Antagonists of PD-1 and PD-L1 in Cancer Treatment

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Abstract

The PD-1 pathway, comprising the immune cell co-receptor Programmed Death 1 (PD-1) and its ligands, PD-L1 (B7-H1) and PD-L2 (B7-DC), mediates local immunosuppression in the tumor microenvironment. Drugs designed to block PD-1 or PD-L1 “release the brakes” on anti-tumor immunity and have demonstrated clinical activity in several types of advanced cancers, validating this pathway as a target for cancer therapy. Two such drugs have recently been approved to treat refractory advanced melanoma, and regulatory approvals in first- and second-line settings for additional cancer types are anticipated. The manageable safety profile of PD-1/PD-L1 blocking drugs identifies them as suitable for outpatient administration and the development of combinatorial therapies. Ongoing studies aim to identify biomarkers to guide patient selection, which would further improve the risk:benefit ratio for these drugs.

INTRODUCTION

The PD-1 pathway includes the inhibitory co-receptor Programmed Death 1 (PD-1) expressed on immune cells such as T, B and NK cells; and its ligands PD-L1 (B7-H1) displayed on cancer and antigen-presenting cells, and PD-L2 (B7-DC) selectively expressed on activated monocytes and dendritic cells. This pathway is a critical mediator of immunosuppression in the local tumor microenvironment (TME). Drugs designed to block PD-1 or PD-L1 “release the brakes” on anti-tumor immunity, enabling endogenous effector
mechanisms. Several different PD-1/PD-L1 blocking antibodies are currently in clinical testing against a wide spectrum of solid and hematologic malignancies. Despite diverse chemical properties (Table 1), each of these drugs has demonstrated anti-tumor activity in the clinic (Table 2), validating the PD-1 pathway as a promising target for cancer therapy.

**MELANOMA**

The annual incidence of melanoma continues to rise worldwide, and despite recent regulatory approvals for ipilimumab and several kinase inhibitors, more effective treatment options for patients with advanced disease are needed. Clinical experience with agents blocking PD-1 and its ligands in melanoma began in 2006 with the first-in-human trial of nivolumab (Opdivo, BMS-936558, MDX-1106, ONO-4538; Bristol-Myers Squibb, Princeton, NJ) involving 39 patients with various advanced, treatment-refractory malignancies. Nivolumab had an acceptable safety profile, and anti-tumor activity was seen not only in patients with melanoma, but also in those with colorectal cancer (CRC) and renal cell carcinoma (RCC), and transiently in non-small-cell lung cancer (NSCLC). Long-term follow-up revealed that tumor regressions were durable. One patient with melanoma achieved a partial response (PR) lasting 16 months after discontinuing nivolumab; at subsequent tumor progression, she was re-treated with nivolumab, resulting in a second PR. Furthermore, one patient each with RCC and CRC remained in complete response (CR) >3 years after completing therapy. Nivolumab was subsequently administered to 107 previously-treated, anti-CTLA-4-naïve patients with melanoma as part of a 306-patient phase I trial with cohort expansion; it was given every 2 weeks for up to 96 weeks. An objective response rate (ORR, PR+CR) of 32% (34/107), evaluated by conventional Response Evaluation Criteria in Solid Tumors (RECIST), was observed. Median response duration was 23 months. Among 21 patients with ORs who discontinued nivolumab for reasons other than progressive disease (PD), 11 (52%) maintained their responses for ≥24 weeks. One-, 2- and 3-year OS rates were 63%, 48% and 41%, respectively, comparing favorably to literature reports of similar patient populations. Fifty-eight patients (54%) experienced a treatment-related immune-mediated adverse event (irAE) of any grade. Of those, only 5 (5%) were grade 3–4.

Several additional studies have tested the efficacy of nivolumab against melanoma. An international phase 3 double-blind trial randomized 418 treatment-naïve patients with BRAF wild type, unresectable stage III-IV melanoma to receive either nivolumab every 2 weeks or dacarbazine chemotherapy every 3 weeks (NCT01721772). The OS rate at 1 year was 73% for patients who received nivolumab and 42% for those who received dacarbazine (P<0.001). Consequently, the trial was unblinded and nivolumab was made available for patients initially enrolled in the dacarbazine group.

Similarly, another phase 3 trial compared nivolumab to dacarbazine or carboplatin/paclitaxel in 405 patients with unresectable or metastatic melanoma, all of whom had previously received ipilimumab, and 18% of whom had previously received a BRAF inhibitor (NCT01721746). Interim analysis revealed an ORR of 32% in the nivolumab group compared to 11% in the chemotherapy group. Thirty-six of 38 (95%) of responses to nivolumab were ongoing at 24 weeks. Grade 3–4 treatment-related AEs were reported in 9%
of patients receiving nivolumab versus 31% of patients who received chemotherapy. Response rates to nivolumab were 44% among patients whose tumors expressed PD-L1 compared with 20% of patients with PD-L1-negative tumors. These data supported the US Food and Drug Administration’s (FDA) 2014 approval of nivolumab as therapy for patients with advanced melanoma refractory to ipilimumab and, for BRAF-mutant tumors, a BRAF inhibitor.

Pembrolizumab (Keytruda, MK-3475, formerly known as lambrolizumab; Merck, Whitehouse Station, NJ) is a distinct anti-PD-1 antibody that was recently FDA-approved in the US for patients with treatment-refractory advanced melanoma. In a phase I study including 135 patients with previously-treated or untreated advanced melanoma, an ORR of 38% was observed across all dose levels (RECIST 1.1). Toxicities were generally tolerable, with grade 3–4 AEs reported in 13% of patients. Subsequently, 173 ipilimumab-refractory patients received pembrolizumab on 2 expansion cohorts in this trial (2 and 10 mg/kg). With a median follow-up of 8 months, toxicity rates were similar to previous reports and the ORR was 26% for both dose levels (21/81 evaluable patients at 2 mg/kg; 20/76 evaluable patients at 10 mg/kg). Based largely on these results, pembrolizumab 2mg/kg every 3 weeks was approved by the FDA in September 2014 for patients with progressive melanoma after ipilimumab and, if BRAF V600 mutation positive, after BRAF inhibitor therapy.

