Pembrolizumab for Treatment of Patients with Advanced or Unresectable Melanoma
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Abstract
From Coley’s toxin to combination immune checkpoint inhibition, strategies to activate the immune system and generate anticancer immunity have been ongoing for well over a century. Over the past decade, the so-called immune checkpoint inhibitors, generally monoclonal antibodies that target key regulators of T-cell activation, emerged as the most effective immune-targeted agents. Pembrolizumab is the first anti-programmed death 1 (PD-1) antibody approved by the FDA for the treatment of metastatic melanoma. With responses seen in 25% to 40% of patients, depending on dose and setting (i.e., before or after ipilimumab), pembrolizumab specifically and anti-PD-1 antibodies generally are revolutionizing the treatment of melanoma. However, in the setting of other recent advances in the field, a number of practical issues are emerging that need to be addressed to optimize the care of patients with melanoma. First, the optimal sequencing of therapy (first-line immunotherapy over molecular targeted therapy, ipilimumab versus pembrolizumab as initial immune checkpoint inhibitor) is unknown and must be evaluated through randomized trials. Second, there is a strong rationale to combine immune checkpoint inhibitors (i.e., anti-PD-1 with ipilimumab) and to combine immune therapies with targeted therapy agents, so determining whether combination therapy is better than direct sequencing is another critical issue that needs to be addressed in carefully carried out studies.

Introduction
In the late 19th century, William Coley began dosing cancer patients with a toxin derived from bacteria in an attempt to promote an immune response against their cancers (1). This early and crude form of immune therapy served as a proof of concept and inspired greater than a century’s worth of investigation. During this time, the pillars of adaptive immune function, namely antigen processing and presentation, antigen recognition, coactivation/anergy, and effector function, were identified and subsequently targeted by various treatments tested in the clinic. One group of molecules, monoclonal antibodies targeting checkpoints of T-cell activation, has recently been developed and demonstrated activity in a number of malignancies, and, to date, has culminated in the regulatory approval of three agents for the treatment of metastatic melanoma.

Melanoma, the fifth and seventh most common malignancy in men and women, respectively, has long been the focus of immunotherapy efforts (2). In the 1960s and 1970s, nonspecific (to cancer) vaccination, typically with Bacillus Calmette–Guerin (BCG), was investigated and shown to be associated with rare responses (3). With the identification and purification of type 1 cytokines, both interferon alpha 2B (IFN alfa2b) and IL2 were proven effective (IFN in the adjuvant setting and IL2 in metastatic setting), with durable response lasting years and even decades seen in 5% to 10% of metastatic melanoma patients treated with high-dose IL2 (4, 5). The standard treatment for metastatic melanoma has changed dramatically over the past 4 years, with the regulatory approval of six therapies, compared with only two agents were approved by the FDA in the preceding 35 years (dacarbazine in 1976, high-dose IL2 in 1998). Specifically, in 2011, the FDA approved ipilimumab, a cytotoxic T-lymphocyte 4 (CTLA-4) inhibitor, based on the results of a randomized phase III trial of a gp100 vaccine, single-agent ipilimumab, or the combination of the vaccine with ipilimumab that demonstrated superiority in overall survival (OS) of ipilimumab (either alone or in combination) compared with single-agent vaccine (6). Soon to follow was the approval of a BRAF inhibitor, vemurafenib, also in 2011, for patients whose tumors harbored a BRAF mutation, and then the BRAF inhibitor dabrafenib and MEK inhibitor trametinib in 2013 for the same patient population (7, 8). In early 2014, the combination of dabrafenib and trametinib was approved for BRAF-mutant melanoma followed by the approval of two inhibitors of the programmed death 1 (PD-1) receptor, pembrolizumab and nivolumab, both in late 2014 (9–11). Immune Checkpoints
The goal of immunotherapy is to harness the power of the human immune system to selectively attack human cancers. Since the time that immune cytokines such as IL2 and interferon alpha were discovered and developed as therapies, the strategy has been to provide a very general stimulus that leads to a systemic inflammatory response that hopefully will trigger tumor antigen recognition, tumor-specific immune activation, and tumor destruction. In the late 1980s and early 1990s, so-called immune checkpoints that regulated T-cell activation were identified and included CTLA-4 and PD-1 and its ligands PD-L1 and PD-L2. These seminal discoveries have ushered in a new generation of
immune therapies that specifically targeted mechanisms of immune regulation that have ultimately translated into more effective therapies for melanoma, as well as a wide range of other malignancies.

