# Nivolumab in melanoma: latest evidence and clinical potential

**Douglas B. Johnson, Chengwei Peng and Jeffrey A. Sosman**

*Ther Adv Med Oncol*

2015, Vol. 7(2) 97–106

DOI: 10.1177/1758834014567469



***Abstract*:** Melanoma has historically been considered a refractory disease with few if any options in the advanced/metastatic setting. Advances in both immune and genetically targeted treatment approaches have revolutionized the spectrum of treatment options for melanoma patients over the last several years. Recently, checkpoint inhibition has become a major focus in the immune-based therapy of cancer, especially melanoma. This concept involves inhibition of regulatory cell surface molecules which act normally to dampen or modulate T-cell activation. Cancer, including melanoma, takes advantage of this physiologic mechanism to turn off T-cell activation and prevent effective T-cell antitumor responses. Checkpoint inhibitors such as anti cytotoxic T-lymphocyte antigen 4 (anti-CTLA-4) and anti programmed death-1 (PD-1) can reverse this immune suppression and release T-cell activation. Nivolumab, a monoclonal antibody to the PD-1 receptor, promotes antitumor immunity by removing this key negative regulator of T-cell activation. In phase I/II studies, promising activity and safety have been observed and ongoing phase III trials are comparing nivolumab with other standard of care therapies (chemotherapy, ipilimumab). Efficacy may be even further increased when used in combination with ipilimumab (albeit with increased toxicity). In contrast to typical short-lived responses with cancer therapy in metastatic solid tumors, many responses induced by nivolumab appear durable. In this review, we discuss the evolution of immune therapy in melanoma leading to the development of nivolumab, the clinical experience with this agent, and its future development and clinical potential.

***Keywords:*** anti-PD-1, checkpoint inhibitor, immunotherapy, melanoma, nivolumab

## Background

Advanced melanoma has been resistant to conventional cytotoxic chemotherapy and has historically lacked effective systemic treatment options [Middleton *et al.* 2000; Korn *et al.* 2008]. These poor results spurred the intensive pursuit of alternative treatment strategies for the last several decades, which in part led to the understanding that melanoma is a particularly immunogenic tumor. Agents targeting the programmed death-1 (PD-1) receptor and its ligand (PD-L1) are a new, promising class of therapeutics which inhibit a critical negative regulator of T-cell activation and thereby promote antitumor immunity [Hirano *et al.* 2005, Ott *et al.* 2013]. Nivolumab (BMS-936558) is a monoclonal antibody to PD-1 being developed for treatment of advanced melanoma and other cancers. Clinical trials have demonstrated promising activity particularly in advanced melanoma, nonsmall cell lung cancer and renal cell carcinoma, as well as a tolerable toxicity profile [Topalian *et al.* 2012, 2014; Weber *et al.* 2013]. In this review, we discuss the evolution of immune therapy in melanoma, the clinical experience with nivolumab, and future directions and potential for this agent in melanoma therapy.

## Evolution of immune therapy in melanoma

Numerous strategies to stimulate an antineoplastic immune response have been explored. Historically, the cornerstones of immune therapy were high-dose interleukin-2 (IL-2) for metastatic melanoma and high-dose interferon-α for resected melanoma (stage II and III) at high risk of recurrence. High-dose IL-2 induces objective responses in approximately 15–20% of patients with metastatic melanoma and 6–8% of treated patients

Correspondence to:
**Douglas B. Johnson, MD, MSCI**
Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, 777 Preston Research Building, 2220 Pierce Avenue, Nashville, TN 37232, USA
**Douglas.b.johnson@vanderbilt.edu**

**Chengwei Peng, BS**
**Jeffrey A. Sosman, MD**
Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, Nashville, TN, USA

experience durable (>3 years) complete remissions [Rosenberg *et al.* 1994; Atkins *et al.* 1999]. Severe acute toxicities including multiorgan dysfunction, hemodynamic compromise and confusion preclude therapy in patients with marginal functional status, organ dysfunction or advanced age [Schwartzentruber, 2001]. Furthermore, intensive monitoring in an inpatient setting at an experienced center is a requisite for IL-2 therapy. Interferon-α, used in the adjuvant setting for resected, high-risk melanoma, has demonstrated improved relapse-free survival compared with observation [Kirkwood *et al.* 1996, 2000, 2001, 2004]. However, the effects on overall survival (OS) remain controversial and are modest at best; meta-analyses have demonstrated a relative improvement in OS of approximately 10% [hazard ratio (HR) = 0.89]. Chronic, dose-limiting toxicities are bothersome to nearly all patients and prevent completion of therapy in some. Despite the activity of these therapies, no consistent survival improvement for any agents had been demonstrated in metastatic melanoma prior to 2010 and the need for more effective immune-based therapies remained a clear priority.

