Therapeutic uses of anti-PD-1 and anti-PD-L1 antibodies

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Abstract

Despite extensive investigation over the past three decades, cancer immunotherapy has produced limited success, with few agents achieving approval by the Food and Drug Administration and even the most effective helping only a minority of patients, primarily with melanoma or renal cancer. In recent years, immune checkpoints that maintain physiologic self-tolerance have been implicated in the down-regulation of anti-tumor immunity. Efforts to restore latent anti-tumor immunity have focused on antibody-based interventions targeting CTL antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1) on T lymphocytes and its principal ligand (PD-L1) on tumor cells. Ipilimumab, an antibody targeting CTLA-4, appears to restore tumor immunity at the priming phase, whereas anti-PD-1/PD-L1 antibodies restore immune function in the tumor microenvironment. Although ipilimumab can produce durable long-term responses in patients with advanced melanoma, it is associated with significant immune-related toxicities. By contrast, antibodies targeting either PD-1 or PD-L1 have produced significant anti-tumor activity with considerably less toxicity. Activity was seen in patients with melanoma and renal cancer, as well as those with non-small-cell lung, bladder and head and neck cancers, tumors not previously felt to be sensitive to immunotherapy. The tolerability of PD-1-pathway blockers and their unique mechanism of action have made them ideal backbones for combination regimen development. Combination approaches involving cytotoxic chemotherapy, anti-angiogenic agents, alternative immune-checkpoint inhibitors, immunostimulatory cytokines and cancer vaccines are currently under clinical investigation. Current efforts focus on registration trials of single agents and combinations in various diseases and disease settings and identifying predictive biomarkers of response.

Keywords: antibody, cancer, immune checkpoint, immunotherapy

Introduction

The field of cancer immunotherapy has made significant strides over the past decade, spurred by the enhanced understanding of the complex interplay between the tumor and the immune system. Though cancer primarily has a genetic etiology, the behavior of cancer cells reflects its ability to exploit the tumor microenvironment, circumvent or harness tissue homeostatic processes and grow, invade and metastasize, thereby leading to its major clinical manifestations. The lack of immunologic control is currently recognized as one of the emerging hallmarks of cancer (1). The cancer immunooediting concept has been proposed as a mechanism by which tumors escape control. The concept involves three phases: elimination (tumor cell eradication), equilibrium (when editing of surviving tumor cells occurs) and escape (when the altered tumor cells progress through the shield of the activated immune response) (2, 3).

When the TCR of a T cell recognizes antigens expressed in the context of the MHC, the immune checkpoint modulates signaling: co-stimulatory molecules such as CD28 on T cells enhance the signal, whereas co-inhibitory molecules suppress it. Recent research has implicated the expression of immunoinhibitory checkpoints such as CTL antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1) as potential mediators of the equilibrium and escape phases of cancer immunooediting described above. These molecules are expressed on activated T cells, but when they bind to ligands either on antigen-presenting cells (CTLA-4 binding to CD80/CD86) or tumor cells (PD-1 binding to PD-L1), they tend to shut down the anti-tumor response. Efforts to use antibodies to target and block these immunoinhibitory interactions have ushered in a new era of immunotherapy.
The anti-CTLA-4 antibody, ipilimumab, has produced durable anti-tumor responses and prolonged survival in patients with advanced melanoma, leading to its Food and Drug Administration (FDA) approval in 2011 (4). While this approach serves as proof of concept for the potential efficacy of checkpoint blockade, ipilimumab, perhaps because of its role in the priming phase of the immune response, appears to also restore immunity against a variety of normal tissues leading to severe immune-related adverse events (irAEs) in a substantial minority of patients. Consequently, more recent efforts have focused on the restoration of anti-tumor immunity selectively within the tumor microenvironment through the use of antibodies that block the PD-1–PD-L1 pathway.

This review describes pre-clinical studies and early clinical trials with the various antibodies targeting the PD-1–PD-L1 pathway in patients with cancer. We describe the anti-tumor activity in patients with a variety of tumor types, the side-effects of the agents and current efforts to develop combination regimens using PD-1 blockade as a backbone and to identify predictive biomarkers of response.

The PD-1 signaling pathway in the immune response

PD-1 is a cell surface receptor that is a member of the CD28 family of T-cell regulators, within the immunoglobulin superfamily of receptors (5). The human PD-1 gene is located at chromosome 9q37, and the full-length PD-1 cDNA encodes a protein with 288 amino acid residues with 60% homology to murine PD-1 (6). It is present on CD4^+CD8^− (double negative) thymocytes during thymic development and is expressed upon activation in mature hematopoietic cells such as T and B cells, NKT cells and monocytes after prolonged antigen exposure (reviewed in ref. 5).

