Anti-PD1 and anti-PD-L1 in the treatment of metastatic melanoma

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Practice points

- Programmed cell death-1 ligand-1 (PD-L1) positivity status cannot be considered as a predictive marker for selecting patients for anti-PD1 therapy because patients who are PD-L1 negative may also respond to treatment.
- Unconventional response (immune-related adverse events) as observed with ipilimumab can also be seen with these compounds.
- Anti-PD1 and anti-PD-L1 treatment is effective regardless of the mutational status of the patient.
- Patients who have interrupted treatment due to causes other than progression continue to respond.
- The safety profile of anti-PD1 and anti-PD-L1 treatment is better compared with that of ipilimumab.
- Treatment algorithms for the management of immune-related adverse events associated with ipilimumab are also valid for anti-PD-1/PD-L1 therapy.

SUMMARY Programmed cell death receptor-1 (PD-1) and programmed cell death-1 ligand-1 (PD-L1) represent promising novel targets in immunotherapy. PD-1 is an inhibitory receptor involved in T-cell regulation that is expressed by activated T cells. Nivolumab and pembrolizumab are anti-PD-1 antibodies that have shown antitumor activity and acceptable tolerability in patients with metastatic melanoma in preclinical development and Phase I/II clinical trials. Several ongoing Phase III studies are further investigating the efficacy and safety of anti-PD-1 therapy in melanoma. Initial data on the combination of anti-PD-1 and anti-cytotoxic T-lymphocyte-associated antigen 4 blockade with nivolumab and ipilimumab also appear promising. Monoclonal antibodies to blockade PD-L1 may also be an effective immunotherapy strategy in melanoma and several anti-PD-L1 antibodies are in development.

Immunotherapy in metastatic melanoma has been the focus of renewed interest and drug development in recent years. The blockade of immune system regulatory checkpoints is the most studied strategy to enhance antimelanoma immunity. Inhibition of the cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) molecule by ipilimumab results in strong and durable antitumoral responses with significantly prolonged overall survival (OS) in patients with metastatic melanoma [1]. Programmed cell death receptor-1 (PD-1) is another inhibitory receptor involved in T-cell regulation that is expressed by activated T cells, including memory T cells and T-regulatory cells, B cells and myeloid cells [1]. Preclinical and Phase I/II clinical trials have shown antitumor activity of anti-PD-1 antibodies in patients with melanoma [2]. In this review, we will focus on the development of anti-PD-1 and anti-programmed cell death-1 ligand-1 (PD-L1) antibodies from preclinical to clinical experience in melanoma.

KEYWORDS

- anti-PD-1 • anti-PD-L1
- immunotherapy
- metastatic melanoma
- MK3475 • nivolumab

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Mechanism of action & preclinical studies

PD-1 is a member of the CD28 family and is a 50–55 kDa type I transmembrane glycoprotein whose extracellular domain shares 21–33% sequence identity with CTLA-4, CD28 and inducible T-cell co-stimulator [2]. It is constitutively expressed on T cells in the thymus and induced on peripheral T and B cells upon activation [3]. Expression of PD-1 contrasts with the restricted expression of other CD28 family members to T cells, suggesting that PD-1 regulates a wider spectrum of immune responses compared with other CD28 family members [2]. Its physiological role is to downregulate T-cell activity upon binding to two receptors, PDL-1 and PDL-2 [3]. PD-L1 and PD-L2 are type I transmembrane glycoproteins that share homology with B7.1 and B7.2 that are ligands for CD28 and CTLA-4 [4]. These ligands are expressed in normal tissues such as placenta, heart, lung, liver, spleen, lymph nodes and thymus [4]. Furthermore, they are constitutively expressed or can be induced on several tumors [4]. Using murine models, Yagita and colleagues discovered that PD-L1 is expressed on resting B cells, T cells, macrophages and dendritic cells and that its expression may be upregulated on these cells by stimulation from cytokines such as IFN γ , IL-4, IL-12 and granulocyte-macrophage colony stimulating factor [5]. The PD-1/PDL-1 pathway has been widely studied in mice models. In particular, studies in PD-1-deficient mice showed hyperactivation of the immune system with the appearance of autoimmune diseases such as glomerulonephritis, lupus-like syndrome, etc. [6]. In accordance with antitumoral immunity, Iwai *et al.* and Hirano *et al.* demonstrated that PD-L1 on tumor cells suppresses the cytolytic activity of CD8 T cells in P815 mastocytoma cell lines [7,8]. Various systems have shown that tumor eradication can be accelerated by PD-1–PD-L blockade. They include antibody blockade of PD-1 and PD-L1, DNA vaccination of the extracellular region of PD-1, Pcd1^{-/-} mice, tumor-specific T-cell clones, TCR transgenic mice and human autologous T cells [9–12]. PD-1 blockade has also been shown to have a role in tumor suppression of different tumor cell lines, including melanoma and colon cancer [13].