Ipilimumab is a fully humanized monoclonal antibody that inhibits cytotoxic T-lymphocyte antigen 4 (CTLA4). CTLA4 engages the antigen presenting cell (APC) receptor B-7.1 and B-7.2 and prevents T cell costimulation, thereby playing a critical modulatory role of immune activation [Leach *et al.* 1996]. Ipilimumab inhibits this interaction and functions to 'remove the brakes' on cellular immune activation, resulting in an antitumor response in some patients. Aberrant T-cell activation against self-antigens may complicate therapy. This was the first agent to demonstrate an improvement in OS in advanced melanoma. In a study of patients progressing on prior therapies, ipilimumab 3mg/kg for 4 doses was compared with a gp100 vaccine [Hodi *et al.* 2010]. A median OS of 10.1 months was identified with ipilimumab compared with 6.4 months with the vaccine (HR for death, 0.68; $p < 0.001$). In the first-line setting, ipilimumab combined with dacarbazine was superior to dacarbazine alone with a median OS of 11.2 months *versus* 9.1 months; 3-year OS was 20.8% compared with 12.2% (HR for death, 0.72; $p < 0.001$) [Robert *et al.* 2011]. The combination of ipilimumab with dacarbazine was limited by frequent elevation of hepatic enzymes preventing continuation of ipilimumab therapy in some patients. These studies led to regulatory approval for ipilimumab as a single agent in the United States and Europe as the first commercially available immune checkpoint inhibitor. Analyses subsequent to approval have demonstrated a 'plateauing' of the OS curve with approximately 20% survival at 3–5 years, suggesting that durable benefit is achieved in a minority of patients [Prieto *et al.* 2012; McDermott *et al.* 2013; Ascierto *et al.* 2014].

Ipilimumab, while representing a substantial advance over previously available therapeutics, has some clear drawbacks. First, classic Response Evaluation Criteria In Solid Tumors (RECIST) responses are rare and often occur after a prolonged duration of therapy, sometimes after initial evidence of progression of disease with new or existing lesions [Ribas *et al.* 2009; Wolchok *et al.* 2009; Hodi *et al.* 2010]. Second, toxicities may occasionally cause substantial morbidity and even mortality, with severe immune-related adverse events occurring in 10–20% of patients [Weber *et al.* 2009; Hodi *et al.* 2010; O'Day *et al.* 2010]. Colitis, endocrinopathies, dermatitis, hepatitis and neurotoxicity are the most common immune-related manifestations of therapy. These adverse events are quite distinct from the toxicity profile of cytotoxic chemotherapy and are generally reversible with corticosteroids.

## Anti-PD-1/PD-L1 therapy

Novel therapeutic targets for immune checkpoint blockade were identified in PD-1 (B7-H1) and its ligand, PD-L1 (B7-DC) [Dong *et al.* 1999; Tseng *et al.* 2001]. PD-1, a receptor expressed on CD4+ and CD8+ T cells (as well as B cells and natural killer cells), binds to PD-L1 and induces functional exhaustion of a cytotoxic immune response. While this interaction does have a physiologic role to promote immune tolerance and suppress autoimmunity, many cancers and chronic viral infections exploit this pathway to achieve immune evasion. The interaction between PD-1 and PD-L1 in cancer causes T-cell apoptosis, limits T-cell expansion and inhibits production of IL-2 and interferon-γ [Dong *et al.* 2002; Pedoeem *et al.* 2014]. PD-L1 is expressed in a variety of tumor types as opposed to B7.1 or B7.2, but also can be found on inflammatory cells such as T lymphocytes and infiltrating mononuclear cells. Inhibiting this immune modulatory axis, therefore, was hypothesized to induce more specific antitumor responses and mitigate autoimmune toxicity. Several antibodies targeting PD-1 [nivolumab, pembrolizumab (MK-3475)] and PD-L1

(MPDL3280A, MEDI4736, others) are in various stages of clinical development for a variety of cancers and all show promising clinical responses.

## Nivolumab

Nivolumab is a fully humanized, monoclonal, immunoglobulin G4 (IgG4) antibody to PD-1. Engaging its target, PD-1, prevents interaction with both PD-L1 and PD-L2. PD-L1 is expressed on approximately 40–50% of melanomas and has limited expression otherwise in most visceral organs with the exception of respiratory epithelium and placental tissue [Dong *et al.* 1999; Petroff *et al.* 2003; Kim *et al.* 2005]. More recently studies have suggested its presence and expression on immune cells infiltrating tumor cells (TIL) [Powderly *et al.* 2013]. Less is known about the expression and role of PD-L2 in immune tolerance and antitumor immunity. In early nivolumab clinical trials, pharmacodynamic studies revealed that receptor binding was largely dose independent over a 30-fold dose increase [Brahmer *et al.* 2010]. PD-1 receptor saturation was maintained for several months even in the absence of continued therapy.

## Phase I clinical trial

The initial phase I clinical trial of nivolumab was conducted in patients with melanoma, lung cancer, renal cell carcinoma and a limited number of other malignancies [Topalian *et al.* 2012]. In this report, 94 patients with melanoma were treated in total with nivolumab, with doses ranging from 0.1 to 10 mg/kg every 2 weeks [cohorts of 0.1, 0.3, 1.0, 3.0 or 10 mg/kg]. Among these patients, the objective response rate (ORR) by RECIST criteria across all dose levels was 28%, with the highest response rate observed in 3 mg/kg (41%). Responses were durable in most patients; in the 21 patients that achieved an objective response, 13 continued to respond for more than 1 year. Of note, 8 responding patients experienced transient tumor regression ranging from 1.9 to 5.6 months. Progression-free survival (PFS) at 24 weeks was 41%.

Overall, nivolumab was well tolerated with low grade fatigue, diarrhea, pruritus, nausea and decreased appetite occurring as the most common adverse events. Grade 3/4 treatment related events occurred in 14% of patients and immune-related adverse events were observed in 6%. Pneumonitis, which has emerged as the most serious toxicity, occurred in nine patients and caused three deaths (none in patients with melanoma).