PD-1 is viewed as a co-inhibitory receptor. Through its binding to its two main ligands PD-L1 (B7-H1) or PD-L2 (B7-DC), PD-1 down-regulates T-cell activation that would typically occur with recognition, by the TCR, of tumor antigens expressed in the context of the MHC on tumor cells. The ligation of PD-L1 or PD-L2 to PD-1 inhibits T-cell proliferation and down-regulates expression of the anti-apoptotic molecule Bcl-xL, cytokine expression and the mTOR pathway in immune cells (7, 8). Thus, induction of PD-1 at the time of T-cell activation in peripheral tissues and the tumor microenvironment, in response to chronic inflammation or tumor antigen expression, and its binding to PD-L1 on either tumor cells or antigen-presenting cells functions as an immune checkpoint that serves to curb persistent immune response and thwarts potential injury to normal tissues.

The PD-1 axis in malignancy

Tumors display a wide variety of antigens that can potentially be exploited by harnessing the adaptive immune response. The T-cell response to these antigens can be dysregulated by tumor cells seeking to evade immunologic detection and destruction by hijacking physiological homeostatic immune-checkpoint signaling pathways. In addition to a variety of mechanisms that can create an immunosuppressive microenvironment (e.g. secretion of inhibitory cytokines, presence of regulatory T cells), many tumor types also express PD-L1 (9, 10).

It is hypothesized that binding of the ligand PD-L1 to PD-1 down-regulates effector anti-tumor T-cell activity and facilitates immune evasion. This is supported by the finding of an association between PD-1/PD-L1 expression and poor prognosis in several tumor types including gastric, ovarian, lung and renal carcinomas (10, 11). In human melanoma, tumor-infiltrating T lymphocytes (TILs) predominantly expressed PD-1 and demonstrated impaired effector cytokine production compared with PD-1− TILs and peripheral blood lymphocytes, which may signify a state of T-cell exhaustion (12). PD-L1-expressing melanoma cells tightly co-localize with TILs and detectable IFN-γ, suggesting that the TILs stimulate PD-L1 expression resulting in self-inhibition, thus inducing immune resistance (13). In aggregate, the data support disruption of the PD-1 axis as a valid cancer therapeutic strategy.

Pre-clinical models of PD-1 blockade

Genetically, PD-1-deficient mice develop a strain-specific, delayed-onset, organ-specific autoimmunity with incomplete penetrance, supporting a role of the PD-1 axis in self-tolerance. In vitro studies of PD-1 blockade by PD-1-specific antibody showed augmentation of cytotoxic T-cell responses to melanoma-specific antigens including increased frequencies of IFN-γ-secreting antigen-specific cells (14). In mouse models, PD-1 blockade inhibited hematogenous dissemination of B16 melanoma cells and CT26 colon cancer cells to the liver and lung, respectively, through an effector T-cell mechanism (15). Animal experiments also lend support to the presence of synergy between PD-1 axis inhibition and chemotherapy or anti-CTLA-4 treatment (16). These experiments fostered interest in clinical investigation of agents that block the PD-1 pathway as cancer immunotherapies.

Clinical studies of PD-1 targeting on oncology

The principal method for inhibiting the PD-1 pathway clinically has been through the development of genetically engineered monoclonal antibodies that inhibit either PD-1 or PD-L1 function. A number of these agents have been developed and are being investigated clinically. These are listed in Table 1 and described in more detail below. Combination therapies are described in a separate section.

Nivolumab (MDX-1106, BMS-936558, ONO-4538)

Nivolumab is a fully human IgG4 subtype antibody to human PD-1 with a stabilizing hinge region mutation resistant to exchange of IgG4 molecules (thereby stabilizing the specificity), with demonstrated in vitro activity on T cells specific for tumor antigens. A first-in-human Phase I study of nivolumab was performed in patients with treatment-refractory solid tumors including melanoma, colorectal cancer (CRC), castration-resistant prostate cancer (CRPC), non-small-cell lung cancer (NSCLC) and renal cell carcinoma (RCC) (17). Nivolumab was administered as a single dose ranging from 0.3 to 10 mg kg⁻¹ and objective responses were noted in one patient each with CRC, melanoma and RCC. There were no dose-limiting toxicities (DLTs) observed within 4 weeks of the initial dose; however, one patient developed a grade 3 colitis after repeated dosing. The serum half-life of nivolumab
Table 1. Antibodies that target the PD-1 axis and are undergoing clinical investigation for cancer