Recently, most attention has focused on the relationship between high expression of PDL-1 and poor prognosis and survival in various cancers. Thompson *et al.* [14] showed that the high expression of PD-L1 on clinical samples of

renal cell carcinoma (RCC) and/or on lymphocytes correlated with poor survival of patients. This correlation has been also shown in other cancers such as ovarian, melanoma, pancreatic carcinoma and hepatocellular carcinoma [15–17].

PD-1–PDL-1-dependent immune inhibition is responsible for immune resistance through tumor cell evasion.

The induction of tumor-specific T-cell energy may be the most important mechanism of immune escape induced by the PD-1/PDL-1 pathway [18]. Thus, PD-1–PD-L blockade may revert the immunocompromised status of tumor-bearing hosts and activate the host immune system to eradicate tumors [18].

To potentiate tumor regression, several studies have focused on the combination of immunotherapeutic strategies with the most interesting combination seeming to be anti-PD1 therapy with anti-CTLA-4. CTLA-4 and PD-1 appear to play complementary roles in regulating adaptive immunity. Although PD-1 contributes to T-cell exhaustion, CTLA-4 inhibits T-cell activation at earlier points. Curran *et al.* [19] showed the most favorable effects of PD-1 and CTLA-4 combination blockade using vaccination with irradiated B16 melanoma cells expressing granulocyte-macrophage colony stimulating factor or Flt3 ligand. The combination of PDL-1 and CTLA-4 blockade resulted in stronger rejection of preimplanted tumors than CTLA-4 blockade alone (65 vs 10%). PD-1 and CTLA-4 blockade increases effector T-cell infiltration, with advantages on T effectors and T regulatory cell ratios within the tumor. This study demonstrated that multiple blockade of co-inhibitory pathways lead to activation of CD8⁺ and CD4⁺ lymphocytes within the tumor and also promotes reduction of suppressor cells, such as T-regs and myeloid-derived suppressor cells, with increased inflammation in the tumor microenvironment [19].

Anti-PD1 antibodies: nivolumab & pembrolizumab (MK3475)

Nivolumab (BMS-936558 or MDX-1106) is a fully human IgG4 monoclonal antibody with high affinity to PD-1. It blocks binding of PD-1 to PD-L1 and PD-L2, stimulating proliferation of T cells and the production of cytokines [20]. Nivolumab has been tested in multiple solid tumors including melanoma, RCC and non-small-cell lung carcinoma (NSCLC). A Phase I clinical trial (NCT00730639) was conducted to evaluate the activity and safety profile of

nivolumab in solid tumors. A total of 306 pretreated patients were enrolled, of whom 129 had NSCLC, 107 melanoma, 34 RCC, 19 colorectal cancer (CRC) and 17 castration-resistant prostate cancer [21]. Nivolumab was administered at different doses (0.1, 0.3, 1, 3 or 10 mg/kg) every 2 weeks for a maximum of twelve 8-week treatment cycles. Responses were evaluated using Response Evaluation Criteria in Solid Tumors 1.0 with a computed tomography scan performed every 8 weeks. Objective responses were observed (21%) in patients with NSCLC, melanoma and RCC, while no responses were observed in patients with CRC or castration-resistant prostate cancer. Early responses were detected in 46% of patients at the first tumor assessment after 8 weeks. Delayed and durable responses (>1 year) were observed in 13 out of 270 (5%) patients with NSCLC, melanoma and RCC after increases in tumor burden. In patients with NSCLC, 1-year and 2-year OS was 42 and 14%, respectively, with a median duration of survival of 9.6 months (95% CI: 7.8–12.4). In melanoma patients, 1-year OS was 62% and 2-year OS was 43%, with a median survival duration of 16.8 months (95% CI: 12.5–31.6; [Table 1](#)). Improved OS was also observed in RCC with a 1-year OS of 70% and 2-year OS of 50% (median duration of over 22 months, 95% CI: 13.6–NE) [21]. Treatment-related adverse events (AEs) of any grade were observed in 75% of patients, with 17% experiencing grade 3–4 events ([Table 2](#)). The most frequent AEs were rash, pruritus, diarrhea, increased levels of thyroid-stimulating hormone and alanine aminotransferase and pneumonitis. Six ongoing Phase III trials with nivolumab in NSCLC, melanoma and RCC are evaluating the impact on OS and the role of potential predictive factors of response.