Long-term follow up from this trial in melanoma patients was recently published including 107 melanoma patients [Topalian *et al.* 2014]; 1 and 2 year survival rates were 62% and 43%, respectively, with a median OS of 16.8 months (Table 1). The updated median PFS was 3.7 months and 27% of patients remained free of progression at 2 years. The updated response rate was similar to the original publication at 31% (33/107) with a Kaplan–Meier estimated median response duration of 2 years (104 weeks). Responding patients also continued to benefit after drug discontinuation; of 17 patients discontinuing therapy for reasons other than disease progression, 12 (71%) had persistent responses lasting $\geqslant$16 weeks. Long-term evaluation of safety was comparable to the original analysis, with 22% of patients experiencing grade 3/4 treatment related events and 5% experiencing grade 3/4 immune-related adverse events. Toxicities were not cumulative and almost exclusively occurred in the first 6 months of therapy. These data were recently updated at the American Society of Clinical Oncology (ASCO) annual meeting in 2014 and demonstrated a 48% survival rate at 2 years and 41% at 3 years [Hodi *et al.* 2014].

## Nivolumab in ipilimumab refractory and naïve patients

Nivolumab was further tested in a second phase I trial to evaluate the impact of prior ipilimumab [Weber *et al.* 2013]. Nivolumab was administered to patients who were ipilimumab refractory (3 cohorts all with 3 mg/kg dose) and naïve (1 mg/kg, 3 mg/kg or 10 mg/kg cohorts) in combination with a peptide vaccine to melanoma antigens gp100, NY-ESO-1 and MART-1. Across all groups, the ORR response was 25% and an additional 21% had stable disease (SD) as their best response (Table 1). In ipilimumab- naïve patients, the ORR was 24% *versus* 26% in ipilimumab refractory patients. Both groups also had similar complete response (CR) + partial response (PR) +SD responses, 45% for ipilimumab naïve patients and 47% for ipilimumab refractory patients. Addition of the vaccine did not appear to alter clinical responses.

The combination of nivolumab and peptide vaccine was generally well tolerated. The most common side effects were reaction at vaccine injection

**Table 1.** Response rate, survival and adverse events in nivolumab trials.

| | Topalian *et al.* 2014 (JCO) | Weber *et al.* 2013 (JCO) | | Wolchok *et al.* 2013 (NEJM) |
|---|---|---|---|---|
| | | Ipilimumab naïve | Ipilimumab refractory | Ipilimumab + Nivolumab concurrent therapy |
| **Patient number** | 107 | 34 | 56 | 53 |
| **Objective response rate (%)** | 31% | 24% | 26% | 40% |
| PDL-1 positive (%)* | 36 | 67 | | 46 |
| PDL-1 negative (%) | 0 | 19 | | 41 |
| **Overall survival at 12 months** | 62% | – | | 85% |
| **Grade 3/4 treatment related adverse events (%)** | 5 | 9 | 5 | 53 |

*Cutoff of 5% staining by IHC.
UHC, immunohistochemistry; PD-L1, programmed death ligand-1.

site and fatigue. In ipilimumab naïve patients, grade 3/4 toxicities included one case of bilateral optic neuritis, one case of fever and one case of pneumonitis, all which resolved with steroid treatment. In ipilimumab refractory patients, grade 3/4 toxicities included one case of rash and one case of pneumonitis, both of which were also responsive to corticosteroids.

PD-L1 expression was investigated as a biomarker for response to therapy (see PD-L1 expression below). Other immune biomarkers were also assessed. Although all patients had at least 10% tumor staining for gp100, NY-ESO-1 or MART-1 for trial inclusion, pretreatment antigen-specific CD8+ T cells for NY-ESO-1 and MART-1 were significantly lower in responders compared with nonresponders. With treatment, CD8+ T cells that recognized MART-1 increased in responders but decreased in nonresponders. Regulatory T cells also decreased in responders and increased in nonresponders at the 12 week assessment.

Early results from a larger phase III clinical trial were also presented at the European Society of Medical Oncology (ESMO) meeting. This study compared nivolumab with cytotoxic chemotherapy (dacarbazine or carboplatin/paclitaxel) in patients who had previously received ipilimumab and BRAF inhibitors if a *BRAF* mutation was identified. In this study, the objective response rate was higher in the nivolumab group compared with chemotherapy at 32% (38 of 120 patients) *versus* 10% (5 of 47). With over 6 months follow up in all patients, 36 of the 38 responding patients had ongoing responses [Weber *et al.* 2014]. In December 2014, nivolumab received approval from the United States Food and Drug Administration for patients who previously received ipilimumab and, if applicable, a BRAF inhibitor.

Results from a randomized phase III trial also became available recently. In this study, previously untreated patients without BRAF mutations were randomized to nivolumab or cytotoxic chemotherapy (dacarbazine). Nivolumab was markedly superior in terms of response rate (40% vs. 13.9%), median PFS (5.1 months vs. 2.2 months), and 1-year overall survival (72.9% vs. 42.1%; all comparisons $p<0.001$). [Robert et al. 2014]

## PD-L1 expression

Since nivolumab inhibits the interaction between PD-1 and its ligand, PD-L1, it was hypothesized that PD-L1 expression by the tumor would be required for response to therapy. To investigate this, 42 pretreatment tumor samples from the phase I trial were assessed by immunohistochemistry (IHC) for expression of PD-L1 (including 18 patients with melanoma). Using a cutoff of 5% IHC as positive expression, 0/17 patients with negative PD-L1 expression achieved an objective response *versus* 9/25 patients with positive PD-L1 expression [Topalian *et al.* 2014]. These findings led to speculation that PD-L1 negative tumors would not respond nivolumab. Subsequent studies, however, have demonstrated that melanomas without detectable PD-L1 levels can also achieve a response, albeit at lower rates [Weber *et al.* 2013]. In the phase II clinical trial, when 5% expression by IHC indicated a positive stain, a 67% ORR was observed in PD-L1(+) tumors compared with a 19% ORR in PD-L1(-) tumors. A statistically significant association between PD-L1 expression and response rate was observed, although as

mentioned, patients with PD-L1(-) tumors still responded. Further follow-up data from the phase I trial assessing OS demonstrated that median survival among 18 PD-L1(+) patients had not been reached and was 12.1 months for 23 patients with PD-L1(-) melanomas [Hodi *et al.* 2014]. These efforts show that PD-L1(-) melanomas can respond to anti-PD-1.

