Published in final edited form as:

Cancer J. 2018 ; 24(1): 7–14. doi:10.1097/PPO.0000000000000299.

Clinical development of PD-1/PD-L1 in Advanced Melanoma

Rodrigo Ramella Munhoz, MD¹ and Michael Andrew Postow, MD^{2,3,*}

¹Melanoma/Sarcoma Group - Oncology Center, Hospital Sírio Libanês

²Melanoma and Immunotherapeutics Service – Memorial Sloan Kettering Cancer Center

³Weill Cornell Medical College

Abstract

The development of new treatment options has dramatically improved the landscape for patients with advanced melanoma. Part of these advances emerged through the identification of the importance of factors that regulate the immune system, including proteins that negatively modulate T cell-mediated responses termed “immune checkpoints.” Indeed, blockade of the *cytotoxic T-lymphocyte associated protein 4* (CTLA-4) immune checkpoint served as a proof of principle that the manipulation of these molecules could induce robust anticancer effects, yet limited to a small percentage of patients. Targeting a distinct checkpoint, the *Programmed Death Receptor 1* (PD-1) yielded improved outcomes and reduced toxicity compared to CTLA-4 blockade and in separate studies, chemotherapy. More recently, combined CTLA-4/PD-1 blockade was shown to result in higher response rates, while accompanied by increased toxicity. In this article, we review the clinical development of anti-PD-1 monotherapy and combinations that may expand the benefit of immunotherapy for patients with advanced melanoma.

Keywords

melanoma; immunotherapy; nivolumab; pembrolizumab; PD-1; immune-checkpoint blockade

Introduction

Although metastatic melanoma is still generally perceived as an aggressive disease with limited treatment options, many new treatment options have emerged over the past decade. These advances have led to a meaningful improvement in response rates and prolonged survival for patients.[1]

The recognition of aberrant activation of the *mitogen-activated protein kinase pathway* (MAPK) resulting mutations of the *V-Raf Murine Sarcoma Viral Oncogene Homolog B* (BRAF) gene paved the way for the development of highly active small molecules,[3,4]

Corresponding author: Michael A. Postow, MD, Memorial Sloan Kettering Cancer Center., 300E 66th Street. New York, USA. Postal code 10065., postowm@mskcc.org.

Conflicts of Interest and Source of funding:

M.A.P. - honoraria (BMS, Merck); advisory role (BMS, Merck, Novartis, Array BioPharma); research funding (BMS)

R.R.M. - honoraria (BMS, MSD, Roche, Novartis); advisory role (Lilly, Merck Serono, MSD, Roche, Novartis); travel expenses (BMS, MSD, Roche, TEVA, Novartis); research involvement (BMS, Lilly, Roche)

resulting in the approval of the BRAF inhibitors vemurafenib and dabrafenib and the *mitogen-activated protein kinases enzymes* (MEK) inhibitors cobimetinib and trametinib for patients with tumors harboring oncogenic BRAF mutations.[4–6]

Advances in the manipulation of the MAPK pathway have been mirrored by the development of monoclonal antibodies capable of improving an antitumor immune response by inhibiting negative modulators of the immune response, or immune checkpoints.[7] Agents targeting the *cytotoxic T lymphocyte associated antigen 4* (CTLA4) and *programmed cell death protein 1* (PD-1) and its ligand (PD-L1) have become important strategies to control tumor growth in patients with advanced melanoma, with now impressive survival gains (Figure 1). In 2011, ipilimumab, a fully human monoclonal antibody against CTLA-4 became the first agent of its class approved for clinical use for patients with advanced melanoma. The approval was based on survival gains in randomized phase 3 clinical trials,[8,9] and provided a new therapy for patients that led to a solid possibility of prolonged antitumor immunity and sustained disease control.[10,11]

Although CTLA-4 was the first immune checkpoint to demonstrate clinical success, other investigations have explored a wide variety of cell-surface receptors distinct from CTLA-4 that are also involved in the modulation of immune responses, including the immune-agonist receptors CD28, OX40, CD27, CD137, HVEM and GITR, and the inhibitory co-receptors (or other checkpoints) PD-1, VISTA, BTLA, TIM-3, and LAG-3.[12,13] Among these newer targets, blockade of PD-1 or its ligand, PD-L1, has been more extensively studied.

Preclinical studies first shed light on the relevance of the PD-1 and PD-L1 in regulating immune responses. Mice lacking PD-1 were shown to develop auto-immune manifestations, including lupus-like arthritis, glomerulonephritis, autoimmune cardiomyopathy and graft-versus-host-like disease, highlighting the role of the PD-1/PD-L1 and PD-L2 axis in the maintenance of tolerance and downregulation of immune responses.[14,15] The interaction of PD-1 and its ligands PD-L1 and PD-L2 was subsequently confirmed as an inhibitory pathway involved in the negative regulation of peripheral T-cell activity and related to evasion mechanisms by tumor cells,[16,17] proposing a potential target for drug development. Indeed, robust and durable tumor regressions have been demonstrated in clinical trials investigating the antitumor effects of anti-PD-1 agents. Currently, the fully human IgG4 monoclonal antibody nivolumab, and the humanized IgG4 antibody pembrolizumab are approved for clinical use and among the standard first-line treatment options for patients with advanced melanoma.[1] More recently, an enhanced antitumor effect was demonstrated with the concurrent use of ipilimumab and nivolumab, and a growing number of other combinations are being investigated.[18,19]

In this article, we review the results of clinical trials that support the use of nivolumab, pembrolizumab and combined checkpoint blockade in clinical practice for patients with advanced melanoma and address open questions related to the clinical use of these agents.

