-L1 Immunohistochemistry Staining and Objective Complete or Partial Response Rate

Agent	Study	Definition of IHC Positivity	Antibody Clone	ORR in PD-L1–Positive Tumors, n (%)	ORR in PD-L1–Negative Tumors, n (%)
Nivolumab	Brahmer et al <sup>29</sup>	≥5% of tumor cells	5H1	3/4 (75)	0/5 (0)
Nivolumab	Topalian et al <sup>30</sup>	≥5% of tumor cells	5H1	9/25 (36)	0/17 (0)
Nivolumab	Taube et al <sup>46</sup>	≥5% of tumor cells	5H1	9/23 (39)	1/18 (6)
Nivolumab	Robert et al <sup>32</sup>	≥5% of tumor cells	28-8 with automated Dako assay	NR/74 (52.7)	NR/136 (33.1)
Nivolumab	Motzer et al <sup>39</sup>	≥5% of tumor cells	28-8	9/29 (31)	14/78 (18)
Nivolumab	Rizvi et al <sup>36</sup>	≥5% of tumor cells	With automated Dako assay	6/25 (24)	7/51 (14)
Nivolumab	Brahmer et al <sup>37</sup>	≥1% of tumor cells	28-8	11/63 (17)	9/54 (17)
Pembrolizumab	Patnaik et al <sup>40</sup>	>5% of tumor cells	22C3	2/2 (100)	0/12 (0)
Pembrolizumab	Garon et al <sup>45</sup>	≥50% of tumor cells	22C3	33/73 (45.2)	20/131 (15.2)
Pembrolizumab	Robert et al <sup>44</sup>	≥1% of tumor cells	22C3	NR	NR
Ipilimumab and nivolumab	Wolchok et al <sup>47</sup>	≥5% of tumor cells	28-8 with automated Dako assay	<i>Concurrent:</i> 6/13 (46) <i>Sequential:</i> 4/8 (50)	<i>Concurrent:</i> 9/22 (41) <i>Sequential:</i> 1/13 (8)
Ipilimumab and nivolumab	Postow et al <sup>48</sup>	≥5% of tumor cells	With automated Dako assay	<i>Combination:</i> 14/24 (58) <i>Ipilimumab alone:</i> 2/11 (18)	<i>Combination:</i> 31/56 (55) <i>Ipilimumab alone:</i> 1/27 (4)

IHC, immunohistochemistry; NR, not reported; ORR, objective response rate; PD-L1, programmed death ligand 1.

not a significant association between TIL expression of PD-L1 and clinical outcome with nivolumab treatment, the findings suggested that the functional profile of TILs determines PD-L1 expression.<sup>46</sup> In an analysis of PD-L1 expression on IHC of a NSCLC expansion cohort from the KEYNOTE-001 trial, PD-L1 expression in at least 50% of tumor cells correlated with the likelihood of a response to treatment.<sup>45</sup> In the CheckMate 063 trial, more objective responses were seen in PD-L1–positive tumors (24% vs 14%), and 30% of samples with unevaluable PD-L1 expression had an objective response, although the small sample size limits the interpretation of these data.<sup>36</sup> Conversely, in the randomized, placebo-controlled trial of nivolumab vs dacarbazine in advanced melanoma, PD-L1 status alone did not predict the likelihood of a response to treatment.<sup>32</sup> Several other studies also found that PD-L1 staining does not correlate with improved PD-1 inhibitor response.<sup>37,47,48</sup> In the KEYNOTE-001 trial original cohort, IHC studies for PD-L1 were also performed on 14 biopsy samples, but the relationship with PD-L1 staining and response could not be analyzed owing to small sample size.<sup>40</sup> The results of PD-L1 IHC biomarker studies are summarized in Table 4.

The inconsistencies in the IHC studies highlight the challenges of using PD-L1 expression as a biomarker. First, there are technical challenges. Multiple antibodies

are available, including 5H1, 22C3, and 28-8, and different methods are used for IHC staining, resulting in differences in the sensitivity and specificity of the assays.<sup>49</sup> Additionally, there is no standard cutoff for PD-L1 positivity; the values have ranged from 1% to 50% in different studies. Finally, there are several cells within the tumor microenvironment, including the tumor cells themselves, the TILs, the endothelial cells, and the myeloid-derived cells, that can stain with PD-L1 IHC. The location of these staining cells can also vary, from within the tumor to on the leading edge. The implication of PD-L1 IHC in these different cell types and locations is not fully understood. A physiologic challenge of PD-L1 as a biomarker is the inducibility of PD-L1 expression. The dynamic nature of PD-L1 expression in the tumor microenvironment results in this potential predictive biomarker being both context- and timing-dependent.<sup>49</sup>