<table>
<thead>
<tr>
<th>Target</th>
<th>Antibody</th>
<th>Molecular structure</th>
<th>Clinical development phase</th>
<th>Tumor types in evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD-1</td>
<td>Nivolumab (BMS-936558)</td>
<td>Fully human IgG4</td>
<td>Phase III</td>
<td>Melanoma, RCC, NSCLC, HNSCC</td>
</tr>
<tr>
<td></td>
<td>Pembrolizumab (MK-3475)</td>
<td>Humanized IgG4</td>
<td>Phase III</td>
<td>Melanoma, NSCLC</td>
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<tr>
<td></td>
<td>Pidilizumab (CT-011)</td>
<td>Humanized IgG1κ</td>
<td>Phase II</td>
<td>HEME, melanoma</td>
</tr>
<tr>
<td>PD-L1</td>
<td>BMS-936559</td>
<td>Fully human IgG4</td>
<td>Phase I</td>
<td>Advanced solid tumors</td>
</tr>
<tr>
<td></td>
<td>MPDL3280A</td>
<td>Fully human IgG1</td>
<td>Phase I</td>
<td>Melanoma, RCC, NSCLC</td>
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<tr>
<td></td>
<td>MEDI4736</td>
<td>Fully human IgG1</td>
<td>Phase II</td>
<td>URO</td>
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<td>Phase II</td>
<td>NSCLC</td>
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<td>Advanced solid tumors</td>
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<td></td>
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<td>Merkel cell carcinoma</td>
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HEME, hematologic malignancies; URO, urothelial carcinoma (bladder).

ranged from 12 to 20 days. By flow cytometry, T-cell PD-1 occupancy was 59–81% after 57 days, suggesting a pharmacodynamic effect that persisted well beyond that predicted by the pharmacokinetics. This proof of principle study established that an anti-PD-1 antibody was both tolerable and had demonstrable anti-tumor activity in the dose range studied across several tumor types.

A multi-dose Phase I dose-escalation trial extended the above findings. In this study, 296 patients with the same advanced cancers were given nivolumab at doses from 0.1 to 10 mg kg\(^{-1}\) every 2 weeks for up to 2 years (9). Objective tumor responses were noted in patients with NSCLC (14 of 76 patients), melanoma (26 of 94 patients) and RCC (9 of 33 patients) with responses lasting 1 year or more in 20 of 31 responding patients followed at least that long. There were no tumor responses observed in patients with CRPC or CRC. Common treatment-related side-effects were fatigue, anorexia, nausea, rash and diarrhea. Grade 3 or 4 toxicities were reported in 14% of patients and evident at all dose levels without obvious dose dependency. However, only 15 patients (5%) discontinued treatment for toxicity reasons. IrAEs of special interest were observed including pneumonitis, colitis, hepatitis, hypophysitis and thyroiditis. Responses in patients with NSCLC, perceived to be a non-immunologically governed tumor type, were of special interest.

Long-term efficacy outcomes and safety have been described for a subset of 107 patients with melanoma (18). The median overall survival (OS) was 16.8 months [95% confidence interval (CI): 12.5–31.6 months] with a 2-year survival rate of 43%. Among 33 objective responders, the median duration of response was 2 years. Prolonged disease stability was observed in 12 of 17 (71%) patients who stopped treatment for reasons other than disease progression, suggesting the potential for durable disease control. Most toxicities developed within the first 6 months and there were few late toxicities, suggesting an absence of cumulative toxicity.

Single-agent trials of nivolumab are ongoing or planned across a spectrum of tumor types including lymphomas (NCT02038946, NCT02038933), NSCLC (NCT01721759, NCT02066636), melanoma after progression on anti-CTLA-4 antibody (NCT02156804) and hepatocellular carcinoma (NCT01658878). Finally, ongoing randomized trials comparing nivolumab to standard of care focus on NSCLC in comparison to chemotherapy (NCT02041533, NCT01673867), melanoma in comparison to ipilimumab (NCT01844505) or dacarbazine (NCT01721772) or other chemotherapy (NCT01721746), RCC in comparison to everolimus (NCT01668874) and in head and neck squamous cell cancer (HNSCC) in comparison to either cetuximab, methotrexate or docetaxel (NCT02105636). In July 2014, nivolumab was approved in Japan for use in advanced melanoma.