Early-phase clinical trials investigating single-agent anti-PD-1 therapy

The development of anti-PD-1 agents in melanoma prompted significant enthusiasm since early-phase clinical trials. In a dose-escalation, phase I study (CA209-003), nivolumab was evaluated at doses ranging from 0.1–10mg/kg of body weight every 2 weeks in 296 patients with a variety of solid tumors, including advanced melanoma, non-small cell lung cancer, prostate cancer, renal cell carcinoma and colorectal cancer. Of note, no maximum tolerated dose (MTD) was achieved, and nivolumab demonstrated a favorable toxicity profile, with an incidence of grade 3 or 4 treatment-related adverse events of 14%. Activity was demonstrated across all doses; objective responses occurred in an impressive 26 of 94 evaluable patients with melanoma (28%).[20] Results of 107 patients with melanoma treated with nivolumab in this phase I study were subsequently updated in 2014: median overall survival (OS) was 16.8 months (95% CI: 12.5–31.6 months), with a 2-year OS rate of 43%. It is important to highlight that 62% of these patients had received two to five prior systemic therapies before enrolling onto this phase I trial. Objective responses (OR) were seen in 31% of patients and, in line with prior observations with ipilimumab, responses were durable.[21] Five-year overall survival analyses were presented in 2016, confirming the possibility of sustained disease control with PD-1 blockade, with 34% of the melanoma patients alive at 5 yearsS.[22]

Similarly, pembrolizumab demonstrated significant antitumor effect and good tolerability in a large-scale, phase I study (KEYNOTE-001) that included 655 patients divided across multiple cohorts and enrolled between December/2011 and September/2013.[23] Pembrolizumab was administered intravenously at 2mg/kg to 10mg/kg every 2 weeks and every 3 weeks. A comprehensive analysis including the 8 study cohorts was published after a median follow-up of 21 months.[24] Once again, single-agent anti-PD-1 therapy was shown to be well tolerated: 14% of the patients experienced a grade 3 or 4 treatment-related adverse event. Objective response rates (ORR) across all cohorts and doses was 33%, and 45% in treatment-naïve patients. Of note, 74% (152/205) of responses were ongoing at the time of data cutoff. Median OS in the total population was 23 months (95% CI: 20–29 months), with a 2-year OS rate of 49%. Median overall survival was 31 months in treatment-naïve patients (95% CI: 24 months - not reached). Three-year overall survival analyses of KEYNOTE-001 were presented in 2016:[25] while the duration of response had not been reached, median OS was 24.4 months in the general population and 32.2 months in treatment-naïve patients.

The spectrum of toxicity of both nivolumab and pembrolizumab in early-phase clinical trials is, as expected, largely attributable to immune-related events, and the most frequent any-grade adverse events were fatigue, rash and/or pruritus, diarrhea and thyroiditis/hypothyroidism.[26] Nevertheless, virtually any organ or tissue may be affected by excessive immunity in the setting of PD-1 blockade, and serious, yet uncommon adverse events include pneumonitis, nephritis, hypophysitis, adrenalitis, myocarditis, hematologic disorders (example: immune mediated hemolytic anemia) and neurologic events (examples: Guillain-Barré syndrome, myasthenic syndromes, and meningitis/meningoencephalitis). Other laboratory abnormalities can occur, including elevation of AST and/or ALT (although severe hepatitis and liver failure are rare) as well as elevations in amylase and lipase. It is important

to highlight that it is possible that late toxicities may ensue after a long duration on treatment, but it does not appear that toxicities are cumulative as treatment duration continues.[21]

Efficacy of single-agent anti-PD-1 agents versus standard therapies in ipilimumab-refractory patients in randomized trials

Due to unprecedented antitumor activity resulting from anti-PD-1 immunotherapy in early-phase clinical studies, randomized registration trials of nivolumab and pembrolizumab were rapidly conducted in order to assess the efficacy and tolerability of these agents initially in ipilimumab (and BRAF inhibitor)-refractory patients.

In the CheckMate-037 trial, patients with advanced melanoma who progressed after ipilimumab (and a BRAF inhibitor for those with a BRAF-V600 mutation) were randomly assigned 2:1 to nivolumab 3mg/kg administered every 2 weeks (n=272) or investigator's choice of chemotherapy (dacarbazine or carboplatin/paclitaxel) (n=133) between December/2012 and January/2014.[27] The primary endpoints were OS and patients were stratified according to the presence or absence of a BRAF mutation, tumor expression of PD-L1 and previous response to ipilimumab. Among the 120 initial patients randomly allocated to nivolumab treatment, ORR was 31.7%, in comparison to 10.6% in the chemotherapy group. Results were updated after a median follow up of approximately 2 years, including the 405 patients initially accrued.[28] ORR (27% versus 10%) and duration of response (32 versus 13 months) were higher with nivolumab compared to chemotherapy. No statistically significant differences in OS were reported (median OS with nivolumab 15.7 months versus 14.4 months with chemotherapy (95.54% CI 0.73–1.24), with 2-year OS rates of 38.7% versus 33.9%. However, 41% of the patients initially treated with chemotherapy received an anti-PD-1 agent subsequently.[28] One additional factor that highlights the need for caution in the interpretation of overall survival results is the proportion of patients who did not receive the assigned treatment after randomization in this open-label study: 23% of the patients in the chemotherapy group versus 2% in the nivolumab group. In this updated analysis, treatment-related adverse events of any grade occurred in 77% and 82% of patients in the nivolumab and chemotherapy groups, respectively, and grade 3 and 4 adverse events in 14% and 34%, respectively; 51% of the patients in the nivolumab group received an immune-modulating agent for the treatment of toxicities, most frequently systemic corticosteroids.[28]

Similarly, the KEYNOTE-002 randomized, phase II trial investigated the efficacy of investigational regimens of pembrolizumab (2mg/kg or 10mg/kg every 3 weeks), with chemotherapy (carboplatin and paclitaxel, carboplatin, paclitaxel, temozolomide or dacarbazine) as the comparator arm.[29] The study primary endpoint was progression free survival (PFS) in the intention to treat (ITT) population. Patients were required to have progressive disease following prior ipilimumab and, if BRAF V600-mutant, also BRAF and/or MEK inhibition, and were stratified by Eastern Cooperative Oncology Group (ECOG) performance status, lactate dehydrogenase concentration (LDH), and BRAF V600 mutation status. Between November/2012 and November/2013, 540 patients were enrolled

and randomized. The use of pembrolizumab was associated with a statistically significant improvement in median PFS, with a 6-months PFS rate of 34% in the pembrolizumab 2mg/kg arm, 38% in the pembrolizumab 10mg/kg arm and 16% in the chemotherapy group ($p<0.001$ for the comparison of both arms versus chemotherapy). Best ORR were 21% and 25% for the 2mg/kg and 10mg/kg arms, respectively, versus 4% in the control arm based on independent central review. The incidence of grade 3 and 4 treatment-related adverse events was 11–14% with pembrolizumab and 26% with chemotherapy. With extended follow up, and replicating what was reported in CheckMate-037, no significant OS benefit was seen with the use of pembrolizumab and 2-year OS rates were 35.9% and 38.2% in pembrolizumab 2mg/kg and 10mg/kg arms, respectively, and 29.7% in the control arm, with 55% of the patients in the control arm crossing over to pembrolizumab.[30]