**Preclinical Rationale for PD-1/PD-L1 Inhibitors**

Preclinical models have predicted both toxicity and efficacy of immune checkpoint blockade. For example, knockout mice for CTLA-4 develop rapidly progressing and lethal autoimmunity within a few weeks after birth (12). In contrast, PD-1 knockout mice develop normally, although these have splenomegaly and increased levels of certain immunoglobulins, while PD-L1 deficient mice have enhanced immunogenicity and susceptibility to develop triggered autoimmunity (such as the experimental autoimmune encephalomyelitis); neither PD-1 nor PD-L1 knockout mice exhibit the severe endogenous autoimmunity of the CTLA-4 knockout mice (13, 14). Inhibition of immune checkpoints with monoclonal antibodies, however, has been associated with promising activity in murine models of cancer. The initial report of antitumor effects in cancer was with an anti–CTLA-4 antibody that was associated with tumor rejection (15). The therapeutic value of anti–PD-1/PD-L1 antibodies on preclinical models of cancer has also been demonstrated (16). Finally, dual monoclonal inhibition of CTLA-4 and PD-1 has been shown to be superior to single-agent CTLA-4 or PD-1 inhibition, in melanoma models (17).

**Clinical Development of PD-1 Inhibitors**

First-in-class: nivolumab

The first anti–PD-1 antibody to be studied in the clinic was nivolumab, a fully human IgG4 monoclonal antibody that was deemed safe in a dose-exploration phase I trial that evaluated 1 to 5 total doses (18). Remarkably, responses were seen in 3 patients despite so few doses being given. The second trial of nivolumab enrolled nearly 300 patients, including those with melanoma (107 patients), non–small cell carcinoma (76), and renal cell carcinoma (33; ref. 19). Responses were seen in each disease type, including in 31% of the melanoma patients. In addition, in the melanoma cohort, the median OS was 16.8 months and the 2-year survival rate was 43%. Of note, prior anti–CTLA-4 antibody therapy was not allowed. Subsequently, there have been two randomized trials of nivolumab compared with chemotherapy that have been reported, one in the first-line setting in patients without BRAF mutations and another following ipilimumab in all patients and also BRAF-targeted therapy in BRAF-mutant patients (10, 20). In the first-line setting, nivolumab was found to be superior than chemotherapy with respect to response rate, progression-free survival (PFS), and OS (10). Similarly, nivolumab was found to be superior to chemotherapy following ipilimumab (20). This latter dataset was responsible for the FDA approval of single-agent nivolumab in patients with metastatic melanoma following ipilimumab therapy in December 2014.

First approved: pembrolizumab

Pembrolizumab, the second anti–PD-1 antibody to be tested in the clinic, is a highly selective, humanized monoclonal IgG4-kappa isotype antibody against PD-1. The phase I trial was a revelation, the results of which led to the FDA approval of pembrolizumab in patients with metastatic melanoma following ipilimumab therapy in September 2014 (10, 21). The initial report of this trial documented the outcomes of 135 patients with metastatic melanoma and includes patients who had and who had not received prior ipilimumab (21). Two doses were tested, 2 and 10 mg/kg, and doses were given every 2 or 3 weeks indefinitely. The objective response rate was 38%, median PFS was 7 months, and, furthermore, over 70% of patients had some regression of disease (based on the so-called waterfall plot; ref. 21). A later report of this trial described the outcomes of a subgroup of 173 patients with ipilimumab-refractory, metastatic melanoma who were randomized to either 2 or 10 mg/kg given every 3 weeks (10). Responses were seen in 26% of patients (although again over 70% had evidence of tumor regression from baseline). PFS was over 7 months, and 1-year survival appeared to be approximately 60% or greater, although interpretation is challenging because the median follow-up at the time of publication was only 8 months.

**Tolerability of pembrolizumab**

With the introduction of the anti–CTLA-4 antibodies ipilimumab and tremelimumab into the clinic, the concept of immune-related adverse events (irAE) became reality. With ipilimumab, common irAEs include dermatitis, colitis, hepatitis, and hypophysitis, while less common toxicities include nephritis, uveitis, and neuritis (e.g., facial nerve palsies), among others. Severe or life-threatening side effects occur in 20% to 30% of patients, and, in most series, the rate of fatal irAEs is 1% to 2%, most commonly related to colonic perforation in the setting of immune-mediated colitis. Anti–PD-1 antibodies are also associated with irAEs, although at lower rates than anti–CTLA-4 antibodies. Table 1 details the rates of these toxicities in both melanoma patients and patients with other solid tumor malignancies.