**Pembrolizumab (MK-3475)**

Pembrolizumab is a humanized monoclonal IgG4 anti-PD-1 antibody consisting of a high-affinity mouse anti-PD-1-derived variable region grafted on to a human IgG4 immunoglobulin molecule with an engineered Fc region for stabilization. Pre-clinical anti-tumor activity was demonstrated in animal models of multiple tumor types. A first-in-human, Phase I dose-escalation study was conducted in patients with advanced refractory malignancies at dose levels 1, 3 and 10 mg kg\(^{-1}\) given intravenously initially and after 4 weeks and then every 2 weeks (19). The maximum observed toxicity was grade 2 pruritus and no drug-related grade 3 or greater AEs were observed. Therefore, the maximum tolerated dose was not reached. The half-life was 13.6–21.7 days and not obviously dose related. Four patients had some tumor regression.

This study was then expanded, with patients receiving pembrolizumab at 10 mg kg\(^{-1}\) every 2 weeks or either 2 or 10 mg kg\(^{-1}\) every 3 weeks in non-randomized cohorts; in total, there were 135 patients with melanoma (20). Enrollment included 48 patients who had received prior ipilimumab but could not have experienced severe irAEs. Though 79% of patients had some AEs, only 13% had severe (grade 3 or 4) drug-related toxicities including skin rash or pruritus, fatigue, diarrhea, abdominal pain and hepatic dysfunction. The highest rate of severe toxicities (23%) was in those receiving the highest dose (10 mg kg\(^{-1}\) every 2 weeks) versus <10% in the less-dose-intensive cohorts. AEs potentially of an autoimmune nature included isolated instances of pneumonitis, kidney injury, hepatitis, diarrhea, hyperthyroidism, hyperthyroidism and adrenal insufficiency.

The overall objective response rate (ORR) based on immune-related response criteria was 38% (44 of 117) with 8 additional patients experiencing unconfirmed responses. A total of 77% had some degree of tumor regression including
8 patients with stable disease for over 24 weeks. The majority of responses were established by the time of the first radiologic assessment at 12 weeks. The median progression-free survival exceeded 7 months. Biopsies of responding tumors showed dense infiltration by CD8+ T cells. Prior ipilimumab exposure did not appear to have an obvious impact on efficacy or toxicity outcomes.

Ongoing trials of pembrolizumab monotherapy are being conducted in patients with advanced solid tumors (NCT01295827), NSCLC (NCT01840579) and hematologic malignancies (NCT01953692). Randomized trials comparing pembrolizumab to standard of care are ongoing in PD-L1+ NSCLC in comparison to combination chemotherapy (NCT02142738) or single-agent docetaxel (NCT01905657) and in ipilimumab-treatment-naive patients with melanoma in comparison to ipilimumab (NCT01866319) and ipilimumab-refractory patients with melanoma in comparison with chemotherapy (NCT01704287). On the basis of the results of the expanded Phase I trial, pembrolizumab was given ‘breakthrough drug’ designation by the FDA and received FDA approval for the treatment of patients with ipilimumab-treated advanced melanoma in September of 2014.

**Pidilizumab (CT-011)**

Pidilizumab is a humanized IgG1κ recombinant anti-PD-1 monoclonal antibody that has demonstrated anti-tumor activity in mouse cancer models. In a first-in-human Phase I dose-escalation study in patients with advanced hematologic cancers, 17 patients were treated with a single dose up to 6 mg kg⁻¹ (21). A maximum tolerated dose was not reached, though patients often had serious leukemia and graft-versus-host disease-related complications. The half-life of pidilizumab ranged from 217 to 410 h. One third of patients had at least stable disease as the best response, with a single complete response (CR) occurring in a patient with follicular lymphoma. Immune activation was detected by the finding of a higher percentage of circulating CD4+ cells in peripheral blood from 24 h to 3 weeks after the initial injection, possibly due to inhibition of T-cell apoptosis.

In a subsequent Phase II trial, pidilizumab was given to 66 patients with non-progressing diffuse large B-cell lymphoma (22). The dose was 1.5 mg kg⁻¹ every 6 weeks for three cycles starting 1–3 months after autologous hematopoietic stem cell transplantation (AH SCT). The study met its primary end-point with 72% of patients being progression free at 16 months which compared favorably to a 52% historical rate for the same investigators in the same patient population. There was no evidence of significant autoimmune toxicity, infusion reactions and no treatment-related deaths. Observed toxicities were typical of patients receiving AH SCT. Consistent with an on-target effect of pidilizumab, treatment was associated with a significant increase in the absolute number of peripheral blood PD-L1+ activated helper T cells (CD4+ CD25+) within 24 h and sustained for over 16 weeks. Although the independent anti-tumor effects of pidilizumab in a post-transplant setting are difficult to gauge in the absence of a control arm, the negligible toxicity and favorable clinical benefit observed was felt to warrant further study.