Efficacy of single-agent anti-PD-1 agents versus standard therapies in ipilimumab-naïve patients in randomized trials

In ipilimumab naïve patients, both nivolumab and pembrolizumab resulted in superior OS and better tolerability when compared to ipilimumab or chemotherapy. In the CheckMate-066 phase III trial, 418 untreated patients without a BRAF mutation were randomized in the front-line setting to receive nivolumab at the standard dose of 3mg/kg every 2 weeks or dacarbazine[31]. Randomization was stratified according to PD-L1 expression status (positive versus negative or indeterminate) and metastasis stage defined according to the staging system of the American Joint Committee on Cancer (AJCC). OS rate at 1 year was 72.9% for nivolumab and 42.1% for dacarbazine (HR 0.42; 99.79% CI 0.25–0.73; $p<0.001$), with objective responses occurring in 40% (including 7.6% of complete responses) and 13.9% of the patients in each arm, respectively (odds ratio 4.06; $P<0.001$). Median PFS was also significantly prolonged with nivolumab (median PFS 5.1 months versus 2.2 months; HR 0.43; 95% CI 0.34–0.56; $p<0.001$). The incidence of treatment-related grade 3 or 4 adverse events was 11.7% among those treated with nivolumab.[31] Long-term data subsequently presented suggested a 2-year OS rate of 57.7% with nivolumab (median OS not reached) and 26.7% with chemotherapy (HR 0.43; 95% CI 0.33–0.57; $p<0.001$).[32] In the first line setting, nivolumab also compared favorably to ipilimumab in the randomized CheckMate-067 trial, which also comprised a third arm consisting of combined ipilimumab and nivolumab and is discussed in more detail below (Section “Anti-PD-1-based combinations”).[19]

Pembrolizumab was also investigated in immunotherapy-naïve patients in the phase III KEYNOTE-006 trial, which randomized 834 patients between September/2013 and March/2014 into three treatment arms: pembrolizumab 10 mg/kg every two weeks or every three weeks, or ipilimumab 3 mg/kg every three weeks for four doses.[33] Patients were stratified based on ECOG performance status, line of therapy, and PD-L1 expression and the primary endpoint was OS. In the final OS analysis after a median follow up of 22.9 months, pembrolizumab resulted in a significant improvement in 2-year OS rates when compared to ipilimumab (55% in both pembrolizumab arms vs 43%; HR 0.68; 95% CI 0.53–0.87; $p=0.0009$ and HR 0.68; 95% CI 0.53–0.87; $p=0.0008$, for 2-week and 3-week schedules compared to ipilimumab, respectively).[34] In a recent update of this study, with additional

11 months of follow up (33.9 months of median follow up in total), median OS in pembrolizumab arms was 32.3 months, and 15.9 months in the ipilimumab group (HR 0.70; 95%CI 0.58–0.86), with revised best ORR of 42% versus 16%; the median duration of response had not been reached in patients treated with pembrolizumab.[35] There appeared to be no significant differences between administering pembrolizumab every 2 or every 3 weeks.

Although these trials allowed subsequent therapies and, as discussed before, crossover to either subsequent anti-PD-1 or ipilimumab were significant, the only trial to specifically address sequencing of single agent nivolumab or ipilimumab was the CheckMate-064 study. [36] In this randomized, phase II trial, patients with advanced melanoma (treatment-naïve or with progression after no more than one previous systemic therapy), were randomly assigned to induction with either nivolumab 3 mg/kg every 2 weeks for six doses followed by ipilimumab 3 mg/kg every 3 weeks for four doses, or the reverse sequence. After induction, both arms received maintenance treatment with nivolumab 3 mg/kg every 2 weeks until disease progression. Upfront treatment with nivolumab followed by ipilimumab resulted in higher ORR at week 25 (41% versus 20%) and prolonged OS when compared to ipilimumab followed by nivolumab (median OS not reached versus 16.9 months; 12-month OS: 76% vs 54%); of note, the incidence of grade 3–5 treatment-related adverse events was similar between groups (50% in the nivolumab followed by ipilimumab group versus 43% among those treated with upfront ipilimumab).[36]

Anti-PD-1 and anti-CTLA-4 based combinations

Based on the results of randomized trials, anti-PD-1 monotherapy rapidly was consolidated among the standard first-line therapies for patients with advanced melanoma (Table 1). Nevertheless, many patients do not respond favorably to anti-PD-1. Based on preclinical models and the availability of clinical agents, the combination of anti-CTLA-4 and PD-1 agents emerged to potentially enhance the activity of immunotherapy.

In a phase I, dose escalation study, the combination of nivolumab and ipilimumab was administered every 3 weeks for 4 doses, followed by maintenance doses of both single-agent nivolumab and the combination. Objective responses occurred in 40% of the patients, although 53% developed grade 3 or 4 treatment related adverse events.[37]

These promising data led to two randomized trials which addressed the role of combined nivolumab and ipilimumab vs. ipilimumab monotherapy. In the phase II CheckMate-069 trial, treatment-naïve patients (n=142) with advanced melanoma were randomized 2:1 to receive either the combination of nivolumab 1mg/kg and ipilimumab 3mg/kg (n = 95) or ipilimumab 3mg/kg plus placebo (n = 47) every 3 weeks for 4 doses, followed by nivolumab 3mg/kg or placebo every 2 weeks until disease progression or unacceptable toxicity.[18] The primary endpoint was the ORR among patients with BRAF wild-type tumors, and was significantly improved in patients treated with the combination: 61% versus 11% (p<0.001), with 22% of the patients achieving a complete response and a median duration of response not reached. In an updated analysis after a median follow up of 24.5 months and including the overall study population, 2-year OS rates were 63.8% in the combination group 53.6% in

the ipilimumab plus placebo group; median OS had not been reached in either group (HR 0.74; 95% CI 0.43–1.26; $p=0.26$). Updated ORR for combination and ipilimumab monotherapy were 59% and 11%, respectively (61% and 11% in BRAF wildtype patients, respectively). Among patients harboring BRAF wild-type tumors, the 2-year OS rate with nivolumab/ipilimumab was 69% and 53% with ipilimumab plus placebo.[38] Notably, after progressive disease, many patients in the ipilimumab plus placebo group initially were able to receive nivolumab. Likely this accounted for the favorable OS in the ipilimumab plus placebo group.