**Table 1. Toxicity of pembrolizumab**

<table>
<thead>
<tr>
<th>Adverse event, %</th>
<th>Any (CTCAE grade)</th>
<th>Grade 3/4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatigue</td>
<td>36</td>
<td>2</td>
</tr>
<tr>
<td>Pruritus</td>
<td>24</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Rash</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>16</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Nausea</td>
<td>12</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Vomiting</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Asthenia</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Cough</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Myalgia</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Headache</td>
<td>8</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>8</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Decreased appetite</td>
<td>7</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>7</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Chills</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Pyrexia</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>ALT increased</td>
<td>5</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Pneumonitis</td>
<td>3</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Colitis</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Total</td>
<td>83</td>
<td>12</td>
</tr>
</tbody>
</table>

NOTE: Summary of investigator-determined, related adverse events (in percent) in 411 patients with melanoma enrolled onto the phase I trial of pembrolizumab. Grading was done according to the common terminology criteria for adverse events (CTCAE) v.4.0.
Clinical use of pembrolizumab. The FDA label of pembrolizumab states that patients must have metastatic or unresectable melanoma, previously received ipilimumab, and received BRAF-targeted therapy if their tumors harbor a BRAF<sup>V600</sup> mutation. These limitations represent the first such conditions placed on an approved drug for melanoma, obviously are a sign of the progress made in the treatment of this disease, and are likely to be removed when the first-line trial comparing pembrolizumab with ipilimumab (NCT01866319) is reported in the near future if pembrolizumab is determined to be superior to ipilimumab. Given this changing landscape, it is worth comparing pembrolizumab with ipilimumab and BRAF-targeted therapy with respect to response rates, rapidity of response, survival, and optimal sequencing of therapy.

The central tenets of immunotherapy, particularly when compared with molecularly targeted therapy, are as follows:

1. Responses are uncommon, particularly after targeted therapy failure.
2. Responses take time, occurring after many weeks or months of therapy.
3. Benefit is long lasting.

These are certainly true with high-dose IL2 and also ipilimumab, which is associated with tumor regression in 10% to 20% of patients that often take months to be realized and tend to be durable, highlighted by the over 20% 5-year survival (22-24). However, with pembrolizumab and other anti–PD-1/PD-L1 antibodies, the first two tenets are being challenged and the final tenet is, as yet, unproven.

With response rates in the 25% to 40% range, depending on dose level and whether given before or after ipilimumab, the data with pembrolizumab clearly oppose the traditionally held belief that immunotherapy responses are uncommon (10, 21). Furthermore, the tumor regression rates (based on waterfall plot analysis) exceed 70% and approach those of the BRAF inhibitor vemurafenib (21, 25) (Figure 1). Amazingly, when patients are treated with pembrolizumab, tumor regression is expected. There are fewer data about response rates in the setting of BRAF inhibitor–resistant or –refractory melanoma with pembrolizumab and other anti–PD-1/PD-L1 inhibitors; however, one dramatic response was shown during the initial presentation of the data with MPDL3280A at the American Society of Clinical Oncology (ASCO) in 2013 (26). In addition, Fig. 3 shows the imaging of a patient with BRAF/MEK inhibitor–resistant disease treated with single-agent pembrolizumab, documenting response to therapy.