Pidilizumab was given to 32 patients with heavily pre-treated rituximab-sensitive follicular lymphoma at a dose of 3 mg kg⁻¹ intravenously every 4 weeks for four cycles with up to eight additional infusions for patients with stable disease or responding to therapy (23). Rituximab was given at a standard dose of 375 mg m⁻² intravenously weekly for 4 weeks beginning 17 days after the first dose of pidilizumab. The commonest side-effects were mild anemia and fatigue, and five patients had a respiratory infection. Nineteen patients (66%) achieved an objective response including 15 (52%) with a CR. This activity was felt to exceed the historical control for such patients re-treated with rituximab alone. There were no severe (grade 3 or 4) autoimmune or treatment-related toxicities. Six patients had a delayed response occurring after 4 months. While pidilizumab appeared to exhibit a favorable risk–benefit profile, once again this was difficult to access in the absence of a control arm receiving rituximab alone.

Atkins and colleagues conducted a randomized Phase II trial of pidilizumab in patients with advanced melanoma (24). Approximately, 50 patients each were treated with single-agent pidilizumab at either 1.5 or 6 mg kg⁻¹ intravenously every 2 weeks for up to 1 year. Half the patients at each dose level had received prior ipilimumab. The ORR for all patients was 5.9% (90% CI: 2.3, 12.0), OS at 12 months was 64.5% (90% CI: 55.6, 72.0), without significant differences between strata or dose levels and irrespective of therapies given before study entry or after study withdrawal. Treatment was extremely well tolerated with only four (4%) treatment-related serious AEs. Despite the low tumor response rate, relative to either nivolumab or pembrolizumab, the robust 12-month survival rate was felt to justify further exploration of pidilizumab in patients with melanoma preferably in combination with other immunotherapeutics.

Overall, pidilizumab appears to have a favorable toxicity profile and a suggestion of anti-tumor activity particularly in patients with hematologic malignancies. However, the solid-tumor response rates appear to be less than those reported with the other anti-PD-1 inhibitors, suggesting that it has a potentially different mechanism of action.

**PD-L1-targeting strategies**

Another approach to targeting the PD-1 pathway is through antibodies that bind to and prevent the activity of PD-L1, its principal ligand. It has been hypothesized that PD-L1 targeting may be accompanied by less toxicity in part by modulating the immune response selectively in the tumor microenvironment. However, since tumor cell PD-L2 and possibly other tumor-associated molecules may play a role in tolerizing PD-1-expressing lymphocytes, it is conceivable that the magnitude of the anti-tumor immune response could also be blunted.

**BMS-936559**

BMS-936559 is a fully human IgG4 antibody that inhibits binding of PD-L1 to PD-1 and CD80 (which binds not only PD-L1 but also CTLA-4 and CD28) with high affinity. In a Phase I trial in patients with advanced solid tumors, BMS-936559 was given to 207 patients in doses ranging from 0.3 to 10 mg...
kg\(^{-1}\) given every 2 weeks in 6-week cycles for 2 years (25). A maximum tolerated dose was not reached. Only 9% (19 of 2017) had severe grade 3 or 4 treatment-related AEs. No obvious dose–toxicity relationship could be discerned except for infusion reactions at the highest dose. Immune-related toxicities were mild (grade 1 or 2) and/or manageable. In 160 patients evaluable for efficacy, objective responses were seen at doses of 1 mg kg\(^{-1}\) or higher in melanoma (9 of 52), kidney cancer (2 of 17), NSCLC (5 of 49) and ovarian cancer (1 of 17). Both complete and durable responses beyond 24 weeks were seen. The overall pattern of responses and spectrum of toxicities were similar to PD-1-based immune-checkpoint inhibitors with a suggestion of a less-intense immune response.

**MPDL3280A**

MPDL3280A is a human IgG1 antibody that targets PD-L1. Its Fc component has been engineered to not activate antibody-dependent cell cytotoxicity. In a recently reported Phase I study, a 21% response rate was noted in patients with metastatic melanoma, RCC or NSCLC (26). An additional 19% of patients had stable disease lasting longer than 24 weeks. Treatment was well tolerated with 13% treatment-related grade 3 or 4 toxicities including only 2% that were immune related.