Another potential biomarker of response to anti-PD-1 therapy is the pretreatment presence of tumor-associated CD8+ T cells. In a study of 46 patients who had advanced melanoma treated with pembrolizumab, the pretreatment biopsy specimens of those patients who experienced a tumor response on therapy had higher CD8+ cell densities at the invasive margin than did the specimens of the patients whose disease progressed on therapy. Over the course of therapy, the biopsy specimens of the group with a tumor



response showed an increase in CD8+ cell density at both the invasive edge and the center of the tumor. This was not seen in the group whose disease progressed on treatment. A predictive model was developed based on this data set to assess the probability of response to anti-PD-1 therapy and accurately predicted the response of 13 of 15 patients in a validation set from a phase 1 study of pembrolizumab in advanced melanoma.<sup>50</sup>

In the investigation of biomarkers for the prototype immune checkpoint blockade agent ipilimumab, high mutational load and neo-epitope signature were found to be associated with clinical benefit.<sup>51</sup> Similar studies have also been done with PD-1 inhibitors and confirmed that a high mutational burden is associated with clinical benefit of PD-1 inhibition. In a study by Rizvi and colleagues, 2 independent cohorts of patients who had NSCLC treated with pembrolizumab were assessed for mutational burden and response to treatment. In the discovery cohort of 16 patients with NSCLC, the patients with a higher mutational burden had improved objective response, durable clinical benefit, and improved PFS, and these findings were confirmed in the validation cohort of 18 patients with NSCLC.<sup>52</sup>

Another biomarker of response to PD-1 inhibitors is mismatch repair (MMR) status. In a study of 41 patients who had progressive metastatic carcinoma treated with pembrolizumab, the association between MMR deficiency and clinical response to treatment was investigated. Of the 41 patients, 32 had colorectal cancer; 11 of these 32 patients had MMR-deficient tumors and 21 had MMR-proficient tumors. Overall, patients in the group with MMR-deficient tumors were found to have more somatic mutations than those in the group with MMR-proficient tumors (mean of 1782 mutations vs mean of 73 mutations,  $P=.007$ ), and the high somatic mutational burden was associated with prolonged PFS (HR, 0.628;  $P=.021$ ) with pembrolizumab treatment. Among the patients who had colorectal cancer treated with pembrolizumab, median PFS and OS were not reached in the cohort with MMR-deficient tumors but were 2.2 and 5.0 months, respectively, in the cohort with MMR-proficient tumors (HR, 0.10 for PFS;  $P<.001$ ; HR, 0.22 for OS;  $P=.05$ ).<sup>53</sup>

## Programmed Death 1 Inhibitors in Combination Therapy

### *Nivolumab and Ipilimumab*

The combination of ipilimumab and nivolumab has been studied in advanced melanoma. Ipilimumab is a CTLA-4 immune checkpoint inhibitor that was approved by the FDA in 2011 as therapy for advanced unresectable melanoma. The approval was based upon several phase 3 trials showing a dramatic improvement in OS in

comparisons with dacarbazine or glycoprotein 100 peptide vaccine.<sup>54,55</sup> Given the complementary roles of PD-1 and CTLA-4 in adaptive immunity and the encouraging results of the combination in preclinical models, a phase 1 study investigating the safety and efficacy of combined therapy in advanced melanoma was performed.<sup>47</sup> Escalating doses of ipilimumab and nivolumab were used to treat 86 patients with unresectable stage III or IV melanoma, either concurrently (n=53) or sequentially (n=33). The ORR was 40% in the concurrent group and 20% in the sequential group. Grade 3 or 4 treatment-related adverse events were seen in 53% of the patients receiving concurrent therapy, including hepatic, gastrointestinal, and renal events. Therapy was discontinued by 21% of the patients owing to treatment-related adverse events. Among the group receiving sequential therapy, 18% of patients had grade 3 or 4 treatment-related adverse events, with elevated lipase the most common event (18%). In the sequential group, 9% discontinued therapy because of treatment-related adverse events.<sup>47</sup> Longer-term follow-up of the patients receiving concurrent therapy revealed a 1-year OS rate of 82%.<sup>56</sup>