MK3475 (pembrolizumab) is a potent and highly selective humanized monoclonal antibody

of the IgG4/κ isotype that blocks the interaction between PD-1 and its ligands PD-L1 and PD-L2 [28]. In an open-label, Phase I dose escalation study to evaluate the safety and clinical activity of MK-3475 monotherapy (NCT01295827), doses of 1, 3 and 10 mg/kg were well tolerated with no dose-limiting toxicities [28]. Preliminary evidence of antitumor activity was observed in patients with melanoma. Hamid *et al.* reported results from the expansion cohort of this study in which pembrolizumab was administered at three different dosage regimens (2 mg/kg every 3 weeks, 10 mg/kg every 2 weeks or 10 mg/kg every 3 weeks) in 135 patients with advanced melanoma either pretreated or not with ipilimumab [29]. The response rate was 38% (95% CI: 25–44), with the highest confirmed response rate observed in the cohort that received 10 mg/kg every 2 weeks (52%; 95% CI: 38–66). The response rate was similar between patients who had received prior ipilimumab treatment and those who had not (overall response rate [ORR]: 38% [95% CI: 23–55] and 37% [95% CI: 26–49], respectively). Responses were durable in the majority of patients (median followup 11 months among patients who had a response). Recently, an update of this study was reported which included an additional 276 patients (total n = 411) randomized to two groups: ipilimumab-refractory and ipilimumab-naïve at two different dosages: 2 mg/kg every 3 weeks or 10 mg/kg every 3 weeks [23]. ORR was 34% (95% CI: 29–39) across all patients, 40% (95% CI: 32–48) in the ipilimumab-naïve group and 28% (95% CI: 22–35) in the ipilimumab-refractory group. The median progression-free survival (PFS) was 5.5 months, with no significant difference between the two groups. The median OS was not reached with a 1-year OS rate of 69% (considering all 411 patients enrolled in the study) and 18 months OS rate of

Table 1. Efficacy summary of anti-PD1 and anti-PD-L1 studies in melanoma.

	Overall response rate (%)	Median progression-free survival (months)	1-year overall survival (%)	2-year overall survival (%)	Ref.
Nivolumab	32	3.7	63	48	[22]
Pembrolizumab	34	5.5	69	62 [†]	[23]
BMS936559	17	6.9	NR	NR	[24]
MPDL3280A	29	NR	NR	NR	[25]
MEDI4736	25	NR	NR	NR	[26]
Ipilimumab + nivolumab	53	>12	94	88	[27]

[†]Overall survival rate at 18 months.
NR: Not reported.

62% (considering only the 135 patients enrolled in the first part of the study). Drug-related AEs (any grade) were reported in 83% of patients, with 12% of grade 3/4 toxicity. Most frequent side effects were fatigue (36%) and pruritus (24%), but less than 1% were grade 3/4. Other frequent side effects included diarrhea (16%), arthralgia (16%) and nausea (12%), but again the rate of grade 3/4 events for all of these was less than 1%. Vitiligo secondary to pembrolizumab, as pneumonitis, was reported in 11% of patients (<1% grade 3–4). Grade 3/4 colitis, hypothyroidism, hyperthyroidism and hepatitis were reported in less than 1% of patients. Other potentially immune-related side effects such as nephritis, uveitis and hypophysitis were also reported in less than 1% of the patients (Table 2).