The CheckMate-069 study, however, was not powered or conceived to test the hypothesis that the combination of ipilimumab and nivolumab would result in an OS improvement, and this was addressed in the phase III CheckMate-067 trial. In this randomized, three-arm trial, patients were assigned 1:1:1 to nivolumab 1 mg/kg every three weeks plus ipilimumab 3 mg/kg every three weeks for four doses, followed by nivolumab 3 mg/kg every two weeks, single-agent nivolumab 3 mg/kg every two weeks or single-agent ipilimumab 3 mg/kg every three weeks for four doses.[19] The study was designed to allow for a direct comparison between any nivolumab-containing arms and ipilimumab with PFS and OS as co-primary endpoints; the comparison between ipilimumab/nivolumab versus single-agent nivolumab, however, was exploratory. Results were updated with a median follow up of approximately 30 months in both nivolumab-containing arms.[39] Objective response rates were 58.9% for ipilimumab plus nivolumab (including 17.2% of complete responses), 44.6% for single-agent nivolumab alone and 19% for single-agent ipilimumab. Updated PFS analyses suggested a 58% relative reduction in the risk of disease progression in favor of the combination when compared to ipilimumab alone (mPFS 11.7 versus 2.9 months; HR 0.42; 95% CI 0.34–0.51; $p<0.001$); median PFS was also prolonged with nivolumab monotherapy compared to ipilimumab alone (mPFS 6.9 versus 2.9 months; HR 0.54; 95% CI 0.45–0.66; $p<0.001$). In an exploratory analysis, median PFS was superior with the combination versus nivolumab monotherapy (HR 0.76; 95% CI 0.62–0.94). Although median OS had not been reached in neither nivolumab-containing arm, 2-year OS rates were 64% for ipilimumab and nivolumab, 59% for single-agent nivolumab, a difference that was not statistically significant at this point in this exploratory analysis (HR 0.88; 95% CI 0.69–1.12), and 45% for single-agent ipilimumab ($p<0.0001$ for both comparisons with ipilimumab and nivolumab – HR 0.55, or single-agent nivolumab – HR 0.63).[39]

It is important to emphasize that, although the combination of nivolumab and ipilimumab indeed resulted in more pronounced objective responses and prolonged PFS compared to ipilimumab, evidence is lacking to support the combination significantly improving OS over nivolumab alone. Also, combined checkpoint blockade is clearly not suitable for all patients due to the increased risk of significant and rarely life-threatening treatment-related adverse events. In CheckMate-069, treatment-related grade 3 or 4 AE occurred in 54% of the patients treated with ipilimumab and nivolumab, resulting in treatment discontinuation in 28 (30%) of 94 patients in the combination group and four (9%) of 46 patients in the ipilimumab group.[38] In the updated safety analysis of CheckMate-067, 58.5% of the patients treated with the combination developed grade 3 or 4 adverse event, versus 20.8% with single-agent nivolumab.[39] A summary of treatment-related adverse events is described in Table 2. Despite the high rate of treatment discontinuation of combination immunotherapy, outcomes among this group of patients nevertheless appear favorable.[40]

In an attempt to minimize the incidence of adverse events, a combination regimen consisting of ipilimumab at a reduced dose and pembrolizumab at the standard dose was investigated in the KEYNOTE-029 study. In this open-label, phase I trial, patients (n=153) were treated with “low dose” ipilimumab (1mg/kg given every 3 weeks for 4 doses) combined with standard dose pembrolizumab (2mg/kg given every 3 weeks).[41] Grade 3 or 4 treatment-related adverse events and discontinuation due to toxicities occurred in 45% and 14% of the patients, respectively; there were no treatment-related deaths. Sixty-one percent of the patients achieved an objective response and 1-year OS rate was 89%. One important point to discuss, however, is that ipilimumab results in antitumor effects related to overall survival in a dose-dependent manner, at least when used as single agent. In a recently published randomized trial, ipilimumab 10mg/kg was associated with an improvement in overall survival compared to ipilimumab 3mg/kg (median OS 15.7m vs 11.5m; HR 0.84; 95% CI 0.70–0.99; p=0.04), with a 3-year overall survival rate of 31.2% versus 23.2%, at a cost of increased toxicity (grade 3–5 treatment-related toxicities of 34.3%, versus 18.5% with ipilimumab 3mg/kg).[42] Therefore, larger, randomized trials, and extended follow up are required to understand the efficacy of combination regimens involving reduced different doses of ipilimumab on overall survival.

Efficacy of anti-PD-1 agents in patients with central nervous system metastases

The presence of central nervous system (CNS) involvement has typically defined a subset of patients with an ominous prognosis. Benefit from ipilimumab in this setting was usually limited to a small percentage of patients and short-lived.[43] Recent evidences, however, suggest the treatment of patients with brain metastases is also being positively affected by the incorporation of anti-PD-1 agents and combined immunotherapy approaches.

In a randomized phase II study, nivolumab resulted in intracranial objective response rate of 20% of asymptomatic patients and with no prior CNS-directed therapies, increasing to an intracranial ORR of 42% with the combination of ipilimumab and nivolumab for patients with the same characteristics, although PFS intervals were limited: intracranial 6-month PFS rate of 28% and 46%, respectively.[44] These data are consistent with efficacy of pembrolizumab for patients with melanoma brain metastases in a single arm, phase II study conducted in a small group of patients. The efficacy of combined ipilimumab and nivolumab in patients with brain metastases was also investigated in the single-arm CheckMate-204 phase II study. Among 75 patients, best overall intracranial response to treatment was complete response in 21% and partial response of 33%, to an ORR of 54% and a 6-month PFS rate of 67%.[45]

Bedside questions unanswered by currently available clinical trials and future directions