This activity has prompted more extensive studies in combination with other agents (see below) and in patients with other diseases. For example, Powles et al. reported the results of MPDL3280A treatment in patients with heavily pre-treated advanced urothelial cancer of the bladder (27). Of 67 patients treated, 2 patients had a CR and the ORR was 25%. Grade 3 toxicity occurred in only 3% of patients. The PD-L1 status of tumor-infiltrating immune cells but not tumor cells correlated with a response. Circulating biomarker changes were representative of expected pharmacodynamic effects such as increases in circulating IFN-γ, IL-18 and activated CD8\(^{+}\) T cells. On the basis of these results, MPDL3280A was designated a ‘breakthrough therapy’ by the FDA and a large confirmatory Phase II trial in patients with advanced bladder cancer is currently ongoing (NCT02108652).

**MEDI-4736**

MEDI-4736 is a human IgG1 antibody that binds specifically to PD-L1. An ongoing Phase 1 dose-escalation study (NCT01693562) of MEDI-4736 given intravenously every 2 (0.1–10 mg kg\(^{-1}\)) or 3 (15 mg kg\(^{-1}\)) weeks was followed by expansion cohorts in eight solid tumors at 10 mg kg\(^{-1}\) every 2 weeks (28). Patients had received a median of four prior treatments. The maximum tolerated dose was not reached for either dosing schedule. Treatment-related AEs occurred in 34% of patients and were all grade 1 or 2, notably diarrhea, fatigue, rash and vomiting. In 26 patients, 4 partial responses (3 in patients with NSCLC and 1 with melanoma) were observed and 5 additional patients exhibited lesser degrees of tumor shrinkage. The disease control rate at 12 weeks was 46%.

Among 151 patients dosed so far in the expansion cohorts, preliminary clinical activity was additionally seen in head and neck, pancreatic and gastric carcinoma with acceptable tolerability. Tumor shrinkage was reported as early as the first assessment at 6 weeks and among the 13 patients with NSCLC, responses were sustained at 10 or more to 14.9 or more months (29). In the NSCLC expansion cohort, the response rate was 16% in 58 evaluable patients and the disease control rate at 12 weeks was 35% with responses seen in all histologic subtypes as well as in a smaller proportion of PD-L1– tumors. Development of antidrug antibodies was exceedingly rare.

On the basis of the favorable toxicity profile and promising activity in a heavily pre-treated NSCLC population, a global Phase III placebo controlled trial using the 10 mg kg\(^{-1}\) biweekly dose has been launched in Stage III patients who have not progressed following chemoradiation (NCT02125461). The primary outcome measures are overall and progression-free survival.

**Combination therapies**

A variety of approaches for combining PD-1 pathway blockers with other agents have been explored over the past few years in an effort to both improve the efficacy of therapy and/or position the treatment regimen for testing in treatment-naive patients with a variety of cancers. Approaches have included combinations with other checkpoint inhibitors (such as ipilimumab), immunostimulatory cytokines (e.g. IFN-γ), cytotoxic chemotherapy, anti-angiogenic inhibitors and small-molecule molecularly targeted therapies many with promising results (reviewed in ref. 30).

The combined use of nivolumab and ipilimumab was initially studied in patients with advanced melanoma with results first reported in 2013 (31). Fifty-three patients received nivolumab at doses ranging from 0.3 to 10 mg kg\(^{-1}\) intravenously concurrently with ipilimumab at 1–3 mg kg\(^{-1}\) every 3 weeks for four doses followed by nivolumab alone every 3 weeks for four doses. Thereafter, the combination treatment was given every 12 weeks for up to eight doses. The ORR was 40% with a disease control rate of 65% at 24 weeks. Of note, 16 patients had tumor reduction of at least 80% by 12 weeks into therapy and 5 patients had a CR. The side-effects were primarily those observed with ipilimumab (skin rash, diarrhea, liver function test and endocrine abnormalities). Grade 3 or 4 treatment-related AEs occurred in 53% of patients (13% involved elevated lipase which was ultimately not felt to be of clinical significance).

The recommended Phase II schedule was nivolumab 1 mg kg\(^{-1}\) with ipilimumab 3 mg kg\(^{-1}\), with this cohort having an ORR of 53% (9 of 17). A recent update reported that 18 of 22 responses were ongoing and estimated the 2-year survival for the concurrently treated cohorts to be a very impressive 79% (32). An additional cohort of 41 patients treated at the recommended Phase 2 dose was reported to have a 43% ORR, confirming the activity of this combination in patients with advanced melanoma. In addition, this combination was explored in patients with metastatic RCC. The combination of ipilimumab (3 mg kg\(^{-1}\)) plus nivolumab (1 mg kg\(^{-1}\)) and the combination of (ipilimumab 1 mg kg\(^{-1}\) plus nivolumab (3 mg kg\(^{-1}\)) were highly active with response rates of 43 and 48%, respectively (33). This combination is being compared to single-agent ipilimumab or nivolumab in patients with advanced
melanoma (NCT01844505) and sunitinib in patients with advanced RCC.