The combination of nivolumab and ipilimumab has been investigated in a Phase I study of 86 patients with metastatic melanoma (NCT01024231) [30]. This study evaluated two regimens: a sequential regimen with at least three cycles of ipilimumab 3 mg/kg before nivolumab 1.0 or 3.0 mg/kg every 2 weeks (n = 33) and a concurrent regimen of escalating doses of intravenous nivolumab (0.3, 1.0, 3.0, 10.0 mg/kg) and ipilimumab (3.0–10.0 mg/kg) administered every 3 weeks for four doses, followed by nivolumab alone every 3 weeks for four doses (n = 53). Among the 33 patients receiving the sequential regimen, AEs of any grade were observed in 29 patients (88%). Treatment-related AEs were observed in 24 patients (73%), with the most common including pruritus (18%) and elevated lipase levels (12%). Grade 3 or 4 AEs were observed in six patients (18%), with an elevated lipase level being the most frequent serious event (6%). Six of 30 patients (20%) receiving sequential therapy had an objective response,

including one with a complete response and four (13%) with tumor reduction of greater than or equal to 80% at 8 weeks [30]. With the concurrent regimen, AEs of any grade were observed in 98% of patients, the most common being rash (55%), pruritus (47%), fatigue (38%) and diarrhea (34%). Grade 3 or 4 events were observed in 72% of patients, with elevated levels of lipase (13%), aspartate aminotransferase (13%) and alanine aminotransferase (11%) being the most frequent. Six of 28 patients (21%) had grade 3 or 4 events that were dose limiting. Serious treatment-related AEs were reported in 49% of patients in the concurrent regimen, including hepatic (15%), gastrointestinal (9%) and renal events (6%). Isolated cases of pneumonitis and uveitis were observed and 11 patients (21%) discontinued therapy because of severe AEs [11]. Doses of nivolumab 1 mg/kg and ipilimumab 3 mg/kg were identified as the maximum doses that were associated with an acceptable level of AEs. In this cohort of 17 patients, nine (53%) had an objective response. Three complete responses were observed and six patients (41%) achieved a tumor reduction of 80% or more. The ORR for all patients in the concurrent regimen group was 40%, with evidence of clinical activity (conventional, unconfirmed or immune-related response or stable disease for ≥ 24 weeks) observed in 65% of patients. Preliminary data showed 1-year OS rate of 82% (95% CI: 69–94) for the concurrent regimen of nivolumab 1 mg/kg and ipilimumab 3 mg/kg [30]. These results suggest that nivolumab and ipilimumab can be administered concurrently with a manageable safety profile, even though AEs were more frequent than with monotherapy. Tumor responses were more rapid, durable and deeper than observed with single agent treatment. Recently, Sznol

Table 2. Percentage of most representative and frequent anti-PD1 and PD-L1 adverse events.					
Any grade (grade 3–4) treatment-related adverse events (%)	Nivolumab [22]	Pembrolizumab [23]	BMS-936559 [24]	MPDL3280A [25]	MEDI4736 [26]
Skin	36.0 (0)	20.0 [†] (<1.0)	39.0 (5.0)	16.0 (0)	–
Gastrointestinal	18.0 (2.0)	16.0 (<1.0)	9.0 (0)	30.0 (2.0)	36.4 (0)
Hepatic	7.0 (1.0)	<1.0 (<1.0)	–	16.0 (7.0)	–
Endocrinopathies	13.0 (2.0)	9.0 (<1.0)	3.0 (0)	–	–
Pneumonitis	4.0 (0)	3.0 (<1.0)	–	–	18.2 [‡] (0)
Fatigue	NR	36.0 (2.0)	–	59.0 (2.0)	36.4 (0)
Nausea	NR	12.0 (<1.0)	–	23.0 (0)	27.3 (0)
[†] This percentage is referred only to cutaneous rash.					
[‡] Bronchitis.					
NR: Not reported.					

et al. [27] reported an update of the 53 patients enrolled in this trial. The updated 1-year OS rate was 85% and the 2-year survival rate was 79%. The ORR was 42%, with confirmed complete responses increasing from 10 to 17%. Nearly 42% of patients had greater than or equal to 80% reduction in overall tumor measurements. The responses were durable and 82% were ongoing at time of analysis, with median duration of response not reached. Clinical responses were seen regardless of tumor BRAF-mutation status or PD-L1 status. With nivolumab 1 mg/kg plus ipilimumab 3 mg/kg, the 1-year OS rate and 2-year OS were 94 and 88% respectively. Grade 3/4 side effects occurred in 58 of the 94 patients (62%). The most common grade 3/4 toxicities were increased lipase and amylase levels, laboratory abnormalities that were easily reversible. A total of 23% of patients discontinued the combination therapy due to treatment-related AEs and most of the toxicity occurred during the induction with concomitant nivolumab and ipilimumab. One drug-related death occurred in the latest cohort, as a consequence of colitis.