Other issues also complicate the routine use of PD-L1 as a biomarker for response. The PD-L1 assay developed alongside nivolumab is an IgG1 monoclonal antibody, clone 5-H1, and is now being developed by Dako® [Taube *et al.* 2012]. Each pharmaceutical company with anti-PD-1/PD-L1 antibodies late in development has distinct companion assays to measure PD-L1 expression. Moreover, PD-L1 expression appears to be dynamic and may be modulated by a number of factors [Akbay *et al.* 2013; Frederick *et al.* 2013]. At this time, therefore, the role of PD-L1 expression in treatment decision making for melanoma patients remains yet to be defined.

## Other anti-PD-1/PD-L1 antibodies

Pembrolizumab (MK-3475) is another antibody to PD-1 which appears to have comparable efficacy and tolerability. This agent was evaluated in a phase I study of 411 patients, of which 190 patients were naïve to ipilimumab [Ribas *et al.* 2014]. In this cohort, a response rate of 40% was observed across dose levels. In addition, in 173 patients previously treated with ipilimumab, 26% experienced objective responses [Robert *et al.* 2014]. Pembrolizumab has recently received approval from the US Food and Drug Administration (FDA) for patients previously treated with ipilimumab and, if harboring a BRAF mutation, BRAF inhibitors.

MPDL3280A is a monoclonal antibody to PD-L1. This agent has also been tested in numerous malignancies and is clinically active in melanoma. Among 35 patients with evaluable responses, 9 (26%) experienced an objective response [Hamid *et al.* 2013b]. Other agents are previously or currently in development (BMS-936559, MEDI4736, CT-011) but are beyond the scope of this review.

## Nivolumab plus ipilimumab

Despite the promising activity of nivolumab as a single agent, less than half of patients achieved a durable response. Nivolumab and ipilimumab remove key negative T-cell regulators at distinct phases of the T-cell activation process, putatively at the level of antigen-presenting cell–T cell interaction, and at the level of effector T cell–tumor interface, respectively. Therefore, it was hoped that combined immune checkpoint blockade would offer superior efficacy compared with either agent alone (albeit tempered by concerns of increased toxicity). Preclinical models demonstrate that these agents in combination decrease regulatory T cells, increase tumor infiltrating effector T cells, and extend survival more effectively than either alone [Curran *et al.* 2010]. The combination of nivolumab and ipilimumab was evaluated in a phase I clinical trial [Wolchok *et al.* 2013]. Patients were enrolled in cohorts that either received both drugs concurrently or in sequence. In the concurrent group, regimen dosing of nivolumab ranged from 0.3 mg/kg to 3 mg/kg and ipilimumab dosing ranged from 1 mg/kg to 3 mg/kg.

The maximum dosing level associated with an acceptable level of adverse events was 1 mg/kg of nivolumab and 3 mg/kg of ipilimumab. With this regimen, the ORR was 53% with tumor reduction of ⩾80% in all 9 responding patients. Moreover, this degree of tumor regression occurred rapidly, at the time of first computerized tomography (CT) scan, in all patients. Across all doses, the ORR was 40% and aggregate clinical activity rate (defined as conventional, unconfirmed, or immune-related responses or SD for ⩾24 weeks) was observed in 65% of patients (Table 1). These results indicate that combination therapy may prove to be more effective than monotherapy with either drug alone.

Concurrent therapy had manageable but significant toxicities, with grade 3/4 treatment related adverse events occurring in 53%, higher than in monotherapy trials. The most common toxicities were asymptomatic elevations in lipase (13%), aspartate transaminase (AST) (13%), and alanine aminotransferase (ALT) (11%). The rate of grade 3/4 gastrointestinal toxicity was 9% and serious pneumonitis occurred in 1 patient. Side effects at the maximum acceptable dosing (nivolumab at 1 mg/kg and ipilimumab at 3 mg/kg) included grade 3 uveitis in one patient and grade 3 elevations in AST and ALT levels in another patient. In the majority of cases, toxicities were manageable with corticosteroids (38%), infliximab (2 patients), or mycophenolate mofetil (1 patient).

PD-L1 expression (threshold of 5% staining by IHC) and absolute lymphocyte count (ALC) during treatment (measured between 5 and 7 weeks after therapy initiation) were evaluated as potential biomarkers. Objective responses were observed in patients with both PD-L1 positive tumor samples (6 of 13 patients) and PD-L1 negative tumor samples (9 of 22 patients), indicating that those without PD-L1 expression on tumors may still derive benefits from treatment. This biomarker, therefore, likely has limited value for combination therapy. Increase in ALC, a significant predictor of ipilimumab monotherapy benefit, did not appear predictive in this trial. The ORR was comparable in patients with a low ALC $< 1000$ cells/cm$^3$ (43%) and normal or high ALC (40%).