Despite significant advances introduced by anti-PD-1 therapy, a broad number of questions applicable to clinical practice continue to emerge. One question relates to the optimal duration of treatment with anti-PD-1 agents. In KEYNOTE-001, among 61 patients with

complete responses who stopped pembrolizumab after a median time on treatment of 23 months and additional 10 months of follow-up off treatment, 97% of the responses were maintained.[25] These are promising results, but they must be interpreted with caution due to the limited follow-up. Additional data on duration of therapy come from the KEYNOTE-006 trial where patients received pembrolizumab for up to 24 months. Those with a complete response treated for at least 6 months could discontinue therapy after receiving at least two doses beyond the determination of complete response.[35] Data on patients treated in KEYNOTE-006 who completed protocol-specified time on treatment and discontinued pembrolizumab (including those with a variety of disease outcomes) were recently presented. Among these patients who discontinued pembrolizumab at 2-years, 91% remained progression-free after a median follow up of 9.7 months. Taken together, these data allow one to hypothesize that shorter treatment duration may be equally beneficial for select patients, and this needs to be further investigated in prospective, randomized discontinuation trials.[35]

Additionally, clinically validated biomarkers remain an unmet need. Since early-phase clinical development, correlations between expression of PD-L1 by immunohistochemistry and response to treatment has been demonstrated.[20] Nevertheless, the determination of PD-L1 involves a series of caveats, including different antibodies used for immunohistochemical staining across distinct clinical trials, lack of standardization or definition of cut off value for positivity/negativity, and intrapatient-intertumoral heterogeneity. More importantly, expression of PD-L1 in melanoma samples has never been shown to be a biomarker ready for treatment decision making among those candidates for single-agent anti-PD-1 therapy; even patients classified as PD-L1 negative may derive benefit from therapy (Table 3). Subgroup analyses from the CheckMate-067 trial suggest, however, that the expression of PD-L1 may be relevant to PFS differences between patients treated with ipilimumab and nivolumab compared to nivolumab alone, but patients have a higher response rate with the combination regardless of their PD-L1 status, making clinical application of this biomarker difficult.[39]

Hopefully, in the near future a better understanding of potentially useful biomarkers (neoantigen load, commensal bacteria, gene expression profiles associated with a pro-inflammatory microenvironment, quality of the immune infiltrate, etc.) will allow for a tailored approach for those who are candidates for immunotherapy. Perhaps equally relevant is the importance of biomarkers in the clinical development of a growing number of potential combinations involving anti-PD-1 agents. The association of anti-PD-1 agents with inhibitors of checkpoints beyond CTLA-4 is being extensively evaluated, and the feasibility of combinations with ICOS, GITR and CD27 agonists, and inhibitory anti-LAG3 antibodies, has been demonstrated, accompanied by promising antitumor activity.[46–49] Targeting different steps involved in the composition of an effective immune response also offers growing opportunities: among these targets, indoleamine 2,3-dioxygenase (IDO), a cytosolic enzyme involved in immunosuppressive effects, downregulation of effector T cells and immune tolerance mechanisms surfaces as a potential candidate.[50] IDO inhibitors, including epacadostat and indoximod, yielded exciting results and apparently a favorable toxicity profile when used in combination with ipilimumab and pembrolizumab in early-phase clinical trials.[51–56]

Also, for the meaningful proportion of patients with activating BRAF mutations, therapeutic options have been largely multiplied following the incorporation of BRAF and MEK inhibitors, and the best treatment sequence/algorithm for these patients who are also candidates for immune-checkpoint blockade is being clarified in ongoing randomized clinical trials. However, the convergence of immunotherapy and targeted-therapy is also of clinical interest. As an example, significant activity was demonstrated in patients with tumors harboring BRAF V600 mutations treated with the triplet combination of a BRAF +MEK inhibitors and the anti-PD-L1 agent, atezolizumab.[57]

Conclusions

While single-agent anti-PD-1 remains a standard of care for a large proportion of individuals who are candidates for immunotherapy, select patients may benefit from combined PD-1 and CTLA-4 blockade, and ongoing clinical trials are expanding combinations of treatment possibilities. As management algorithms continue to evolve, the incorporation of reliable, validated biomarkers and the rational investigation of combinations are crucial for the enhancement of the benefits that derived from the incorporation of immunotherapy for patients with advanced melanoma.

Acknowledgments

Supported in part by NIH/NCI Cancer Center Support Grant P30 CA008748.

Funding: No funding received

References

1. Luke JJ, Flaherty KT, Ribas A, Long GV. Targeted agents and immunotherapies: optimizing outcomes in melanoma. *Nat Rev Clin Oncol*. 2017; 14(8):463–82. [PubMed: 28374786]
2. Curtin JA, Fridlyand J, Kageshita T, et al. Distinct sets of genetic alterations in melanoma. *N Engl J Med*. 2005; 353:2135–47. [PubMed: 16291983]
3. The Cancer Genome atlas Network. Genomic Classification of Cutaneous Melanoma. *Cell*. 2015; 161(7):1681–96. [PubMed: 26091043]
4. Robert C, Karaszewska B, Schachter J, et al. Improved overall survival in melanoma with combined dabrafenib and trametinib. *N Engl J Med*. 2015; 372:30–39. [PubMed: 25399551]
5. Ascierto PA, McArthur GA, Dréno B, et al. Cobimetinib combined with vemurafenib in advanced BRAFV600-mutant melanoma (coBRIM): updated efficacy results from a randomised, double-blind, phase 3 trial. *Lancet Oncol*. 2016; 17(9):1248–1260. [PubMed: 27480103]
6. Long GV, Stroyakovsky DL, Gogas H, Levchenko E, de Braud F, Larkin J, et al. COMBI-d: A randomized, double-blinded, Phase III study comparing the combination of dabrafenib and trametinib to dabrafenib and trametinib placebo as first-line therapy in patients (pts) with unresectable or metastatic BRAF^{V600E/K} mutation-positive cutaneous melanoma. *J Clin Oncol*. 2014; 32(suppl) abstr 9011.
7. Leach DR, Kummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. *Science*. 1996; 271(5256):1734–6. [PubMed: 8596936]
8. Hodi FS, O'Day SJ, McDermott DF, et al. Improved Survival with Ipilimumab in Patients with Metastatic Melanoma. *N Engl J Med*. 2010; 363(8):711–723. [PubMed: 20525992]
9. Robert C, Thomas L, Bodarenko I, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. *N Engl J Med*. 2011; 364(26):2517–26. [PubMed: 21639810]