Another anti-PD-1 in development, both in hematology and in melanoma is pidilizumab (CT-011), a humanized anti-PD-1 IgG1k that was initially studied in a Phase II trial enrolling patients with diffuse large B-cell lymphoma (DLBCL) [31]. Recently, Atkins *et al.* reported the results of a Phase II study to evaluate the safety and efficacy of pidilizumab at two dose levels, 1.5 or 6 mg/kg iv. every 2 weeks ($\times 27$) in patients with metastatic melanoma [32]. In 103 patients, ORR using immune-related response criteria was 5.9% (90% CI: 2.3–12.0) with 1.5 mg/kg and 10.0% (90% CI: 1.8–28.3) for those pretreated with ipilimumab. These patients had higher irSD (53.7 vs 20.5%) and slightly longer median PFS (2.8 vs 1.9 months). OS at 12 months was 64.5% (90% CI: 55.6–72.0), without differences between the different subgroups. The most frequent AEs were fatigue (43%), diarrhea (22.5%), arthralgia (21%) and serious AEs reported were pneumonia (5%) and dyspnea (3%).

Nivolumab & pembrolizumab: ongoing Phase II & III clinical trials

Previous early antitumor activity in metastatic melanoma has opened the way to further evaluation of anti-PD-1 therapies in this population. In particular, two ongoing randomized clinical trials are investigating the efficacy of anti-PD-1 in

ipilimumab-pretreated patients with metastatic melanoma compared with chemotherapy. In the Phase III BMS-CA209-037 (NCT01673867) trial, a direct comparison of the OS of nivolumab versus chemotherapy with dacarbazine is being investigated. If this primary end point is reached, this study could support the approval of nivolumab in patients with advanced melanoma progressing on ipilimumab. A randomized Phase II study of pembrolizumab (MK-3475; NCT01295828) is also exploring this topic, and will provide safety and efficacy data on two different blinded doses of pembrolizumab (2 and 10 mg/kg) administered every 3 weeks versus investigator-chosen best chemotherapy. This study allows crossover from the chemotherapy arm in case of progression and PFS is the main end point. Pembrolizumab was recently approved by the US FDA (September 2014) at the dosage of 2 mg/kg every 3 weeks for the treatment of unresectable or metastatic melanoma and disease progression following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor.

The BMS-CA209-066 (NCT01721746) is another interesting randomized Phase III trial which compares the efficacy of nivolumab versus dacarbazine in untreated metastatic melanoma patients with BRAF wild-type. Its primary objective is to compare the OS, while the secondary end point is to evaluate whether PD-L1 expression is a predictive biomarker for OS and ORR. However, a recent press release informed that this Phase III trial was stopped early because the analysis conducted by the independent Data Monitoring Committee showed evidence of superior OS in patients who received nivolumab compared with the control arm (dacarbazine) [33].

Another important randomized Phase III trial is BMS-CA209-067 (NCT01844505), a three-arm double-blind study which is comparing the combination of nivolumab and ipilimumab with single agent therapy (nivolumab or ipilimumab alone). In the combination arm, nivolumab is administered at the dose of 1 mg/kg in association with ipilimumab administered at the dose of 3 mg/kg for four doses every 3 weeks, followed by nivolumab at the dose of 3 mg/kg every 2 weeks until progression or unacceptable toxicity. The study population includes untreated metastatic melanoma patients regardless of BRAF mutational status, although status is documented since it is one of the stratification criteria. Primary end point of the trial is OS and it is hoped the results

will support the approval of the combination as first-line therapy for metastatic melanoma.

Furthermore, a randomized Phase III study to compare the efficacy of pembrolizumab with ipilimumab in untreated metastatic melanoma patients is ongoing (NCT01866319). In this trial, MK3475 is administered with two different schedules, one at a dose of 10 mg/kg every 2 weeks and the other at the same dose every 3 weeks.