Data from this clinical study were recently updated and included an additional 41 patients treated at the nivolumab 1mg/kg plus ipilimumab 3mg/kg dose level [Sznol *et al*. 2014]. The OS at 1 and 2 years was 85% and 79%, respectively, essentially unprecedented results in melanoma trials (albeit in a small sample size). ORR (42% with 17% CR rate) and rate of grade 3/4 adverse events (61%) both slightly increased from the previous analysis (with 1/94 treated patients dying from a treatment-related adverse event). Median duration of response, OS or PFS had not been reached at the time of analysis. Also of note, aggregate clinical activity rate appeared similar between BRAF mutant (60%, $n = 10$) and BRAF wild type melanoma (73%, $n = 26$).

## Future directions and therapeutic potential

Nivolumab and other anti-PD-1/PD-L1 directed therapies represent a major step forward in melanoma therapeutics. While randomized phase III trials have not yet been completed with nivolumab, response rates and 2 year OS observed in early trials have been superior to previously developed immune therapies and molecularly targeted agents. In addition, the incidence of immune-related adverse events appears significantly lower than those observed with anti-CTLA4 directed therapies. Given the favorable efficacy and toxicity profile, nivolumab (along with pembrolizumab) will almost certainly become a standard first-line option for patients with advanced melanoma. The initial regulatory approval of nivolumab, however, may be for previously treated patients (as with pembrolizumab).

One unresolved question currently is whether nivolumab, pembrolizumab, MPDL3280A, or another PD-1/PD-L1-directed therapy earlier in development is the most effective in advanced melanoma. In the initial phase I studies, the ORR of pembrolizumab appears to be somewhat higher than nivolumab (40% and 29%, respectively) [Topalian *et al.* 2012, Hamid *et al.* 2013a]. On the contrary, the response rate in patients treated following ipilimumab appeared possibly higher in nivolumab (32% and 26%) [Robert *et al.* 2014; Weber, 2014]. These agents have not been compared directly and were evaluated in different populations. Moreover, long-term survival has not been evaluated and, therefore, additional studies will likely clarify the relative efficacy of these agents.

Nivolumab in combination with ipilimumab may have still more activity than single-agent nivolumab, although at the cost of increased adverse events [Wolchok *et al.* 2013]. This combination produced rapid and dramatic responses in a phase I trial assessing this combination and OS was 79% at 2 years. Currently, a three-arm phase III trial is comparing nivolumab, ipilimumab and the combination of nivolumab and ipilimumab [ClinicalTrials.gov identifier: NCT01844505]. This study will give insight into whether combination therapy or either agent alone is the more appropriate upfront treatment option.

With the early success of nivolumab plus ipilimumab, additional immune modulators are also being evaluated in combination with nivolumab. Currently, ongoing early-phase clinical trials (see Table 2) include nivolumab plus either lirilumab (anti-KIR) [ClinicalTrials.gov identifier: NCT01714739], BMS-986016 (anti-LAG-3) [ClinicalTrials.gov identifier: NCT01968109] and BMS-982470 (IL-21) [ClinicalTrials.gov identifier: NCT01629758]. These agents aim to synergize with nivolumab to reverse neoplastic immune evasion through natural killer (NK) cell activation (anti-KIR), dual T-cell checkpoint blockade (anti-LAG-3) or a broad range of immune functions including CD8+ T cell and NK cell activation (IL-21) [Woo *et al.* 2012; Kohrt *et al.* 2014; Spolski and Leonard, 2014]. These combinatorial approaches may play a major role, particularly in tumors in which the PD-1/PD-L1 axis is only a partial contributor to neoplastic immune evasion.

Combining nivolumab with targeted therapies (BRAF and MEK-directed therapies) has also

**Table 2.** Ongoing combination studies involving nivolumab.

| Combination | Combination Partner | Trial Phase | ClinicalTrials.gov identifier |
|---|---|---|---|
| N *versus* N + ipilimumab *versus* ipilimumab | Anti-CTLA4 | III | NCT01844505 |
| N + ipilimumab *versus* ipilimumab | Anti-CTLA4 | II | NCT01927419 |
| N + ipiliumab | Anti-CTLA4 | I (biomarker) | NCT01621490 |
| N + lirilumab | Anti-KIR | I (solid tumors) | NCT01714739 |
| N + BMS-986016 | Anti-LAG-3 | I (solid tumors) | NCT01968109 |
| N + BMS-982470 | IL-21 | I (solid tumors) | NCT01629758 |
| N + ipilimumab | Anti-CTLA4 | Expanded access | NCT02186249 |

KIR, killer cell Ig-like receptors; LAG-3, lymphocyte-activation gene 3; IL, interleukin; N, nivolumab.

generated significant interest [Hu-Lieskovan *et al.* 2014]. In preclinical models, these agents (particularly BRAF inhibitors) influence the tumor microenvironment, tumor antigen expression and T-cell function. Extinction of BRAF by RNA interference or MEK inhibitors modulated cytokine expression in the tumor microenvironment, specifically decreasing production of immune-suppressive cytokines (IL-6, IL-10, vascular endothelial growth factor) [Sumimoto *et al.* 2006]. BRAF inhibitors increase melanoma-lineage antigen expression (e.g. MART1, gp100, tyrosinase) which may lead to enhanced immunogenicity and T-cell recognition [Boni *et al.* 2010; Frederick *et al.* 2013]. PD-L1 expression may also be influenced by BRAF inhibition although this relationship appears complex [Jiang *et al.* 2012; Frederick *et al.* 2013]. In addition, tumor infiltrating lymphocytes (CD8+ T cells) appear to increase with selective BRAF inhibitor therapy [Wilmott *et al.* 2012; Liu *et al.* 2013; Frederick *et al.* 2013].