Pembrolizumab was also tested in a phase 2 study which enrolled 540 patients with advanced melanoma whose disease had progressed after BRAF inhibition (if BRAF-mutant) and ipilimumab. Subjects were randomized to receive pembrolizumab at 2mg/kg (n=180) or 10mg/kg (n=181), or investigator’s choice chemotherapy (n=179). ORRs were 21%, 25% and 4%, respectively. The six-month progression-free survival (PFS) rates were 34%, 38% and 16%, respectively (P<0.0001 for pembrolizumab vs. chemotherapy).

Pidelizumab, another anti-PD-1 drug (CT-011, CureTech, Yavne, Israel), was tested in a phase II study of 103 patients with metastatic melanoma. Only 6% of patients demonstrated objective responses, although OS at 12 months was 65%.

Pre-clinical evidence supports therapeutic approaches combining anti-PD-1 with blockade of other immune checkpoints. A phase I study of nivolumab plus ipilimumab was designed to test concurrent or sequential administration in patients with unresectable stage III or IV melanoma. When administered concurrently, nivolumab (1 mg/kg) plus ipilimumab (3 mg/kg) demonstrated ORs in 9 of 17 patients (53%). Despite a 62% rate of grade 3–4 AEs, a phase 3 study is ongoing to compare the efficacy and safety of nivolumab, ipilimumab, or the combination in previously-untreated advanced melanoma (NCT01844505). Additional combinatorial approaches include anti-PD-1 plus cancer vaccines. For example, Weber and colleagues performed a phase I trial of nivolumab with or without a multipeptide vaccine in HLA-A*0201-positive patients with advanced melanoma. The vaccine did not appear to affect the anti-tumor activity or safety profile of nivolumab. A similar trial in the adjuvant setting (surgically resected stage IIIC and IV melanoma) demonstrated a tolerable safety profile and a relapse rate of 30% among 33 patients, with a median follow-up of 32 months. Correlative immunologic phenomena such as increases in circulating vaccine-specific CD8+ T cells were also observed among study participants.
Antibodies blocking PD-L1, the primary ligand for PD-1, are also being tested in patients with melanoma, although there is less experience with this approach compared to anti-PD-1. BMS-936559 (Bristol-Myers Squibb) was administered to 52 evaluable patients with advanced melanoma in a 207-patient phase I trial involving multiple tumor types. The ORR in melanoma was 17%, and prolonged stable disease was seen in 14 additional patients (27%). Overall, a 9% rate of grade 3–4 drug-related toxicities was observed.

MPDL3280A (Genentech/Roche, South San Francisco, CA) was administered to 43 patients with advanced melanoma as part of a 277-patient phase I trial. Objective responses were seen in 30% (13/43) of patients, with 4 additional patients (9%) demonstrating stable disease for ≥24 weeks. Drug-related grade 3–4 AEs occurred in 13%. An ongoing study is combining MPDL3280A with vemurafenib in patients with BRAF V600E melanoma (NCT01656642).

Finally, the PD-L1 antibody MEDI4736 (MedImmune/AstraZeneca, Gaithersburg, MD) has demonstrated preliminary clinical activity and an acceptable safety profile in patients with melanoma. Development of MEDI4736 as monotherapy and in combination regimens for melanoma is underway.

**NON-SMALL-CELL LUNG CANCER**

Unlike melanoma, historically, lung cancer has not been considered to be an immunogenic tumor. Signals of activity seen in early phase studies of agents targeting PD-1 and PD-L1 have been both surprising and exciting, with the potential for durable disease control for the first time in patients with advanced NSCLC. NSCLC is responsible for almost one-third of cancer-related deaths in the US and is the leading cause of cancer mortality for both men and women worldwide. Despite recent progress in targeting specific driver mutations in subgroups of patients, most notably in EGFR and ALK, the majority of patients develops resistance to currently approved chemotherapeutics or molecularly targeted agents within one year of commencing therapy. The median survival for patients with molecularly-unselected, advanced NSCLC ranges from 10 to 12 months.

The first anti-PD-1 drug to be tested in NSCLC was nivolumab. The first-in-human trial of this drug in 39 patients with advanced treatment-refractory cancers included 6 patients with NSCLC, one of whom had a transient response to therapy which did not meet RECIST criteria for a PR. Nevertheless, this preliminary signal of activity prompted further exploration in a multicenter phase Ib study of nivolumab, which enrolled 129 patients with advanced NSCLC. All patients were pre-treated, and 54% had received ≥3 prior lines of prior systemic therapy. Interim results for the response and safety profile of nivolumab in NSCLC were published in 2012, and were updated and expanded with long-term survival data in 2014. Patients received nivolumab (1, 3 or 10 mg/kg) every 2 weeks for up to 96 weeks. The ORR among 129 NSCLC patients was 17% (3%, 24%, and 20% for the 1, 3 and 10 mg/kg dose levels, respectively). The ORR was similar across histologic subtypes (squamous cell lung cancer 17%; non-squamous, 18%) and in EGFR-mutant, KRAS-mutant or wild type NSCLC. Responses were durable, with a median of 74 weeks (19 months). In addition to patients with traditional response patterns by RECIST, six patients had durable
immune-related responses that were not included in ORR calculations, while 10% of patients had stable disease lasting ≥6 months. The median OS for NSCLC patients was 9.9 months and did not vary significantly by histologic subtype. In the Phase I trial, drug-related grade 3–4 AEs occurred in 14% of patients primarily related to immune activation, and were generally manageable. However, 3% (n=4) of patients had grade 3–5 pneumonitis, and 3 of these patients expired. Specific treatment algorithms have been developed for the management of immune-related toxicities in patients receiving anti-PD-1/PD-L1 therapy.24