In a Phase I study in 46 chemotherapy-naive patients with NSCLC, four cohorts of patients received ipilimumab (3mg kg\(^{-1}\)) plus nivolumab for four cycles followed by nivolumab 3mg kg\(^{-1}\) intravenously every 2 weeks (34). In patients with at least 4 months of follow-up, grade 3-4 toxicities occurred in 22 of 46 (48%) and led to discontinuation in 16 patients. Three treatment-related deaths were due to respiratory failure, bronchopulmonary hemorrhage and toxic epidermal necrolysis. The overall ORR was 22% and did not correlate with PD-L1 status. Unexpected excessive toxicities led to an amendment, adding a 1 + 1 mg kg\(^{-1}\) cohort of 30 patients, leaving the safe dose undetermined for further study.

In another Phase I study, 56 patients with advanced NSCLC were assigned based on histology to four cohorts to receive nivolumab (5–10mg kg\(^{-1}\)) intravenously every 3 weeks plus one of four concurrent standard ‘platinum doublet’ chemotherapy regimens (35). No dose de-escalation was required for dose-limiting toxicity but grade 3 or 4 treatment-related AEs were reported in 45% including pneumonitis, fatigue and acute renal failure. The ORR was 33–50% across arms and the 1-year OS rates were promising at 59–87%.

In patients with metastatic RCC who were given nivolumab (dose escalated from 2 to 5mg kg\(^{-1}\) every 3 weeks) in combination with approved doses of the anti-angiogenic agents, sunitinib or pazopanib, there were no DLTs observed in the sunitinib arm (total n = 33) (36). However, four DLTs [elevated ratio of alanine transaminase (ALT) to aspartate transaminase in three; fatigue in one] were observed in the pazopanib arm, leading to its termination. Grade 3- to 4-related AEs were observed in 24 of 33 patients (73%) receiving sunitinib, including elevated ALT, hypertension and hyponatremia and led to therapy discontinuation in 8 of 33 patients (24%). The ORR was 52% (17 of 33) in combination with sunitinib and 45% (9 of 20) with pazopanib, numerically exceeding their known single-agent activity. The progression-free survival was 78% at 24 weeks for sunitinib and 55% for pazopanib. This established the feasibility of treating kidney cancer with a combination of an immune-checkpoint inhibitor and sunitinib. However, given the toxicity and lack of increased CR rates, the value of this combination relative to the administration of agents in sequence remains to be determined.

Other combination regimens being tested include nivolumab in patients with NSCLC with chemotherapy, bevacizumab, erlotinib or ipilimumab (NCT01454102); in advanced colon cancer with ipilimumab (NCT02060188); in chronic myeloid leukemia with dasatinib (NCT02011945); and in advanced solid tumors with IL-21 (NCT01629758). Ongoing combination therapy trials with pembrolizumab include co-administration with cisplatin + pemetrexed or carboplatin + paclitaxel in NSCLC (NCT01840579), pegylated IFN-α2b or ipilimumab in patients with kidney cancer or melanoma (NCT02089685), axitinib in patients with RCC, lenalidomide and dexamethasone in patients with multiple myeloma (NCT02036502) and trametinib and dabrafenib in patients with melanoma (NCT02130466). In addition, MPDL3280A is being studied in combination with bevacizumab in patients with RCC and in combination with cytotoxic chemotherapy in patients with NSCLC.

### Predictive biomarkers

Biomarker development is critical to a personalized-medicine approach through identification of patient subsets that are the main beneficiaries in terms of durable responses or a survival benefit. Tumor-associated PD-L1 expression has been proposed as a potential biomarker for PD-1 pathway expression. Preliminary data suggested a possible tight correlation in the multi-dose Phase I trial with 9 of 25 patients with PD-L1+ tumors compared with 0 of 17 patients with PD-L1- tumors (P = 0.006) exhibiting a response to nivolumab (9). Among 15 patients with advanced NSCLC receiving nivolumab monotherapy, 9 tumors were PD-L1+ with an ORR of 67% with no responses observed in the 6 patients in the PD-L1- group (37).