This type of combination strategy is attractive clinically and suggests the possibility of combining the frequent and rapid responses of targeted therapy with the durability of an immune response. In early clinical trials, experience with immune/targeted regimens gives reason for cautious optimism for nivolumab/BRAF inhibitor combinations. Vemurafenib and ipilimumab induced intolerable transaminitis in the few patients that were treated [Ribas *et al.* 2013]. A phase I trial of ipilimumab plus dabrafenib ± trametinib, however, appears to have a reasonable side effect profile in recently presented data for the ipilumumab/dabrafenib arm [Puzanov *et al.* 2014]. The triplet arm, though, led to unacceptable gastrointestinal toxicity and has been discontinued. At this time, no trials combining nivolumab with BRAF or MEK inhibitors are ongoing, although early phase studies of pembrolizumab with dabrafenib/trametinib [ClinicalTrials.gov identifier: (NCT02130466] and MPDL3280A with cobimetinib [ClinicalTrials.gov identifier: NCT01988896] and vemurafenib [ClinicalTrials.gov identifier: NCT01656642[ respectively are enrolling.

## Conclusion

Nivolumab and other antibodies blocking PD-1 will almost certainly play a central role in melanoma therapeutics in the future. The potential for durable antitumor immune responses coupled with a favorable toxicity profile makes nivolumab an attractive therapeutic option as a single agent. Furthermore, the comparative lack of toxicities likely will lead to combination strategies with other immune-stimulatory and genetically targeted agents. Identifying predictive biomarkers to assist in therapeutic decision making and determining the most appropriate partners for melanoma therapy are critical next steps in harnessing the potential of nivolumab.

## Funding

D.B.J. was supported by NIH grant K12 CA 0906525.

## Conflict of interest statement

J.A.S. is on the advisory board for Bristol Myers Squibb and Roche.

## References

Akbay, E., Koyama, S., Carretero, J., Altabef, A., Tchaicha, J., Christensen, C. *et al.* (2013) Activation of the PD-1 pathway contributes to immune escape in EGFR-driven lung tumors. *Cancer Discov* 3: 1355–1363.

Ascierto, P., Simeone, E., Sileni, V., Pigozzo, J., Maio, M., Altomonte, M. *et al.* (2014) Clinical experience with ipilimumab 3 mg/kg: real-world efficacy and safety data from an expanded access programme cohort. *J Transl Med* 12: 116.

Atkins, M., Lotze, M., Dutcher, J., Fisher, R., Weiss, G., Margolin, K. *et al.* (1999) High-dose recombinant interleukin 2 therapy for patients with metastatic melanoma: analysis of 270 patients treated between 1985 and 1993. *J Clin Oncol* 17: 2105–2116.

Boni, A., Cogdill, A., Dang, P., Udayakumar, D., Njauw, C., Sloss, C. *et al.* (2010) Selective BRAFV600E inhibition enhances T-cell recognition of melanoma without affecting lymphocyte function. *Cancer Res* 70: 5213–5219.

Brahmer, J., Drake, C., Wollner, I., Powderly, J., Picus, J., Sharfman, W. *et al.* (2010) Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. *J Clin Oncol* 28: 3167–3175.

Curran, M., Montalvo, W., Yagita, H. and Allison, J. (2010) PD-1 and CTLA-4 Combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors. *Proc Natl Acad Sci U S A* 107: 4275–4280.

Dong, H., Strome, S., Salomao, D., Tamura, H., Hirano, F., Flies, D. *et al.* (2002) Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. *Nat Med* 8: 793–00.

Dong, H., Zhu, G., Tamada, K. and Chen, L. (1999) B7-H1, a Third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. *Nat Med* 5: 1365–1369.

Frederick, D., Piris, A., Cogdill, A., Cooper, Z., Lezcano, C., Ferrone, C. *et al.* (2013) BRAF inhibition is associated with enhanced melanoma antigen expression and a more favorable tumor microenvironment in patients with metastatic melanoma. *Clin Cancer Res* 19: 1225–1231.

Hamid, O., Robert, C., Daud, A., Hodi, F., Hwu, W., Kefford, R. *et al.* (2013a) Safety and tumor responses with lambrolizumab (anti-PD-1) in melanoma. *N Engl J Med* 369: 134–144.

Hamid, O., Sosman, J., Lawrence, D., Sullivan, R., Ibrahim, N., Kluger, H. *et al.* (2013b) Clinical activity, safety, and biomarkers of MPDL3280A, an engineered PD-L1 antibody in patients with locally advanced or metastatic melanoma (MM). *J Clin Oncol* 31: 9010.

Hirano, F., Kaneko, K., Tamura, H., Dong, H., Wang, S., Ichikawa, M. *et al.* (2005) Blockade of B7-H1 and PD-1 by monoclonal antibodies potentiates cancer therapeutic immunity. *Cancer Res* 65: 1089–1096.

Hodi, F., O'Day, S., McDermott, D., Weber, R., Sosman, J., Haanen, J. *et al.* (2010) Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* 363: 711–723.

Hodi, F., Sznol, M., Kluger, H., McDermott, D., Carvajal, R., Lawrence, D. *et al.* (2014) Long-term survival of ipilimumab-naïve patients (pts) with advanced melanoma (MEL) treated with nivolumab (anti-PD-1, BMS-936558, ONO-4538) in a phase I trial. *J Clin Oncol* 32: 9002.

Hu-Lieskovan, S., Robert, L., Homet Moreno, B. and Ribas, A. (2014) Combining targeted therapy with immunotherapy in braf-mutant melanoma: promise and challenges. *J Clin Oncol* 32: 2248–2254.