Based on these encouraging results, 2 trials evaluating the concurrent administration of ipilimumab and nivolumab against monotherapy were completed. Postow and colleagues compared nivolumab plus ipilimumab with ipilimumab alone in 142 patients who had previously untreated metastatic melanoma. In this phase 2 randomized, double-blind study, the ORRs in patients with *BRAF* wild-type tumors were 61% in the combination therapy arm and 11% in the ipilimumab monotherapy arm. A complete response was seen in 22% of the patients in the combination therapy arm and in no patients in the ipilimumab arm. As in the phase 1 study, there was a high rate (54%) of grade 3 or 4 treatment-related adverse events in the combination arm, but a rate of only 24% in the ipilimumab arm.<sup>48</sup> CheckMate 067, a phase 3 randomized, double-blind trial, was also conducted to compare combination nivolumab plus ipilimumab with nivolumab monotherapy and ipilimumab monotherapy. In this study, 945 patients with untreated, advanced-stage melanoma were randomly assigned to each arm in a 1:1:1 manner and stratified by tumor PD-L1 status, *BRAF* mutation status, and American Joint Committee on Cancer metastatic stage. The 2 primary endpoints of this trial were OS and PFS, with the trial powered to compare combination therapy vs ipilimumab alone. PFS times were 11.5, 2.9, and 6.9 months in the combination arm, ipilimumab arm, and nivolumab arm, respectively. OS data remain immature. Additionally, in patients with PD-L1-negative tumors, the combination of ipilimumab and nivolumab was more effective than either agent administered alone. In

the combination therapy arm, the rate of grade 3 or 4 treatment-related adverse events was 55%, whereas it was 16.3% in the nivolumab monotherapy arm and 27.3% in the ipilimumab monotherapy arm.<sup>57</sup> The increase in treatment-related adverse events with the combination of nivolumab and ipilimumab may be a challenge for the tolerability of this regimen and also raises concerns for the future regarding toxicity with other possible immunologic combination therapies. Based upon the results of the phase 2 trial by Postow and colleagues, the FDA approved the combination of nivolumab and ipilimumab on September 30, 2015 for the treatment of advanced melanoma harboring wild-type *BRAF*.

### Future Directions for Combination Therapy

Other combination therapies are being investigated based on the hypothesis that tumors that may not be immunogenic initially could be treated with a combination therapy in order to create an immunogenic tumor microenvironment that would respond to immune checkpoint therapy.<sup>8,58</sup> The combination of nivolumab and ipilimumab is being investigated in metastatic RCC (NCT02231749), persistent or recurrent ovarian cancer (NCT02498600), and other advanced or metastatic solid tumors (NCT01928394). Additionally, the combination of PD-1 inhibitors with other checkpoint inhibitors, such as lymphocyte activation gene 3 (LAG-3) inhibitor (NCT01968109), indoleamine dioxygenase 1 (IDO1) inhibitor (NCT02178722), and CD27 inhibitor (NCT02335918), and with other immunotherapy agents, such as vaccines (NCT02263508), are being investigated. The combination of PD-1 inhibitors with checkpoint stimulatory agents, such as CD137 agonists (NCT02253992, NCT02179918), is also under investigation.<sup>59</sup> Trials combining checkpoint inhibitors with conventional therapies, such as chemotherapy, radiation, genomically targeted therapies, cytokines, and antiangiogenic therapies, are ongoing.<sup>8</sup>

### Conclusion

Nivolumab and pembrolizumab are the 2 FDA-approved PD-1 immune checkpoint inhibitors that have documented efficacy in melanoma and NSCLC, as well as promising results in other tumor types. Other PD-1 and PD-L1 inhibitors are currently under investigation. Despite the major advances with this immunotherapy, many questions remain, including the role of PD-1 inhibitors in the adjuvant or neoadjuvant setting, the optimal partner therapeutic agents to use in combination with PD-1 inhibitors, and the efficacy of PD-1 inhibitors in cancers other than melanoma and NSCLC. The refinement of biomarkers to select

patients who would most benefit from PD-1 therapy is also a critical field of study. Given the complexity of the regulation of immune checkpoints, it is not likely that a single biomarker will be predictive of response. Rather, there will be a need to assess multiple components within the tumor microenvironment.<sup>8</sup> With numerous ongoing studies of PD-1 inhibitors and the PD-1 pathway, many developments in the use of these immunologic agents are likely to occur in the future.

### Disclosures

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