On the heels of these promising early results, further development of nivolumab for patients with advanced NSCLC has progressed rapidly. Enrollment has been completed to two large randomized studies of nivolumab versus docetaxel chemotherapy in the second-line treatment of patients with advanced squamous and non-squamous tumors (NCT01642004, NCT01673867). Furthermore, a large phase 1 study combining nivolumab with several different agents known to be active in NSCLC is ongoing (NCT01454102). Preliminary results from one arm of this study combining nivolumab (5 or 10 mg/kg every 3 weeks) concurrently with first-line platinum doublet chemotherapy (cisplatin/pemetrexed, carboplatin/paclitaxel, or cisplatin/gemcitabine), followed by maintenance nivolumab until disease progression, were presented in 2014.25 At the time of that report, 56 patients had been treated, and grade 3–4 AEs were reported in 45% of patients, the most common being pneumonitis (4 patients), fatigue and acute renal failure (3 patients each). The ORR across the treatment arms ranged from 33–47%, and one-year OS rates varied from 50–87%.

Data on the first-line use of nivolumab monotherapy in patients with advanced NSCLC have also recently been reported.26 Among 20 patients receiving nivolumab at 3 mg/kg every 2 weeks, the ORR was 30% and the median PFS was 36 weeks at first analysis. Treatment was well tolerated overall, with 20% of patients experiencing grade 3–4 toxicities, all of which resolved with appropriate management. This efficacy signal is encouraging and similar to or better than that expected with chemotherapy in the first-line setting. Efficacy in treatment-naïve patients also appears to be greater than that seen with nivolumab monotherapy in pre-treated patients with NSCLC, a finding which is being further evaluated in ongoing studies. In the first-line setting, accrual has recently commenced to a phase 3 study of nivolumab vs. investigator’s choice chemotherapy for patients with NSCLC whose tumors express the PD-L1 protein by immunohistochemistry (IHC, see Biomarkers section for details) (NCT02041533).

Testing of pembrolizumab anti-PD-1 therapy in patients with NSCLC has focused on patients whose pre-treatment tumor specimens contain at least 1% PD-L1+ cells. Data were recently reported from a large study of pembrolizumab monotherapy (2 mg/kg every 3 weeks, or 10 mg/kg every 2 or 3 weeks until disease progression) in patients with PD-L1+ advanced NSCLC.27 Seventy-eight percent of screened patients (57/73) had PD-L1+ tumors, including 45 patients with measurable disease who were enrolled on the study. Treatment was well tolerated with only 3/45 patients experiencing grade 3–4 AEs (one each with elevated creatine phosphokinase, pericardial effusion and pneumonitis). The ORR by RECIST v1.1 (central review) for this cohort was 26%, while the immune-related criteria response rate was 47% (investigator review). The median duration of response was not yet reached at a median follow-up of 36 weeks.
Pembrolizumab (10 mg/kg every 2 or 3 weeks) has also demonstrated activity as a single agent in the second- and subsequent-line advanced NSCLC setting, with a 20% ORR among 194 patients not selected by PD-L1 status. Among patients with PD-L1+ tumors the ORR was 23%, while among patients with PD-L1(−) tumors it was 9%. Treatment was generally well tolerated with 10% of patients experiencing grade 3–5 toxicities. Randomized phase 3 studies of pembrolizumab monotherapy versus standard chemotherapy are planned or ongoing in the first- and second-line settings for patients with PD-L1+ NSCLC (NCT02220894, NCT01905657).

Three anti-PD-L1 drugs have also demonstrated activity: BMS-936559, MEDI4736, and MPDL3280A in patients with NSCLC. With BMS-936559, an ORR of 10% among 49 patients treated in the second-line or higher setting was reported, although this drug is not currently being further developed in NSCLC. For MEDI4736, approximately 450 patients with advanced solid tumors have been treated with monotherapy or combination therapies to date, including 155 patients with NSCLC. Grade 3–4 toxicities occurred in 4% of these patients, with arthralgia being the most common (1%). The ORR in 58 evaluable patients with NSCLC was 16%, with a higher ORR noted in patients with PD-L1+ tumors (25%, vs. 3% in PD-L1 negative). The disease control rate (DCR, OR + stable disease ≥12 weeks) was 35% overall (45% in PD-L1+, 24% PD-L1 negative patients). Finally, MPDL3280A was administered to 53 heavily pre-treated patients with NSCLC from a total of 277 patients with advanced solid tumors who enrolled in a phase I study. Patients received doses of up to 20 mg/kg with no MTD or dose-limiting toxicities reported; there were no cases of grade ≥3 pneumonitis. Low-grade pyrexia was reported in 21% of all patients but was uncommon after the first cycle. The ORR among the NSCLC patients was 21%. The authors reported a trend towards a higher ORR in former/current smokers versus non-smokers (42% versus 10%; P=0.4229) which requires elucidation in a larger cohort. PD-L1 expression on tumor-infiltrating lymphocytes (TILs), assessed by IHC, appeared to predict response: the ORR was 83% in a small number of patients with high level PD-L1 expression, while it was 14–20% among those with lower or absent expression. Interestingly, in this study, tumor cell PD-L1 expression did not correlate with treatment response. Responses were durable, with a 24-week PFS rate of 45%. Studies of MPDL3280A and MEDI4736 in NSCLC are ongoing.

UROLOGIC MALIGNANCIES

Renal cell carcinoma

Approximately 64,000 new cases and 14,000 deaths from kidney cancer are expected each year in the US. The main systemic treatment options for patients with advanced kidney cancer, in particular clear-cell renal cell carcinoma (ccRCC), include inhibitors of the vascular endothelial growth factor (VEGF) and mammalian target of rapamycin (mTOR) pathways. While these drugs have had a significant impact on ccRCC management, durable disease control is rare. Only high-dose interleukin-2 induces durable disease control in a small proportion of patients, but toxicity limits its application.