Although other studies with nivolumab and those with other PD-1 pathway blockers (pembrolizumab and MPDL3280A) showed a trend for enhanced activity favoring PD-L1+ expressing tumors, responses were still seen in patients with PD-L1- tumors. Further, in some tumor types such as RCC, the tumor expression of PD-L1 occurred in such a minority of patients (20–30%) that despite the higher response rate for patients with PD-L1+ tumors, a greater absolute number of patients with PD-L1- RCC exhibited a tumor response (38).

Recent data have further highlighted some of the limitations of using PD-L1 expression as a requirement for patients receiving PD-1-based inhibitors. First off, the assays for PD-L1 expression are difficult to perform, use distinct antibodies, different cutoff points and measure expression on different cells within the tumor microenvironment (See Table 2 for a description of assays). There are currently no data to determine how the various assays compare with each other, if at all.

Further, PD-L1 is an inducible molecule and tumors are frequently heterogeneous. Therefore, with as little as 1% of cell expression being considered positive, it is uncertain how reproducible a particular assay would be even in an individual patient’s tumor. For example, discordance between primary tumor and metastases for PD-L1 positivity in both directions has been observed in kidney cancer in 15% (5 of 34) of patients (40). Finally, PD-L1 expression appears to be

### Table 2. Diversity of PD-L1 immunohistochemistry and clinical outcome in kidney cancers in selected studies

<table>
<thead>
<tr>
<th>Agent (target)</th>
<th>ORR in PD-L1+</th>
<th>ORR in PD-L1-</th>
<th>Antibody</th>
<th>Cell stained</th>
<th>Criteria for positivity</th>
<th>Study (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nivolumab (PD-1)</td>
<td>2 of 4 (50%)</td>
<td>0 of 1 (0%)</td>
<td>Murine SH1</td>
<td>Tumor</td>
<td>≥5% of cells</td>
<td>Topalian et al. 2012 (9)</td>
</tr>
<tr>
<td>MPDL3280 (PD-L1)</td>
<td>2 of 10 (20%)</td>
<td>2 of 21 (10%)</td>
<td>Genentech/Roche</td>
<td>Immune cell</td>
<td>Any cell</td>
<td>Cho et al. 2013 (38)</td>
</tr>
<tr>
<td>Nivolumab (PD-1)</td>
<td>4 of 18 (22%)</td>
<td>3 of 38 (8%)</td>
<td>Dako (28.8)</td>
<td>Tumor</td>
<td>≥5% of cells</td>
<td>Choueiri et al. 2014 (39)</td>
</tr>
</tbody>
</table>
less relevant in combination immunotherapy regimens. For example, the response to the combination of ipilimumab and nivolumab mentioned above appeared to be unrelated to PD-L1 expression.

Considering all of these factors, there is a critical need for standardization of PD-L1 assays before they can be used for selecting patients for PD-1 pathway inhibitor therapy. In the meantime, it has been recommended that PD-L1 expression, preferably in a biopsy of a metastatic lesion proximate to the time of treatment, could be used to stratify patients in randomized trials involving agents that block the PD-1 pathway or to select tumor types for investigation of PD-1 pathway inhibitors. Further, given the mechanism of PD-L1 expression, it is conceivable that some measure of immune infiltration or tumor antigen recognition either by themselves or in combination with PD-L1 expression may ultimately be a better predictor of sensitivity to the PD-1 pathway.

Conclusion
Cancer immunotherapeutics has made a remarkable journey from bench to bedside over the past decade. The discovery and antibody targeting of immune regulatory mechanisms such as the PD-1–PD-L1 pathway has led to clinically meaningful anticancer results. Rapid advances are being made in a broad array of tumor types resulting in the first regulatory approvals for this class of agents in patients with advanced melanoma based on meeting high safety and efficacy standards. Numerous ongoing studies are expected to establish the worth of PD-1 pathway inhibitors in other tumor types as well as in combinations with approved agents.

However, given that only a subset of patients will likely benefit from such treatment, and the likely expense of such therapies, it is imperative to accelerate biomarker development in order to focus treatment on those most likely to benefit and facilitate, through clinical trial enrichment, the approval of these agents in the treatment-naive setting. It is hoped that successes in management of advanced tumors will lead to more effective use of these agents in earlier stage cancers, alone in or in combination with other immunotherapeutic agents or other modalities, such as surgery or radiation, to substantially improve cure rates. Better elucidation of the interface of tumor immune control with other established hallmark biologic processes of cancer will advance our fundamental understanding of cancer development and progression and may ultimately lead to even greater advances in cancer treatment and control.

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References