10. Maio M, Grob JJ, amdal S, et al. Five-Year Survival Rates for Treatment-Naive Patients With Advanced Melanoma Who Received Ipilimumab Plus Dacarbazine in a Phase III Trial. *J Clin Oncol*. 2015; 33(10):1191–1196. [PubMed: 25713437]
11. Schadendorf D, Hodi FS, Robert C, et al. Pooled analysis of long-term survival data from phase II and phase III trials of ipilimumab in unresectable or metastatic melanoma. *J Clin Oncol*. 2015; 33:1889–94. [PubMed: 25667295]
12. Mellman I, Coukos G, Dranoff G. Cancer immunotherapy comes of age. *Nature*. 2011; 480(7378):480–489. [PubMed: 22193102]
13. Chen L, Flies DB. Molecular mechanisms of T cell co-stimulation and co-inhibition. *Nat Rev Immunol*. 2013; 13(4):227–242. [PubMed: 23470321]
14. Nishimura H, Nose M, Miao H, et al. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. *Immunity*. 1999; 11(2):141–51. [PubMed: 10485649]
15. Nishimura H, Okazaki T, Tanaka Y, et al. Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. *Science*. 2001; 291(5502):319–22. [PubMed: 11209085]
16. Agata Y, Kawasaki A, Nishimura H, et al. Expression of the PD-1 antigen on the surface of stimulated mouse T and B lymphocytes. *Int Immunol*. 1996; 8:765–72. [PubMed: 8671665]
17. Freeman GJ, Long AJ, Iwai Y, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J Exp Med*. 2000; 192:1027–34. [PubMed: 11015443]
18. Postow MA, Chesney J, Pavlick AC, et al. Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. *N Engl J Med*. 2015; 372:2006–17. [PubMed: 25891304]
19. Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. *N Engl J Med*. 2015; 373:23–34. [PubMed: 26027431]
20. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune-correlates of anti-PD-1 antibody in cancer. *N Engl J Med*. 2012; 366:2443–54. [PubMed: 22658127]
21. Topalian SL, Sznol M, McDermott DF, et al. Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. *J Clin Oncol*. 2014; 32(10):1020–30. [PubMed: 24590637]
22. Hodi FS, Kluger H, Sznol M, et al. Durable, long-term survival in previously treated patients with advanced melanoma (MEL) who received nivolumab (NIVO) monotherapy in a phase I trial. Presented during AACR Annual Meeting; 2016;
23. Hamid O, Robert C, Daud A, et al. Safety and Tumor Responses with lambrolizumab (Anti-PD-1) in melanoma. *N Engl J Med*. 2013; 369:134–44. [PubMed: 23724846]
24. Ribas A, Hamid O, Daud A, et al. Association of pembrolizumab with tumor response and survival among patients with advanced melanoma. *JAMA*. 2016; 315(15):1600–09. [PubMed: 27092830]
25. Robert C, Ribas A, Hamid O, et al. Three-year overall survival for patients with advanced melanoma treated with pembrolizumab in KEYNOTE-001. *J Clin Oncol*. 2016; 34(15_suppl):9503.
26. Postow MA, Callahan MK, Wolchok JD, et al. Immune Checkpoint Blockade in Cancer Therapy. *J Clin Oncol*. 2015; 33(17):1974–82. [PubMed: 25605845]
27. Weber JS, D'Angelo SP, Minor D, et al. Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4-treatment (CheckMate 037): a randomised, controlled, open-label, phase 3 trial. *Lancet Oncol*. 2015; 16(4):375–384. [PubMed: 25795410]
28. Larkin J, Minor D, D'Angelo S, et al. Overall survival in patients with advanced melanoma who received nivolumab versus investigator's choice chemotherapy in CheckMate 037: A randomized, controlled, open-label phase III trial. *J Clin Oncol*. 2017 Jul 3. [Epub ahead of print].
29. Ribas A, Puzanov I, Dummer R, et al. Pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory melanoma (KEYNOTE-002): a randomised, controlled, phase 2 trial. *Lancet Oncol*. 2015; 16:908–18. [PubMed: 26115796]
30. Hamid O, Puzanov I, Dummer R, et al. Final OS analysis for KEYNOTE-002: pembrolizumab vs investigator-choice chemotherapy for ipilimumab-refractory melanoma. *Ann Oncol*. 2016; 27(6):379–400. [PubMed: 26681681]