Most of the clinical experience with PD-1 pathway inhibition in ccRCC stems from trials of nivolumab. In a phase 1 study, 34 previously-treated patients with metastatic RCC (mRCC) received nivolumab (1 or 10 mg/kg every 2 weeks) for up to two years, producing an ORR
Fifty percent of patients were alive at 2 years. Given its encouraging activity and acceptable tolerability, a phase 2 dose-ranging nivolumab trial was initiated in patients with clear cell mRCC who had previously received agents targeting the VEGF pathway. All 168 patients had received prior systemic therapies including VEGF receptor (VEGFR) TKIs (98%), mTOR inhibitors (38%) and immunotherapy (24%), and 70% had received ≥2 therapies. At interim analysis, 21% (35/168) of patients had an OR, and 54% of responses lasted ≥1 year. The ORR was similar across the three nivolumab doses tested (0.3, 2 and 10 mg/kg). Median PFS was 2.7, 4.0 and 4.2 months, and median OS was 18.2, 25.5 and 24.7 months for the 0.3, 2 and 10 mg/kg dose cohorts, respectively. Grade 3–4 treatment-related AEs occurred in 11% of patients, and no drug-related deaths or evidence of high-grade pneumonitis were observed. An ongoing phase 1 trial is evaluating pharmacodynamic and biologic properties of nivolumab in patients with mRCC (NCT01358721). In an interim analysis, the ORR in patients with or without prior treatment was 17% (15/90), and the PFS rate at 24 weeks was 36%.

The PD-L1 blocking antibody MPDL3280A was tested in 56 patients with advanced RCC, administered every 3 weeks at doses of 10, 15, or 20 mg/kg for up to 1 year. Eighty-seven percent of patients had ccRCC, 7% had papillary, and 4% had sarcomatoid histologies. Grade 3–4 treatment-related AEs were reported in 13% of patients, although no dose-limiting toxicities or pneumonitis was observed. Among 47 evaluable patients, the ORR was 13% (20% in PD-L1+, 10% in PD-L1 negative patients).

To potentially increase the efficacy of PD-1 pathway blockade in mRCC, combinations with inhibitors of the VEGF pathway are being pursued. In a phase 1 trial evaluating the safety and tolerability of nivolumab plus either-sunitinib or pazopanib in treatment-naïve or previously-treated patients (NCT01472081), nivolumab was initiated at 2 mg/kg every 3 weeks with planned escalation to 5 mg/kg; sunitinib was administered in a four-weeks-on, two-weeks-off schedule; and pazopanib was administered daily. In an interim report, both combinations showed evidence of antitumor activity (ORR 52% with sunitinib and 45% with pazopanib). However, grade 3–4 treatment-related AEs were observed in 82% (27/33) of patients receiving nivolumab/sunitinib and in 70% (14/20) receiving nivolumab/pazopanib. The most common treatment-related grade 3–4 AEs included hypertension and elevated ALT (18% each), and hyponatremia and increased lymphocyte count (15% each) with nivolumab/sunitinib; and diarrhea and elevated ALT/AST (20% each) and fatigue (15%) with nivolumab/pazopanib. Grade 3–4 treatment-related AEs led to therapy discontinuation in 36% and 25% of patients receiving the sunitinib and pazopanib combination regimens with nivolumab, respectively. Studies combining pembrolizumab with either pazopanib or axitinib are also ongoing (NCT02014636, NCT02133742).

Finally, results from a phase 1 trial evaluating the combination of nivolumab with ipilimumab in patients with mRCC were recently presented (NCT01472081). Most patients (80%; 35/44) had had prior systemic therapy. Grade 3–4 treatment-related AEs occurred in 45% of patients, including elevated lipase (20%), ALT (14%), AST (7%) and amylase (5%), and diarrhea (9%). In the nivolumab 1 mg/kg plus ipilimumab 3 mg/kg arm, 26% (6/23) of patients discontinued treatment due to AEs, versus 10% (2/21) in the nivolumab 3 mg/kg plus ipilimumab 1 mg/kg arm. ORR ranged from 43–48% among the
different cohorts. The majority of responses (80%; 16/20) were ongoing at the time of analysis. While early analysis of the anti-tumor effects of this combination immunotherapy have led to further development in patients with RCC, efforts to improve its safety profile in this patient population also appear to be indicated.

**Bladder Cancer**

Approximately 75,000 newly diagnosed cases and 15,000 deaths from bladder cancer occur in the US annually. The mainstay of therapy for metastatic disease is palliative cisplatin-based chemotherapy. Response rates to second line therapy are generally poor, in the 5–15% range. The PD-L1 targeting antibody MPDL3280A was administered at 15 mg/kg every 3 weeks for up to one year in a phase I study of 68 pre-treated bladder cancer patients. It was generally well tolerated, with only 4% grade 3–4 toxicities and no high-grade pneumonitis. Among 67 evaluable patients, the ORR was 26% at 6 weeks. Thirty patients whose tumors expressed PD-L1 had a response rate of 43%, versus 11% in 35 PD-L1 negative patients. Based on these findings, in 2014 the FDA granted Breakthrough designation for the clinical development of MPDL3280A in advanced bladder cancer.

Recently reported results from the bladder cancer cohort of a phase 1b trial of pembrolizumab in patients with PD-L1+ advanced solid tumors (NCT01848834) support the further development of PD-1 pathway blockade in this malignancy. PD-L1 expression in stromal cells or ≥1% of tumor cells was required for study entry. Patients received pembrolizumab 10 mg/kg every 2 weeks. ORR by central review was 24% (7/29 evaluable patients), with 3 (10%) complete responses. Responses were durable, ranging from 16–40+ weeks, with most responses ongoing at the time of analysis.

**Prostate Cancer**

More than 233,000 new cases of prostate cancer and almost 30,000 related deaths are expected in the US annually. Patients with metastatic prostate cancer have been shown to benefit from a dendritic cell vaccine; however immunotherapies with checkpoint inhibitors have so far failed to produce significant results. In a phase 1 study with the PD-1 inhibitor nivolumab, no ORs were observed in 17 patients with castrate resistant prostate cancer, thus discouraging further investigations of anti-PD-1 monotherapy in this patient population. It is possible that combination therapies will be needed to overcome immune resistance in prostate cancer.