A candidate predictive biomarker for anti-PD1 therapy: the role of PD-L1

Tumor cell expression of PD-L1 has been explored as a potential biomarker for anti-PD-1 therapy. In cancer cells *in vitro*, PD-L1 is upregulated by immune cytokines critical for T-cell functioning, such as IFN- γ [34], which may positively feedback to enhance immune tolerance *in vivo*. Blocking the PD-1/PD-L1 pathway delays tumor progression [34] and adoptive transfer of tumor-specific PD-1-deficient T-cell receptor transgenic T cells can reject tumors. PD-L1 is expressed on melanoma cells and the levels of PD-L1 expression positively correlate with higher ORR and longer PFS when patients are treated with anti-PD-1, while data about a possible correlation with OS are not still mature. Furthermore, tumor cell expression of PD-L1 may be driven by constitutive oncogenic pathways. Recent data also suggest that PD-L1 expression may reflect an adaptive immune resistance in response to an endogenous anti-tumor immune response, which may remain in check unless it is unleashed through blockade of the PD1/PD-L1 pathway [35].

Topalian *et al.* demonstrated that PD-L1 expression evaluated by immunohistochemistry on melanoma biopsy samples was correlated with response to nivolumab treatment, while no responses were observed in PD-L1-negative tumors [36]. Similarly, Grosso *et al.* reported that median OS and PFS were higher in patients with PD-L1-positive tumor biopsy samples compared with PD-L1-negative tumors (21.1 vs 12.5 months, and 9.1 vs 2.0 months, respectively) in melanoma patients treated with anti-PD-1 [37]. Moreover, ORR was 44% (7/16) in PD-L1-positive tumors versus 17% (3/18) in PD-L1-negative tumors. Response was also correlated with PD-L1 status in the Phase I trial of nivolumab (BMS-CA209-003), in which ORR was 41% (7/17) in patients with PD-L1-positive tumors compared with 14% (3/21) in patients

with PD-L1-negative tumors. In the concurrent nivolumab and ipilimumab (BMS CA209-004) trial, ORR was 46% (6/13) in patients with PD-L1-positive tumors and 41% (9/22) in patients with PD-L1-negative tumors. Thus, in the combination study, positive and negative PD-L1 staining of the pretreated tumors had similar percentages of response.

Similar results were observed in a trial with the anti-PDL-1, MPDL3280A, where ORR in 38 patients with melanoma was 27% (4/15) in PD-L1 positive and 20% (3/15) in PDL-1-negative tumors, while best disease control rate (complete and partial response plus stable disease) was in favor of PD-L1-positive tumors (87 vs 20%) [38]. These findings are summarized in Table 3. Debate concerning the correlation of response to anti-PD-1 and PD-L1 expression still remains, since responses in patients with negative PD-L1 tumors have been demonstrated. As such, additional studies are needed to define the role of PD-L1 as a potential predictive marker of response to anti-PD-1 therapy.

Anti-PD-L1

PD-L1 is the main PD-1 ligand that is upregulated in solid tumors, where it can inhibit cytokine production and the cytolytic activity of PD-1 positive, tumor-infiltrating CD4⁺ and CD8⁺ T cells [41]. These characteristics make PD-L1 a promising target for cancer immunotherapy.

BMS-936559 is a high-affinity, fully human, PD-L1-specific, IgG4 (S228P) monoclonal antibody that inhibits the binding of PD-L1 to both PD-1 and CD80 [24]. A Phase I dose-escalation study was conducted in 207 patients with solid tumors which included advanced pretreated NSCLC (n = 75), melanoma (n = 55), CRC (n = 18), RCC (n = 17), ovarian cancer (n = 17), pancreatic cancer (n = 14), gastric cancer (n = 7) and breast cancer (n = 4) [24]. Anti-PD-L1 antibody was administered at doses of 0.3, 1, 3 and 10 mg/kg given as a 60-min intravenous infusion on days 1, 15, and 29 of each cycle. Patients continued treatment for up to 16 cycles unless they had unacceptable toxic effects or disease progression. The median duration of therapy was 12 weeks (range 2–111 weeks). A maximum tolerated dose was not reached. AEs of any grade were reported in 188 of 207 patients (91%), with fatigue, infusion reactions, diarrhea, arthralgia, rash, nausea, pruritus and headache being the most frequent. Treatment-related