31. Robert C, Long GV, Brady B, et al. Nivolumab in Previously Untreated Melanoma without BRAF Mutation. *N Engl J Med*. 2015; 372:320–330. [PubMed: 25399552]
32. Atkinson V, Ascierto PA, Long GV, et al. Two-Year Survival and Safety Update in Patients (pts) with Treatment-Naïve Advanced Melanoma (MEL) Receiving Nivolumab (NIVO) or Dacarbazine (DTIC) in CheckMate-066. Presented during the Society for Melanoma Research International Congress; 2015;
33. Robert C, Schachter J, Long GV, et al. Pembrolizumab versus Ipilimumab in Advanced Melanoma. *N Engl J Med*. 2015; 372:2521–32. [PubMed: 25891173]
34. Schachter J, Ribas A, Long GV, et al. Pembrolizumab versus ipilimumab for advanced melanoma: final overall survival results of a multicentre, randomised, open-label phase 3 study (KEYNOTE-006). *Lancet Oncol*. 2017 Aug. [Epub ahead of print].
35. Robert C, Long GV, Schachter J, et al. Long-term outcomes in patients (pts) with ipilimumab (ipi)-naïve advanced melanoma in the phase 3 KEYNOTE-006 study who completed pembrolizumab (pembro) treatment. *J Clin Oncol*. 2017; 35(suppl) abstr 9504.
36. Weber JS, Gibney G, Sullivan RJ, et al. Sequential administration of nivolumab and ipilimumab with a planned switch in patients with advanced melanoma (CheckMate 064): an open-label, randomised, phase 2 trial. *Lancet Oncol*. 2016; 17:943–55. [PubMed: 27269740]
37. Wolchok JD, Kluger H, Callahan MK, et al. Nivolumab plus ipilimumab in advanced melanoma. *N Engl J Med*. 2013; 369:122–33. [PubMed: 23724867]
38. Hodi FS, Chesney J, Pavlick AC, et al. Combined nivolumab and ipilimumab versus ipilimumab alone in patients with advanced melanoma: 2-year overall survival outcomes in a multicentre, randomised, controlled, phase 2 trial. *Lancet Oncol*. 2016; 17(11):1558–68. [PubMed: 27622997]
39. Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Overall survival results from a phase III trial of nivolumab combined with ipilimumab in treatment-naïve patients with advanced melanoma (CheckMate 067). *Proc of the Am Assoc Cancer Res*. 2017:58.
40. Schadendorf D, Wolchok JD, Hodi FS, et al. Efficacy and Safety Outcomes in Patients With Advanced Melanoma Who Discontinued Treatment With Nivolumab and Ipilimumab Because of Adverse Events: A Pooled Analysis of Randomized Phase II and III Trials. *J Clin Oncol*. 2017 Aug 25. [Epub ahead of print].
41. Long GV, Atkinson V, Cebon JS, et al. Standard-dose pembrolizumab in combination with reduced-dose ipilimumab for patients with advanced melanoma (KEYNOTE-029): an open-label, phase 1b trial. *Lancet Oncol*. 2017 Jul 17. [Epub ahead of print].
42. Ascierto PA, Del Vecchio M, Robert C, et al. Ipilimumab 10 mg/kg versus ipilimumab 3 mg/kg in patients with unresectable or metastatic melanoma: a randomised, double-blind, multicentre, phase 3 trial. *Lancet Oncol*. 2017; 18(5):611–622. [PubMed: 28359784]
43. Margolin K, Ernstoff MS, Hamid O, et al. Ipilimumab in patients with melanoma and brain metastases: an open-label, phase 2 trial. *Lancet Oncol*. 2012; 13(5):459–65. [PubMed: 22456429]
44. Long GV, Atkinson V, Menzies AM, et al. A randomized phase II study of nivolumab or nivolumab combined with ipilimumab in patients (pts) with melanoma brain metastases (mets): The Anti-PD1 Brain Collaboration (ABC). *J Clin Oncol*. 2017; 35(15_suppl):9508.
45. Tawbi HAH, Forsyth PAJ, Algazi AP, et al. Efficacy and safety of nivolumab (NIVO) plus ipilimumab (IPI) in patients with melanoma (MEL) metastatic to the brain: Results of the phase II study CheckMate 204. *J Clin Oncol*. 2017; 35(15_suppl):9507–9507.
46. Burris HA, Callahan MK, Tolcher AW, et al. Phase 1 safety of ICOS agonist antibody JTX-2011 alone and with nivolumab (nivo) in advanced solid tumors; predicted vs observed pharmacokinetics (PK) in ICONIC. *J Clin Oncol*. 2017; 35(suppl) abstr 3033.
47. Siu LL, Steeghs N, Menjawy T, et al. Preliminary results of a phase I/IIa study of BMS-986156 (glucocorticoid-induced tumor necrosis factor receptor–related gene [GITR] agonist), alone and in combination with nivolumab in pts with advanced solid tumors. *J Clin Oncol*. 2017; 35(suppl) abstr 104.
48. Sanborn RE, Pishvaian MJ, Kluger HM, et al. Clinical results with combination of anti-CD27 agonist antibody, varlilumab, with anti-PD1 antibody nivolumab in advanced cancer patients. *J Clin Oncol*. 2017; 35(suppl) abstr 3007.

49. Ascierto PA, Melero I, Bhatia S, et al. Initial efficacy of anti-lymphocyte activation gene-3 (anti-LAG-3; BMS-986016) in combination with nivolumab (nivo) in pts with melanoma (MEL) previously treated with anti-PD-1/PD-L1 therapy. J Clin Oncol. 2017; 35(suppl) abstr 9520.
50. Moon YW, Hajar J, Hwu P, Naing A. Targeting the indole- amine 2,3-dioxygenase pathway in cancer. J Immunother Cancer. 2015;3. [PubMed: 25767716]
51. Gibney GT, Hamid O, Gangadhar TC, Lutzky J, Olszanski AJ, Gajewski T, et al. Preliminary results from a phase 1/2 study of INCB024360 combined with ipilimumab (ipi) in patients (pts) with melanoma. J Clin Oncol. 2014;32.
52. Gangadhar TC, Hamid O, Smith DC, et al. Epacadostat plus pembrolizumab in patients with advanced melanoma and select solid tumors: Updated phase 1 results from ECHO-202/ KEYNOTE-037. Ann Oncol. 2016; 27(suppl_6):1110PD.
53. Hamid O, Bauer TM, Spira AI, et al. Safety of epacadostat 100 mg bid plus pembrolizumab 200 mg Q3W in advanced solid tumors: Phase 2 data from ECHO-202/KEYNOTE-037. J Clin Oncol. 2017; 35(suppl) abstr 3012.
54. Perez RP, Riese MJ, Lewis KD, et al. Epacadostat plus nivolumab in patients with advanced solid tumors: Preliminary phase I/II results of ECHO-204. J Clin Oncol. 2017; 35(suppl) abstr 3003.
55. Zakharia Y, Drabick JJ, Khleif S, et al. Updates on phase1b/2 trial of the indoleamine 2,3-dioxygenase pathway (IDO) inhibitor indoximod plus checkpoint inhibitors for the treatment of unresectable stage 3 or 4 melanoma. J Clin Oncol. 2017; 34(15_suppl):3075.
56. Zakharia, Y., McWilliams, R., Shaheem, M., et al. Interim Analysis of the Phase 2 Clinical Trial of the IDO Pathway Inhibitor Indoximod in Combination With Pembrolizumab for Patients With Advanced Melanoma. Presented during the 107th Annual Meeting of the American Association for Cancer Research (AACR); 2017; Abstract CT117
57. Sullivan RJ, Gonzalez R, Lewis K, et al. Atezolizumab (A) + cobimetinib (C) + vemurafenib (V) in BRAFV600-mutant metastatic melanoma (mel): Updated safety and clinical activity. J Clin Oncol. 2017; 35(suppl) abstr 3063.
58. Puzanov I, Dummer R, Schachter J, et al. Efficacy based on tumor PD-L1 expression in KEYNOTE-002, a randomized comparison of pembrolizumab (pembro; MK-3475) versus chemotherapy in patients (pts) with ipilimumab-refractory (IPI-R) advanced melanoma (MEL). J Clin Oncol. 2015; 33(15_suppl):3012–3012.
59. Daud A, Blank CU, Robert C, et al. KEYNOTE-006 study of pembrolizumab (pembro) versus ipilimumab (ipi) for advanced melanoma: Efficacy by PD-L1 expression and line of therapy. J Clin Oncol. 2016; 34(suppl) abstr 9513.