**GYNECOLOGIC AND BREAST CANCERS**

**Gynecologic Cancers**

Ovarian cancer has long been recognized as an immunogenic tumor. TIL-rich and TIL-poor ovarian cancers have 5-year survival rates of 38% and 4.5%, respectively. High PD-L1 expression by ovarian cancer cells is inversely associated with numbers of intraepithelial CD8+ TILs and is associated with a poor prognosis. Nivolumab was tested in 15 patients with relapsed platinum-resistant ovarian cancer, with a 23% ORR and a 54% DCR (CR + PR + stable disease). A phase I study of BMS-936559 anti-PD-L1 documented one objective response in 17 ovarian cancer patients. Evaluation of the PD-1 pathway in
Breast Cancer

Breast cancer is increasingly recognized as an immunogenic tumor. In randomized trials of contemporary adjuvant chemotherapy, either stromal TILs or intratumoral and stromal TILs were identified as independent prognostic factors for survival in triple negative breast cancer (TNBC). Brisk TILs also predicted trastuzumab benefit in HER-2+ breast cancer. Tissue microarray (TMA) analysis of 660 breast cancers showed that infiltration by PD-1+ TILs is associated with worse survival in the luminal B and basal-like subtypes. Likewise, TMA analysis of 650 breast cancers showed that PD-L1 protein is expressed by tumor cells in 23.4% of breast cancers, and is associated with worse survival in patients with luminal B, HER-2+ and basal-like breast cancers. Breast tumor cell PD-L1 expression was strongly correlated with the presence of PD-1+ TILs, suggesting that some breast cancers may respond to PD-1 blockade.

Early results from two trials testing PD-1 pathway blockade in patients with TNBC were recently presented. The first, a phase 1b study of pembrolizumab (10 mg/kg administered every 2 weeks), enrolled 32 patients with advanced TNBC whose tumors expressed PD-L1. Of all patients screened, 58% had PD-L1+ tumors. Five grade 3–4 toxicities and one treatment-related death due to disseminated intravascular coagulation were observed. Among 27 evaluable patients, the ORR was 19%, including 1 CR and 4 PRs. The second trial, a phase 1a study of MPDL3280A (15 or 20 mg/kg administered every 3 weeks), has enrolled patients with metastatic TNBC regardless of PD-L1 expression status. In an interim report, 12 patients with PD-L1+ tumors were evaluable for safety, 9 of whom were also evaluable for clinical response. Of all patients screened, 23% had PD-L1+ tumors. The ORR was 33%, including 1 CR and 2 PRs. As enrollment on this study continues, multiple additional clinical trials are testing PD-1 pathway blockade as treatment for patients with breast cancer.

OTHER TUMOR TYPES

Squamous Head and Neck Cancer

Squamous cell carcinoma of the head and neck (SCCHN) is the fifth commonest cancer worldwide, and median survival for patients with advanced disease is approximately 13 months. Both human papilloma virus (HPV)-associated and non-HPV-associated head and neck tumors have prominent lymphoid infiltrates, and there is evidence in HPV-associated tumors for adaptive immune resistance, with dysfunctional TILs which express PD-1 and tumor cells with high levels of PD-L1 expression.

Antitumor activity has been reported with 3 PD1 pathway blockers in patients with refractory SCCHN. A phase 1b study of pembrolizumab (10 mg/kg administered every 2 weeks) enrolled patients whose tumors expressed PD-L1. Among 104 patients screened, 81 (78%) were PD-L1+ and 61 were eligible for enrollment [36 HPV(−), 23 HPV+, and 2

endometrial and cervical cancers has been limited. One study of 115 cervical cancers demonstrated PD-L1 expression by 19% of cervical tumors, with PD-1 expression in about 50% of TILs (independent of tumor cell PD-L1 expression). PD-L1 expression in cervical cancer was not associated with survival outcomes.
with undetermined HPV status]. Among 56 patients evaluable for response, the ORR was 20%, which was similar between HPV+ and HPV(−) tumors. In a phase 1b multi-arm expansion study, 54 patients with SCCHN received MEDI4736 monotherapy at 10 mg/kg every 2 weeks for up to one year. The ORR among 22 evaluable patients at the time of report was 14%. Finally, in a large phase I study of the anti-PD-L1 drug MPDL3280A, one durable partial response to treatment was reported among six evaluable patients with SCCHN.18

Hematologic Malignancies

PD-1 and PD-L1 inhibition are active areas of investigation in several different types of hematologic cancers. In 2014, nivolumab received FDA Breakthrough designation for the treatment of Hodgkin lymphoma (HL) following autologous stem cell transplant and brentuximab vedotin therapy (anti-CD30 antibody-drug conjugate). This designation was based on results from a phase 1 dose escalation study in relapsed or refractory hematologic malignancies in which patients received nivolumab at 1 or 3 mg/kg every 2 weeks. In a phase II expansion cohort of patients with relapsed refractory HL, the ORR was 87%. Four of 23 patients (17%) had a CR, one of whom had failed both brentuximab vedotin and autologous transplantation. The rate of progression-free survival at 24 weeks was 86%. In a subset of 10 patients assessed for PD-L1 and PD-L2 copy numbers in Reed-Sternberg cells, all had copy gains of PD-L1 and PD-L2 as a result of either polysomy or amplification, with associated increases in protein expression. Similarly, pembrolizumab was well-tolerated and associated with clinical benefit when administered to patients with HL. Nivolumab has demonstrated efficacy in patients with diffuse large B cell lymphoma, follicular lymphoma or mycosis fungoides, with ORRs of 36% (4/11), 40% (4/10) and 15% (2/13), respectively. No ORs were observed in patients with multiple myeloma. (See chapter 8 for more details)