Jiang, X., Zhou, J., Giobbie-Hurder, A., Wargo, J. and Hodi, F. (2012) The paradoxical activation of MAPK in melanoma cells resistant to BRAF inhibition promotes PD-L1 Expression that is reversible by MEK and PI3K inhibition. *Clin Cancer Res* 19: 598–609.

Kim, J., Myers, A., Chen, L., Pardoll, D., Truong-Tran, Q., Lane, A. *et al.* (2005) Constitutive and inducible expression of B7 family of ligands by human airway epithelial cells. *Am J Respir Cell Mol Biol* 33: 280–289.

Kirkwood, J., Ibrahim, J., Sondak, V., Richards, J., Flaherty, L., Ernstoff, M. *et al.* (2000) High- and low-dose interferon alfa-2B in high-risk melanoma: first analysis of intergroup trial E1690/S9111/C9190. *J Clin Oncol* 18: 2444–2458.

Kirkwood, J., Ibrahim, J., Sosman, J., Sondak, V., Agarwala, S., Ernstoff, M. *et al.* (2001) High-dose interferon alfa-2b significantly prolongs relapse-free and overall survival compared with the GM2-KLH/QS-21 vaccine in patients with resected stage IIb-Iii melanoma: results of intergroup trial E1694/S9512/C509801. *J Clin Oncol* 19: 2370–2380.

Kirkwood, J., Manola, J., Ibrahim, J., Sondak, V., Ernstoff, M. and Rao, U. (2004) A pooled analysis of Eastern Cooperative Oncology Group and intergroup trials of adjuvant high-dose interferon for melanoma. *Clin Cancer Res* 10: 1670–1677.

Kirkwood, J., Strawderman, M., Ernstoff, M., Smith, T., Borden, E. and Blum, R. (1996) Interferon alfa-2b adjuvant therapy of high-risk resected cutaneous melanoma: the Eastern Cooperative Oncology Group trial EST 1684. *J Clin Oncol* 14: 7–17.

Kohrt, H., Thielens, A., Marabelle, A., Sagiv-Barfi, I., Sola, C., Chanuc, F. *et al.* (2014) Anti-KIR antibody enhancement of anti-lymphoma activity of natural killer cells as monotherapy and in combination with anti-CD20 antibodies. *Blood* 123: 678–686.

Korn, E., Liu, P., Lee, S., Chapman, J., Niedzwiecki, D., Suman, V. *et al.* (2008) Meta-analysis of phase

II cooperative group trials in metastatic stage IV melanoma to determine progression-free and overall survival benchmarks for future phase II trials. *J Clin Oncol* 26: 527–534.

Leach, D., Krummel, M. and Allison, J. (1996) Enhancement of Antitumor immunity by CTLA-4 blockade. *Science* 271: 1734–1736.

Liu, C., Peng, W., Xu, C., Lou, Y., Zhang, M., Wargo, J. *et al.* (2013) BRAF inhibition increases tumor infiltration by T cells and enhances the antitumor activity of adoptive immunotherapy in mice. *Clin Cancer Res* 19: 393–403.

McDermott, D., Haanen, J., Chen, T., Lorigan, P., O'Day, S. and MDX010-20 Investigators (2013) Efficacy and safety of ipilimumab in metastatic melanoma patients surviving more than 2 years following treatment in a phase III trial (MDX010-20). *Ann Oncol* 24: 2694-2698.

Middleton, M., Grob, J., Aaronson, N., Fierlbeck, G., Tilgen, W., Seiter, S. *et al.* (2000) Randomized phase III study of temozolomide *versus* dacarbazine in the treatment of patients with advanced metastatic malignant melanoma. *J Clin Oncol* 18: 158–166.

O'Day, S., Maio, M., Chiarion-Sileni, V., Gajewski, T., Pehamberger, H., Bondarenko, I. *et al.* (2010) Efficacy and Safety of Ipilimumab monotherapy in patients with pretreated advanced melanoma: a multicenter single-arm phase II study. *Ann Oncol* 21: 1712–1717.

Ott, P., Hodi, F. and Robert, C. (2013) Ctla-4 and Pd-1/Pd-L1 Blockade: New Immunotherapeutic modalities with durable clinical benefit in melanoma patients. *Clin Cancer Res* 19: 5300–309.

Pedoeem, A., Azoulay-Alfaguter, I., Strazza, M., Silverman, G. and Mor, A. (2014) Programmed death-1 pathway in cancer and autoimmunity. *Clin Immunol* 153: 145–152.

Petroff, M., Chen, L., Phillips, T., Azzola, D., Sedlmayr, P. and Hunt, J. (2003) B7 Family molecules are favorably positioned at the human maternal-fetal interface. *Biol Reprod* 68: 1496–1504.

Powderly, J., Koeppen, H., Hodi, F., Sosman, J., Gettinger, S., Desai, R. *et al.* (2013) Biomarkers and associations with the clinical activity of PD-L1 blockade in a MPDL3280A study. *J Clin Oncol* 31: 3001.

Prieto, P., Yang, J., Sherry, R., Hughes, M., Kammula, U., White, D. *et al.* (2012) CTLA-4 blockade with ipilimumab: long-term follow-up of 177 patients with metastatic melanoma. *Clin Cancer Res* 18: 2039–2047.

Puzanov, I., Callahan, M., Linette, G., Patel, S., Luke, J., Sosman, J. *et al.* (2014) Phase 1 study of the BRAF inhibitor dabrafenib (D) with or without the MEK inhibitor trametinib (T) in combination with ipilimumab (IPI) for V600E/K mutation–positive unresectable or metastatic melanoma (MM). *J Clin Oncol* 32: 2511.