Table 3. PD-L1 expression and response rate.

	n	PDL-1+	PDL-1-	Ref.
Nivolumab, Topalian <i>et al.</i>	42	9/25 (36%)	0/17 (0%)	[36]
Nivolumab, Weber <i>et al.</i>	44	8/12 (67%)	6/32 (19%)	[39]
Nivolumab, Grosso <i>et al.</i>	34	7/16 (44%)	3/18 (17%)	[37]
MPDL3280A, Hamid <i>et al.</i>	30	4/15 (27%)	3/15 (20%)	[25]
Nivolumab/ipilimumab, Callahan <i>et al.</i>	27	4/10 (40%)	8/17 (47%)	[40]

AEs with potential immune-related causes were observed in 81 patients (39%) and included rash, hypothyroidism, hepatitis and one case each of sarcoidosis, endophthalmitis, diabetes mellitus and myasthenia gravis. These AEs were predominantly grade 1 or 2 and were manageable (Table 2). Clinical activity was observed at all doses of 1 mg/kg or higher. Objective responses (confirmed complete or partial responses) were observed in patients with melanoma, NSCLC, RCC and ovarian cancer, but not in patients with gastric or pancreatic cancers. Many of these responses were durable. In patients with melanoma, there were nine objective responses among 52 patients receiving 1, 3 and 10 mg/kg doses, with response rates of 6, 29 and 19%, respectively. Three patients with melanoma achieved a complete response. All nine patients who had a response started treatment at least 1 year before data analysis, of whom five had an objective response lasting at least 1 year and 27% of patients had stable disease lasting at least 24 weeks.

MPDL3280A is another human monoclonal antibody that targets PD-L1, and which contains an engineered Fc designed to avoid antibody-dependent cellular cytotoxicity. In a Phase I trial with a melanoma expansion cohort of 38 patients, an ORR of 29% was reported with a 24-week PFS rate of 43% (Table 1) [25]. No treatment-related deaths were observed and the majority of AEs were transient grade 1–2 events not requiring intervention (Table 2). No reports of pneumonitis or colitis were described. MPDL3280A appeared generally well tolerated in metastatic melanoma, except in combination with vemurafenib for which toxicities were consistently observed.

MEDI4736 is a human IgG1k antibody anti-PD-L1 with an engineered Fc domain. Preclinical studies showed MEDI4736 overcomes PD-L1-mediated inhibition of human T cells *in vitro* and demonstrates T-cell-dependent antitumor activity. A Phase I study in patients with advanced solid tumors evaluating safety, activity and pharmacokinetics of MEDI4736

at doses of 0.1, 0.3 and 1.0 mg/kg every 2 or every 3 weeks is ongoing. No drug-limiting toxicities have been observed to date, with no grade 3–4 AEs nor any grade of pneumonitis, colitis or hyperglycemia being observed. The only AEs reported have been grade 1–2 events such as diarrhea, vomiting and dizziness (Table 2) [26]. Clinical activity was observed in patients with melanoma and NSCLC in particular and was reported to occur early, after 6 weeks of treatment. Another ongoing Phase I trial (NCT01693562) is studying MEDI4736 given intravenous every 2 or 3 weeks in a 3+3 dose escalation with a 28-day (every 2 weeks) or 42-day (every 3 weeks) dose-limiting toxicity window, followed by expansion in eight solid tumors [42]. Twenty-six patients (13 NSCLC, 8 melanoma, 5 other) were enrolled in the dose-escalation phase. Treatment-related AEs occurred in 34% of patients, all grade 1–2 with none leading to discontinuation of study drug. The most frequent treatment-related AEs were diarrhea, fatigue, rash and vomiting (12% each). Four PRs (three NSCLC, one melanoma) and five additional patients with tumor shrinkage not meeting PR were observed. Disease control rate (PR + SD $\geq$ 12 weeks) was 46%. Tumor shrinkage, as early as 6 weeks, was seen at all dose levels and benefit was durable. Eleven patients remained in the study as of data cut-off time (2+ to 14.9+ months). The expansion cohort was opened using a 10 mg/kg dose every 2 weeks. A total of 151 patients have been dosed, with the goal to enroll 600. Preliminary clinical activity has been observed with acceptable safety across a range of tumors including SCCHN, pancreatic, gastric, NSCLC and melanoma.