Colorectal cancer

While a durable CR was noted in one patient with metastatic CRC in the first-in-human study of nivolumab, subsequent results were disappointing with no further responses seen in patients enrolled in a large phase 1b study. The sole exceptional responder had a microsatellite instability (MSI)-high tumor. Efforts are now focusing on patients whose tumors are MSI-high, as these tumors generally have high frequencies of somatic mutations and, in turn, novel antigens that may incite immune responses. Currently a phase 2 study of pembrolizumab is enrolling patients with MSI-high CRC, with immune-related PFS at 20 weeks as the primary endpoint (NCT01876511). A similar trial of nivolumab alone or in combination with ipilimumab is underway (NCT02060188). Another strategy being employed to enhance the impact of PD-1 pathway blockade in advanced CRC is combinatorial therapy with bevacizumab and multidrug chemotherapy (MPDL3280A; NCT01633970). MPDL3280A monotherapy recently demonstrated anti-tumor activity in a patient with CRC.18

Pancreatic and Gastric Cancers

Individual responses have been reported in heavily pre-treated patients with advanced pancreatic or gastric cancer in an ongoing phase 1 expansion study of MEDI4736 in
refractory solid tumors. Among a small cohort, MPDL3280A also demonstrated activity in a patient with gastric cancer. Results from a phase Ib study of pembrolizumab in patients with advanced PD-L1+ adenocarcinoma of the stomach or gastroesophageal junction demonstrated objective anti-tumor responses in 31% (12/39) of subjects. Given extremely limited treatment options for patients with chemotherapy-refractory pancreatic and gastric cancers, these responses are of significant interest. Early phase clinical trials testing several different antibodies are enrolling patients with both tumor types.

BIOMARKERS

PD-1/PD-L1 checkpoint blockade has demonstrated durable objective tumor regressions and prolonged disease stabilization in significant proportions of patients with multiple cancer types, and has also been associated with “unconventional” activity profiles such as delayed responses, apparent disease progression before regression, and the possibility of a second response following re-induction therapy for disease progression. Although the PD-1 pathway blockers tested to date have been generally well tolerated, grade 3–5 adverse events have occurred. For these reasons, as well as the significant expense of these therapies, the identification of molecular markers that could guide the selection of patients most likely to respond to therapy and least likely to develop serious complications is highly desirable.

Because scientific evidence suggests that the tumor itself is the major site of action for PD-1 pathway blockade, pre-treatment tumor specimens have been examined for biomarkers of response. The initial suggestion that tumor cell surface (“membranous”) PD-L1 protein expression was related to anti-PD-1 activity was reported in a subgroup of 9 patients from the first-in-human nivolumab trial. This preliminary finding was then extended in the follow-up phase Ib trial of nivolumab, studying 61 tumor specimens from 42 patients with advanced melanoma, RCC, NSCLC, CRC, or prostate cancer. There was a highly significant correlation between PD-L1 expression and clinical response: 36% of patients with PD-L1+ tumors demonstrated an OR (nearly twice the ORR of the overall population), while none whose tumors were PD-L1(−) responded to nivolumab. Importantly, for many of these patients, multiple tumor specimens were assessed and a patient was considered “PD-L1+” if any specimen was positive.

Taube and colleagues next expanded these investigations to assess other factors in the TME predictive of response to anti-PD-1, evaluating 68 pretreatment tumor specimens from 41 patients receiving nivolumab at a single institution. Features studied included tumor type; expression of PD-1, PD-L1, and PD-L2 (the second ligand for PD-1) in the TME; and quantification of immune cell subsets using IHC. Among these parameters, tumor cell PD-L1 expression was the single factor most strongly associated with response to anti-PD-1 therapy. Although the presence of tumor cell PD-L1 expression is often associated with TILs and may indicate an ongoing host response to tumor (a phenomenon termed “adaptive immune resistance”), the presence of CD3+ TILs alone was not predictive of clinical response, suggesting that the functional profile of TILs is an important variable. Indeed, Tumeh and colleagues recently highlighted the role of CD8+ TILs in melanoma, and nominated CD8+ T lymphocyte density at the advancing tumor edge as a predictive biomarker of response to anti-PD-1.
Both constitutive and adaptive mechanisms of PD-L1 expression in the TME have been proposed. Patterns of PD-L1 expression tend to vary by tumor type and may reflect the relative contributions of these mechanisms. When PD-L1 is expressed in melanoma, it tends to demonstrate a focal “adaptive immune resistance” pattern of expression on tumor cells; in contrast, CRCs tend to display PD-L1 on immune infiltrates, with minimal tumor cell expression; and constitutive translocation/oncogene-driven tumor cell PD-L1 expression has been reported in glioblastoma multiforme and some lymphomas. 

Anti-PD-1/PD-L1 therapies theoretically protect tumor-specific PD-1+ TILs from inhibition by PD-L1 expressed on neighboring cells in the TME, and recent findings suggest that any cell type expressing PD-L1 may play this role. Taken together, these findings suggest that PD-L1 expression, when observed in association with TILs, may be a useful guide to prioritizing tumor types most likely to benefit from anti-PD-1/PD-L1 therapy. It remains to be determined whether these therapies will be effective tumors in that express PD-L1 in the absence of TILs.

Multiple laboratories have now substantiated the finding that PD-L1 expression in pre-treatment tumor specimens enriches for response to PD-1/PD-L1 blockade, in various tumor types (Table 3). These studies have employed different PD-L1 antibodies, IHC techniques, intervals between specimen acquisition and treatment initiation, scoring systems (PD-L1+ tumor vs. infiltrating immune cells), and numerical thresholds for defining PD-L1 positivity (Table 4). The finding that PD-L1 expression in the TME is associated with an increased likelihood of response to anti-PD-1/PD-L1 therapies has remained remarkably consistent despite these methodological differences. Importantly, however, the vast majority of these studies do not show an absolute association between PD-L1 expression and clinical response: a proportion of PD-L1(−) patients demonstrates clinical response to PD-1 pathway blockade, and conversely, a significant proportion of PD-L1+ patients do not achieve an OR. This lack of a strict association calls into question the use of PD-L1 expression as a single biomarker for patient selection for anti-PD-1/PD-L1 therapies. Ongoing work aims to study tumor PD-L1 expression in larger cohorts of patients, and to examine additional factors in the tumor or in the blood, at the level of DNA, mRNA and/or protein expression, which may be used independently or in multifactorial analyses to improve algorithms for rational patient selection.