Ribas, A., Chmielowski, B. and Glaspy, J. (2009) Do we need a different set of response assessment criteria for tumor immunotherapy? *Clin Cancer Res* 15: 7116–7118.

Ribas, A., Hodi, F., Callahan, M., Konto, C. and Wolchok, J. (2013) Hepatotoxicity with combination of vemurafenib and ipilimumab. *N Engl J Med* 368: 1365–1366.

Ribas, A., Hodi, F., Kefford, R., Hamid, O., Daud, A., Wolchok, J. *et al.* (2014) Efficacy and safety of the Anti-PD-1 monoclonal antibody MK-3475 in 411 patients (pts) with melanoma (MEL). *J Clin Oncol* 32: LBA9000.

Robert, C., Long, GV., Brady B., Dutriaux, C., Maio, M., L, Mortier *et al.* (2014) Nivolumab in previously untreated melanoma without BRAF mutation. *N Engl J Med*. DOI: 10.1056/NEJMoa1412082.

Robert, C., Ribas, A., Wolchok, J., Hodi, F., Hamid, O., Kefford, R. *et al.* (2014) Anti-programmed-death-receptor-1 treatment with pembrolizumab in ipilimumab-refractory advanced melanoma: a randomised dose-comparison cohort of a phase 1 trial. *Lancet*: 384: 1109–1117.

Robert, C., Thomas, L., Bondarenko, I., O'Day, S., Weber, J., Garbe, C. *et al.* (2011) Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N Engl J Med* 364: 2517–2526.

Rosenberg, S., Yang, J., Topalian, S., Schwartzentruber, D., Weber, J., Parkinson, D. *et al.* (1994) Treatment of 283 Consecutive patients with metastatic melanoma or renal cell cancer using high-dose bolus interleukin 2. *JAMA* 271: 907–913.

Schwartzentruber, D. (2001) Guidelines for the safe administration of high-dose interleukin-2. *J Immunother* 24: 287–293.

Spolski, R. and Leonard, W. (2014) Interleukin-21: a double-edged sword with therapeutic potential. *Nat Rev Drug Discov* 13: 379–395.

Sumimoto, H., Imabayashi, F., Iwata, T. and Kawakami, Y. (2006) The BRAF-MAPK signaling pathway is essential for cancer-immune evasion in human melanoma cells. *J Exp Med* 203: 1651–1656.

Sznol, M., Kluger, H., Callahan, M., Postow, M., Gordon, R., Segal, N. *et al.* (2014) Survival, response duration, and activity by BRAF mutation (MT) status of nivolumab (NIVO, anti-PD-1, BMS-936558, ONO-4538) and ipilimumab (IPI) concurrent therapy in advanced melanoma (MEL). *J Clin Oncol* 32: LBA 9003.

Taube, J., Anders, R., Young, G., Xu, H., Sharma, R., Mcmiller, T. *et al.* (2012) Colocalization of inflammatory response with B7-H1 expression in human melanocytic lesions supports an adaptive resistance mechanism of immune escape. *Sci Transl Med* 4: 127ra137.

Topalian, S., Hodi, F., Brahmer, J., Gettinger, S., Smith, D., McDermott, D. *et al.* (2012) Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med* 366: 2443–2454.

Topalian, S., Sznol, M., McDermott, D., Kluger, H., Carvajal, R., Sharfman, W. *et al.* (2014) Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. *J Clin Oncol.* DOI: 10.1200/JCO.2013.53.0105.

Tseng, S., Otsuji, M., Gorski, K., Huang, X., Slansky, J., Pai, S. *et al.* (2001) B7-DC, a new dendritic cell molecule with potent costimulatory properties for T cells. *J Exp Med* 193: 839–846.

Weber, J., Minor, DR., D'Angelo, S., Hodi, FS., Gutzmer, R., Neyns B. *et al.* (2014) A phase 3 randomized, open-label study of nivolumab *versus* investigator's choice of chemotherapy in patients with advanced melanoma after prior anti-CTLA4 therapy. *ECCO Annual Congress*: abstract LBA3.

Weber, J., Kudchadkar, R., Yu, B., Gallenstein, D., Horak, C., Inzunza, H. *et al.* (2013) Safety, efficacy, and biomarkers of nivolumab with vaccine in ipilimumab-refractory or -naïve melanoma. *J Clin Oncol* 31: 4311–4318.

Weber, J., Thompson, J., Hamid, O., Minor, D., Amin, A., Ron, I. *et al.* (2009) A Randomized, double-blind, placebo-controlled, phase II study comparing the tolerability and efficacy of ipilimumab administered with or without prophylactic budesonide in patients with unresectable stage III or IV melanoma. *Clin Cancer Res* 15: 5591–5598.

Wilmott, J., Long, G., Howle, J., Haydu, L., Sharma, R., Thompson, J. *et al.* (2012) Selective BRAF inhibitors induce marked T-cell infiltration into human metastatic melanoma. *Clin Cancer Res* 18: 1386–394.

Wolchok, J., Hoos, A., O'Day, S., Weber, J., Hamid, O., Lebbe, C. *et al.* (2009) Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. *Clin Cancer Res* 15: 7412–7420.

Wolchok, J., Kluger, H., Callahan, M., Postow, M., Rizvi, N., Lesokhin, A. *et al.* (2013) Nivolumab plus ipilimumab in advanced melanoma. *N Engl J Med* 369: 122–133.

Woo, S., Turnis, M., Goldberg, M., Bankoti, J., Selby, M., Nirschl, C. *et al.* (2012) Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. *Cancer Res* 72: 917–927.