When faced with initial treatment decision, the rapidity of response is a critical factor in determining which agent to select. For example, BRAF-targeted therapy (either single-agent BRAF inhibitors or the combination of BRAF-MEK inhibitor therapy) is rightly touted as being associated with near-immediate improvement in tumor-associated symptoms. Although this is not always the case, most patients do feel better within a day or two after commencing therapy, and [218F]fluoro-2-deoxy-D-glucose (FDG)-PET imaging within 1 to 2 weeks show dramatic resolution of FDG avidity (27). Additionally, patients with elevated lactate dehydrogenase (LDH; typically those with rapidly growing disease and worst outcomes) were associated with similar early benefit to those who had a normal LDH in the randomized trials of these agents (25). This is quite different from the ipilimumab and IL2 data that show patients with an elevated LDH are less likely to benefit (and in particular respond) to these therapies than those with a normal LDH (6, 28, 29). The data with anti–PD-1 antibodies are less clear, as randomized data are just beginning to be reported, but analysis of the survival curve from the first-line nivolumab versus chemotherapy study is illustrative (30). In this trial of BRAF wild-type patients, the OS was significantly better in patients receiving nivolumab than chemotherapy (HR, 0.42; 95% CI, 0.25–0.73; P < 0.001); and the OS curves were inseparable in the first 3 months, suggesting that there is no benefit to a therapy that offers “quicker responses” such as chemotherapy. With pembrolizumab, responses may be seen earlier than at the time of first assessment (typically 12 months on clinical trials; ref. 21). Figure 2 shows the radiographic improvement of a patient treated with pembrolizumab after rapid progression following two doses of ipilimumab. Notably, the patient’s quality of life was impaired by pulmonary involvement of disease that was causing dyspnea, cough, and fatigue. Within a
week after the commencement of pembrolizumab, the patient noted improvement in respiratory symptoms and by 12-weeks, marked radiographic regression of disease. Although this is one dramatic case, the bottom line is that anti-PD-1 antibodies, such as pembrolizumab, may be associated with rapid responses and are likely to be as good an option as chemotherapy in patients who need a quick response.

Refinement of patient selection
Preliminary evidence supports equal efficacy for PD-1/PD-L1 antibodies in patients with or without BRAF mutations (10). With nivolumab having demonstrated a particularly striking response rate, PFS and OS impact in the first-line setting, that trial was confined to patients who are BRAF wild-type (30). As more evidence emerges for PD-1/PD-L1 antibodies in the first-line setting, we expect that they would be readily considered as a first-line treatment option for patients, regardless of the underlying driver oncogene. Disease burden, the presence of symptoms, and serum LDH will likely remain factors that influence decision-making regarding the use of BRAF/MEK combination therapy or a PD-1/PD-L1 antibody in the first-line setting until such time as predictive biomarkers are better elucidated and incorporated into clinical practice.

In the development of nivolumab, pembrolizumab, and MPDL3280A, the predictive value of PD-L1 expression on melanoma cells and infiltrating lymphocytes (in the case of MPDL3280A) has been investigated (30–32). In each case, it has been possible to identify low thresholds of expression of PD-L1 on tumor cells and/or tumor-infiltrating lymphocytes that largely, but not completely, discriminate between responders and non-responders. Specifically, response rates of 40% to 50% have been observed in biomarker-positive patients versus 10% or less in biomarker-negative patients (30–32). Whether this imperfect negative predictive value is a consequence of the dynamic nature of PD-L1 expression in tumors or the necessity to account for immunogenicity of tumors and robustness of baseline T-cell recognition and homing is uncertain. However, even with the available evidence, clinicians may consider the use of PD-L1

Figure 2.
Pre- (A) and 12-week (B) scans of a 76-year-old man with melanoma treated with pembrolizumab.

Figure 3.
Pre- (A) and 12-week (B) scans of a 67-year-old woman with BRAF-mutant melanoma treated with pembrolizumab after BRAF/MEK inhibitor therapy. Regression of pleural effusions (white arrows) and pericardiac mass (red arrows) is shown. The white dotted arrow indicates that the effusion has resolved.
expression in treatment decision-making when confronted with a patient whose tumor harbors a BRAF mutation, where BRAF/MEK combination therapy may be much more attractive than PD-1/PD-L1 antibody therapy for a biomarker-negative patient.

**Concluding Thoughts**

Pembrolizumab, the first anti–PD-1 antibody approved by the FDA, is associated with unprecedented response rates in melanoma and, along with other anti–PD-1/PD-L1 agent, represents a class of agents that has activity in other diseases formally not considered susceptible to immunotherapy, such as non–small cell lung cancer and bladder cancer. Although a number of unanswer questions regarding patient selection, sequencing, and combinatorial remain, the addition of pembrolizumab to the war chest against melanoma perhaps represents the most important therapeutic advance in this disease to date.

**References**


**Disclosure of Potential Conflicts of Interest**

K.T. Flaherty is a consultant/advisory board member for Merck. No potential conflicts of interest were disclosed by the other author.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): R.J. Sullivan

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K.T. Flaherty

Writing, review, and/or revision of the manuscript: R.J. Sullivan, K.T. Flaherty

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): R.J. Sullivan

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