CONCLUSIONS

Early evidence of significant and durable clinical activity of PD-1 pathway blocking drugs across a wide spectrum of cancer types has ushered in a new age of cancer immunotherapy and has firmly established this treatment modality in the oncologic armamentarium. Following recent regulatory approvals for pembrolizumab and nivolumab for patients with treatment refractory advanced melanoma, approvals for additional cancer types are anticipated. The generally manageable safety profile of anti-PD-1/PD-L1 drugs supports the development of combinatorial therapies, which are predicted by preclinical models to be able to increase the efficacy of PD-1 pathway antagonists. Studies identifying tumor PD-L1 protein expression as a factor associated with enhanced responsiveness to PD-1 pathway blockade are only scratching the surface of potential biomarkers which might guide patient selection. Such biomarkers hold the promise of further enhancing the risk:benefit ratio for
these drugs and increasing our understanding of the mechanistic underpinnings of this key pathway in tumor biology.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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**REFERENCES**


Table 1

PD-1 pathway blocking drugs currently in clinical testing

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Alternative names</th>
<th>Target</th>
<th>Source</th>
<th>Isotype and characteristics</th>
<th>Regulatory approval</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMS-936559</td>
<td>MDX-1105</td>
<td>PD-L1</td>
<td>Bristol-Myers Squibb</td>
<td>Fully human IgG4</td>
<td>pending</td>
</tr>
<tr>
<td>MEDI0680</td>
<td>AMP-514</td>
<td>PD-1</td>
<td>MedImmune/ AstraZeneca</td>
<td>Information not available</td>
<td>pending</td>
</tr>
<tr>
<td>MEDI4736</td>
<td>none</td>
<td>PD-L1</td>
<td>MedImmune/ AstraZeneca</td>
<td>Fc-modified human IgG1</td>
<td>pending</td>
</tr>
<tr>
<td>MPDL3280A</td>
<td>RG7446</td>
<td>PD-L1</td>
<td>Genentech/ Roche</td>
<td>Fc-modified human IgG1</td>
<td>pending</td>
</tr>
<tr>
<td>MSB0010718C</td>
<td>none</td>
<td>PD-L1</td>
<td>EMD Serono</td>
<td>Fully human IgG1</td>
<td>pending</td>
</tr>
<tr>
<td>Nivolumab</td>
<td>Opdivo; BMS-936558; MDX-1106; ONO-4538</td>
<td>PD-1</td>
<td>Bristol-Myers Squibb; Ono Pharmaceuticals</td>
<td>Fully human IgG4</td>
<td>Treatment-refractory unresectable melanoma (US and Japan)</td>
</tr>
<tr>
<td>Pembrolizumab</td>
<td>Keytruda; MK-3475; lambrolizumab</td>
<td>PD-1</td>
<td>Merck</td>
<td>Humanized IgG4</td>
<td>Treatment-refractory unresectable melanoma (US)</td>
</tr>
<tr>
<td>Pidilizumab</td>
<td>CT-011</td>
<td>PD-1</td>
<td>CureTech</td>
<td>Humanized IgG1</td>
<td>pending</td>
</tr>
</tbody>
</table>
Table 2

Objective response rates (PR + CR by RECIST criteria) in patients with advanced solid tumors receiving PD-1 pathway blocking drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Melanoma</th>
<th>NSCLC</th>
<th>RCC</th>
<th>Bladder</th>
<th>Prostate</th>
<th>Ovarian</th>
<th>SCCHN</th>
<th>Breast</th>
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<tr>
<td><strong>Anti-PD-1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nivolumab</td>
<td>32% (n=107)(^3)</td>
<td>17% (n=129)(^3,21)</td>
<td>30% (n=20)(^36)</td>
<td>29% (n=34)(^3)</td>
<td>21% (n=168)(^3)</td>
<td>NR</td>
<td>0% (n=17)(^7)</td>
<td>23% (n=15)(^32)</td>
</tr>
<tr>
<td>Pembrolizumab</td>
<td>38% (n=135)(^6)</td>
<td>26% (n=42)(^27)</td>
<td>NR</td>
<td>NR</td>
<td>24% (n=29)(^37)</td>
<td>NR</td>
<td>NR</td>
<td>19.6% (n=56)(^53)</td>
</tr>
<tr>
<td>Pidilizumab</td>
<td>6% (n=85)(^11)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td><strong>Anti-PD-L1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMS-936559</td>
<td>17% (n=52)</td>
<td>10% (n=49)</td>
<td>12% (n=17)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>6% (n=17)</td>
<td>NR</td>
</tr>
<tr>
<td>MEDI4736</td>
<td>NR</td>
<td>16% (n=58)(^29)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>14% (n=22)(^20)</td>
<td>NR</td>
</tr>
<tr>
<td>MPDL3280A</td>
<td>30% (n=43)(^18)</td>
<td>23% (n=53)(^18)</td>
<td>14% (n=56)(^38)</td>
<td>26% (n=65)(^36)</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>33% (n=9)(^51)</td>
</tr>
</tbody>
</table>

(a) Alternative drug names are listed in Supplementary Table S1.

* Only patients whose pre-treatment tumor specimens expressed PD-L1 were enrolled.