Discussion

PD1 and PD-L1 represent very promising novel targets in immunotherapy. Results of long-term followup of patients treated with nivolumab show high and prolonged responses with very good OS. Experience with pembrolizumab (MK3475) also reports high response rates and excellent

durability. These data suggest that the efficacy of the anti-PD-1 molecules nivolumab and pembrolizumab appear to be superior to that of ipilimumab, although we must await the results of two Phase III studies (NCT01844505 and NCT01866319) for confirmation. However, anti-PD-1 therapy has already shown superior efficacy to chemotherapy, given the recent approval of pembrolizumab by FDA for the treatment of melanoma patients after ipilimumab failure and analysis conducted by the independent Data Monitoring Committee which showed evidence of superior OS in patients receiving nivolumab [33].

Patterns of tumor regression observed with anti-PD-1 are consistent with immune-related patterns of response. Index lesions often respond as previously undetected lesions that become detectable, a finding that is possibly related to lymphocyte infiltration of previously unknown small nests of tumor cells. Although the full effect of these unconventional response patterns remains to be defined in randomized trials with survival end points, such findings are similar to those observed in patients treated with ipilimumab who achieved a significant extension of OS. However, unlike anti-CTLA-4 and despite anti-PD-1 blockade being an immunotherapy, treatment may result in responses according to Response Evaluation Criteria in Solid Tumors 1.1 criteria comparable to those of immune-related response criteria.

Anti-PD-1 safety profiles seem quite manageable with a lower incidence of immune-related AEs than ipilimumab. Pneumonitis represents an immune-related AE of special interest. Although three deaths occurred in a Phase I trial, mild-to-moderate pneumonitis was managed successfully with either observation or glucocorticoids. As seen with ipilimumab, guidelines for managing AEs associated with anti-PD-1 therapy will be useful in clinical practice.

In addition, initial data on the combination of anti-PD-1 and anti-CTLA-4 (nivolumab and ipilimumab) are very promising with an ORR of over 50%. Moreover, the quality of the responses obtained with the combination of ipilimumab and nivolumab seem to be superior to monotherapy with a higher percentage of complete remission. The safety profile shows a higher percentage of serious AEs (about 62% [27]) compared with monotherapy, however, treatment was manageable using algorithms for the treatment of immune-related toxicity. More definitive

efficacy and safety data for the combination will be provided by the ongoing BMS-CA209-067 Phase III trial.

A particular challenge in cancer immunotherapy is the identification of predictive biomarkers that can identify candidates for such treatment. To date, most studies have shown a positive correlation between tumor expression of PD-L1 and response, although this requires further prospective exploration for its clinical use in selecting patients for immunotherapy with anti-PD-1 antibody.

Monoclonal antibodies to blockade PD-L1 may be another effective immunotherapy strategy in melanoma. In particular, this is a direct immunotherapy on tumor cells, even though resistance due to the loss of PD-L1 may be a limiting factor and requires further research. However, BMS936559, MPDL3280A and MEDI4736 represent novel and promising antibodies for the treatment of some solid tumors, in particular melanoma.

Conclusion & future perspective

PD-1 and PD-L1 represent promising novel targets in immunotherapy and look likely to become increasingly important in the future treatment of metastatic melanoma. Data so far suggest the anti-PD-1 molecules nivolumab and pembrolizumab may be more effective than ipilimumab, although this requires confirmation. However, anti-PD-1 therapy has already shown superior efficacy to chemotherapy. Anti-PD-1 safety profiles appear to be manageable with a lower incidence of immune-related AEs than with ipilimumab. As with ipilimumab, guidelines for managing AEs associated with anti-PD-1 therapy will be important in clinical practice. Combination therapy of anti-PD-1 and anti-CTLA-4 also appears to be very promising and may be superior to monotherapy with either immunotherapy alone. More definitive efficacy and safety data for the combination are required. The use of other novel combined therapy strategies are also likely to be increasingly important in the treatment of melanoma. Better selection of patients through the use of predictive biomarkers should also hopefully improve the utility of these novel treatments.

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