Figure 1.

Overall survival rates at 2 years across randomized clinical trials investigating anti-PD-1 agents in advanced melanoma.

2-year overall survival rates across randomized clinical trials investigating anti-PD-1 agents in monotherapy or in combination with anti-CTLA-4 agents in advanced melanoma; blue bars represent the results of clinical trials that investigated anti-PD-1-containing regimens in ipilimumab-naïve patients.

Table 1
Select randomized clinical trials investigating anti-PD-1 agents in patients with advanced melanoma

Study	Setting	N	Treatments	ORR (%)	Median PFS (m)	OS (m or %)
Anti-PD-1 monotherapy						
KEYNOTE-002 [30]	IPI-refractory	540	PEMBRO 2mg/kg q3w (A) vs PEMBRO 10mg/kg q3w (B) vs ICC	22.2% (A) vs 27.6% (B) vs 4.5% (C)	2.9 (A) vs 3.0 (B) vs 2.8 (C)	Median: 13.4 (A) vs 14.7 (B) vs 11.0 (C) 2-year OS: 35.9% (A) vs 38.2% (B) vs 29.7% (C)
KEYNOTE-006 [35]	IPI-naïve	834	PEMBRO 10mg/kg q2w (A) vs PEMBRO 10mg/kg q3w (B) or IPI 10mg/kg x4 (C)	42% (A+B) vs 15% (C)	8.3 (A+B) [#] vs 3.3 (C)	32.3 (A+B) vs 15.9 (C)
CheckMate-037 [28]	IPI-refractory	405	NIVO 3mg/kg q2w (A) vs ICC (B)	27% (A) vs 10% (B)	3.1 (A) vs 3.7 (B)	Median: 15.7 (A) vs 14.4 (B) 2y OS: 38.5% (A) vs 33.9% (B)
CheckMate-066 [32]	IPI-naïve (BRAF wild-type only)	418	NIVO 3mg/kg q2w (A) vs DTIC (B)	42.9% (A) vs 14.4% (B)	5.4 (A) vs 2.2 (B)	Median: NR (A) vs 11.2 2y OS: 57.7% (A) vs 26.7% (B)
Sequential therapy						
CheckMate-064 [36]	IPI-naïve	140	IPI 3mg/Kg q3w x4->NIVO 3mg/Kg q2w x6 (A) vs NIVO 3mg/Kg q2w x6 - >IPI 3mg/Kg q3w x4 (B)	31% vs 56%	40% (A) vs 62% (B) progression-free at week 25	Median: 16.9 (A) vs NR (B)
Anti-PD-1 and Anti-CTLA-4 combination						
CheckMate-069 [38]	IPI-naïve	142 (109 BRAF wild-type)	IPI 3mg/kg + NIVO 1mg/kg -> NIVO 3mg/kg (A) vs IPI 3mg/kg	61% (A) vs 11% (B) **	NR (A) vs 3.0 (B)	Median: NR (A and B) 2-year OS: 63.8% (A) vs 53.6% (B)
CheckMate-067 [39]	Untreated	945	NIVO 3mg/kg (A) vs NIVO 1mg/kg + IPI 3mg/kg > NIVO 3mg/kg (B) vs IPI 3mg/kg (C)	44.6% (A) vs 58.9% (B) vs 19% (C)	6.9 (A) vs 11.7 (B) vs 2.9 (C)	Median: NR (A and B) vs 20 (C) 2-year OS: 59% (A) vs 64% (B) vs 45% (C)

ORR – overall response rate (complete response + partial response); PFS – median progression-free survival in months; OS –overall survival; ICC – investigator choice of chemotherapy; DTIC – dacarbazine; IPI – Ipilimumab; NIVO – Nivolumab; PEM – Pembrolizumab; NR – not reached;

** - In patients with BRAF wild-type tumors

Table 2

Incidence of any-grade and grade 3 and 4 treatment related adverse events across randomized clinical trials investigating anti-PD-1 agents in advanced melanoma.

	Treatment	Any grade adverse events* (%)	Grade 3 or 4 adverse events* (%)	Treatment discontinuation due to toxicities (%)
CheckMate 037 [27]	Nivolumab	68%	9%	3%
KEYNOTE-002 [29]	Pembrolizumab	68–74%	11–14%	8%
CheckMate 066 [31]	Nivolumab	74.3%	11.7%	6.8%
KEYNOTE-006 [33]	Pembrolizumab	72.9–79.5%	10.1–13.3% ^{**}	4.0–6.9%
CheckMate 069 [38]	Ipilimumab + Nivolumab	92%	54%	30%
CheckMate 067 [39]	Nivolumab	86.3%	20.8%	11.5%
CheckMate 067 [39]	Ipilimumab + Nivolumab	95.8%	58.5%	39.6%

*
- treatment related

**
- grade 3–5

Table 3

Positivity of PD-L1 expression by immunohistochemistry across randomized clinical trials investigating anti-PD1 agents in advanced melanoma.

	Cut-off value for PD-L1 positivity	N (%) of patients with PD-L1 positive tumors treated with single-agent anti-PD1	ORR (%) to single-agent anti-PD-1 in PD-L1 positive/PD-L1 negative patients
CheckMate 037 [27]	At least 5% of tumor cells	134 (49%)	44%/20%
KEYNOTE-002 [58]	At least 1% of tumor cells	193 (67%) [*]	26%/15%
CheckMate 066 [31]	At least 5% of tumor cells	74 (35.2%)	52.7%/33.1%
KEYNOTE-006 [59]	At least 1% of tumor cells	446 (80.2%)	39%/25%
CheckMate 067 [19]	At least 5% of tumor cells	80 (25.3%)	57.5%/41.3%

^{*} - among 286 evaluable of 361 enrolled patients treated with pembrolizumab.