NR, not reported.
Table 3

PD-L1 expression in pre-treatment solid tumor specimens enriches for response to anti-PD-1/PD-L1

<table>
<thead>
<tr>
<th>Tumor types</th>
<th>N=</th>
<th>MEL</th>
<th>NSCLC</th>
<th>RCC</th>
<th>Solid Tumor&lt;sup&gt;a&lt;/sup&gt;</th>
<th>H/N</th>
<th>MEL</th>
<th>NSCLC</th>
<th>NSCLC</th>
<th>Bladder</th>
<th>MEL</th>
<th>NSCLC</th>
<th>Solid Tumors&lt;sup&gt;b&lt;/sup&gt;</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>44</td>
<td>34</td>
<td>210</td>
<td>17</td>
<td>68</td>
<td>107</td>
<td>42</td>
<td>55</td>
<td>113</td>
<td>27</td>
<td>194</td>
<td>150</td>
<td>49</td>
</tr>
<tr>
<td>Response Rates (%)</td>
<td>All patients</td>
<td>32</td>
<td>29</td>
<td>40</td>
<td>29</td>
<td>15</td>
<td>23</td>
<td>21</td>
<td>18</td>
<td>40</td>
<td>19</td>
<td>21</td>
<td>9</td>
</tr>
<tr>
<td>PD-L1 +</td>
<td>67</td>
<td>44</td>
<td>53</td>
<td>50</td>
<td>15</td>
<td>31</td>
<td>36</td>
<td>46</td>
<td>49</td>
<td>57</td>
<td>23</td>
<td>22</td>
<td>25</td>
</tr>
<tr>
<td>PD-L1(−)</td>
<td>19</td>
<td>17</td>
<td>33</td>
<td>0</td>
<td>14</td>
<td>18</td>
<td>0</td>
<td>11</td>
<td>13</td>
<td>5</td>
<td>9</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Ref. #</td>
<td>14</td>
<td>67</td>
<td>6</td>
<td>26</td>
<td>23</td>
<td>32</td>
<td>3</td>
<td>53</td>
<td>68</td>
<td>69</td>
<td>28</td>
<td>20</td>
<td>29</td>
</tr>
</tbody>
</table>

<sup>a</sup>Castration-resistant prostate carcinoma, colorectal carcinoma, kidney cancer, melanoma, and NSCLC.

<sup>b</sup>Colorectal carcinoma, kidney cancer, melanoma, NSCLC. These numbers include the melanoma and NSCLC data shown in the immediately adjacent columns.

H/N, head and neck squamous cell cancer; MEL, melanoma; NSCLC, non-small-cell lung cancer; RCC, renal cell carcinoma.
Table 4

Immunohistochemical methods for evaluating tumor PD-L1 expression

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>JHU</th>
<th>Bristol-Myers Squibb</th>
<th>Merck</th>
<th>Roche/Genentech</th>
</tr>
</thead>
<tbody>
<tr>
<td>mAb clone</td>
<td>5H1</td>
<td>28–8</td>
<td>22C3</td>
<td>SP142</td>
</tr>
<tr>
<td>Automated</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Staining location scored</td>
<td>Membranous</td>
<td>Membranous</td>
<td>Membranous</td>
<td>Membranous</td>
</tr>
<tr>
<td>Cell type scored</td>
<td>Tumor</td>
<td>Tumor</td>
<td>Tumor and/or infiltrating immune cells</td>
<td>Infiltrating immune cells</td>
</tr>
<tr>
<td>&quot;positive&quot; cutoff(a)</td>
<td>&gt; 5%</td>
<td>&gt;5%</td>
<td>&gt;1%</td>
<td>≥1% and &lt; 5% (&quot;IHC 1&quot;); ≥5% and &lt; 10% (&quot;IHC 2&quot;); ≥10% (&quot;IHC 3&quot;)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Melanoma, NSCLC, Renal, Colorectal, Prostate</th>
<th>Melanoma</th>
<th>Melanoma</th>
<th>NSCLC</th>
<th>NSCLC</th>
<th>KCC</th>
<th>HNSCC</th>
<th>Melanoma</th>
<th>NSCLC</th>
<th>Bladder</th>
<th>Melanoma</th>
<th>NSCLC</th>
<th>Solid Tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD-L1 positive patients/total patients (%)</td>
<td>25/42 (60)</td>
<td>12/44 (27)</td>
<td>17/38 (45)</td>
<td>74/210 (35)</td>
<td>9/15 (60)</td>
<td>33/68 (49)</td>
<td>29/107 (27)</td>
<td>81/104 (78)</td>
<td>89/125 (71)</td>
<td>159/194 (82)</td>
<td>55/205 (27)</td>
<td>15/30 (50)</td>
<td>48/184 (26)</td>
</tr>
<tr>
<td>Reference #</td>
<td>3</td>
<td>14</td>
<td>67</td>
<td>6</td>
<td>26</td>
<td>23</td>
<td>32</td>
<td>53</td>
<td>68</td>
<td>32</td>
<td>36</td>
<td>70</td>
<td>18</td>
</tr>
</tbody>
</table>

PD-L1 expression in formalin-fixed, paraffin embedded (FFPE) tumor specimens was evaluated using distinct immunohistochemical assays and scoring methods. Some references report results from different cut-offs for positive scoring in the same cohorts. Final methods and cut-off values are likely to be determined after additional clinical follow-up and analysis of ongoing studies.

\(a\) Positive cutoff, definition of PD-L1 positive specimen according to threshold percent of cells staining with PD-L1 specific mAb.

\(b\) Updated scoring system for NSCLC defines a PD-L1+ specimen as having ≥1% tumor cells expressing cell surface PD-L1, and subdivides "strong" positives (≥50% staining) vs. "weak" positives (<50% staining).\(28\)

\(c\) PD-L1+ defined as IHC 2 and IHC 3.

\(d\) PD-L1+ defined as ≥25% infiltrating cells or tumor cells expressing PD-L1.

\(e\) PD-L1+ defined as "tumors with infiltrating immune cells that stain for PD-L1 using Genentech/Roche Ex IHC".

JHU, Johns Hopkins University.