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EXHIBIT A

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ORIGINAL ARTICLE

PD-1 Blockade in Tumors with Mismatch-Repair Deficiency

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Abstract

Article

References

The programmed death 1 (PD-1) pathway is a negative feedback system that represses Th1 cytotoxic immune responses and that, if unregulated, can damage the host.¹⁻³ It is up-regulated in many tumors and in their surrounding microenvironment. Blockade of this pathway with antibodies to PD-1 or its ligands has led to remarkable clinical responses in patients with many different types of cancer, including melanomas, non-small-cell lung cancer, renal-cell carcinoma, bladder cancer, and Hodgkin's lymphoma.⁴⁻¹⁰ The expression of PD-1 ligands (PD-L1 or PD-L2) on the surface of tumor cells or immune cells is an important — but not a definitive — predictive biomarker of response to PD-1 blockade.^{4,6-8,11}

In reports of the effects of PD-1 blockade in human tumors, only 1 of 33 patients with colorectal cancer had a response to this treatment, in contrast to substantial fractions of patients with melanomas, renal-cell cancers, and lung tumors who have a response.^{10,12} What was different about this single patient? We hypothesized that this patient had mismatch-repair deficiency, because mismatch-repair deficiency occurs in a small fraction of advanced colorectal cancers,^{13,14} somatic mutations found in tumors can be recognized by the patient's own immune system,¹⁵ and mismatch repair-deficient colorectal cancers have 10 to 100 times as many somatic mutations as mismatch repair-proficient colorectal cancers.¹⁶⁻¹⁸ Moreover, mismatch repair-deficient cancers contain prominent lymphocyte infiltrates, a finding consistent with an immune response.¹⁹⁻²² In addition, two of the tumor types that were most responsive to PD-1 blockade in a study by Topalian et al.¹⁰ had high numbers of somatic mutations as a result of exposure to cigarette smoke (lung cancers) or ultraviolet radiation (melanomas).^{23,24} Our hypothesis was correct: the tumor of the single patient with colorectal cancer who had a response to PD-1 blockade was mismatch repair-deficient.²⁵ Therefore, we hypothesized that mismatch repair-deficient tumors are more responsive to PD-1 blockade than are mismatch repair-proficient tumors.

To test this hypothesis, we initiated a phase 2 clinical trial to evaluate immune checkpoint blockade in patients whose tumors had or did not have mismatch-repair deficiency. Because mismatch-repair deficiency in tumors arises through two routes,²⁶⁻²⁸ we recruited patients with hereditary nonpolyposis colorectal cancer (also known as the Lynch syndrome), which results from an inherited germline defect in one of four mismatch-repair genes followed by a second inactivating somatic change in the remaining wild-type allele. We also recruited patients with sporadic mismatch repair-deficient tumors, in which both alleles of a mismatch-repair gene are inactivated by somatic mutations or by epigenetic silencing.²⁹ In either case, the neoplasms that arise harbor hundreds or thousands of mutations.^{16,18}

METHODS

Patients

Patients with treatment-refractory progressive metastatic cancer were recruited from three centers for this phase 2 study (Table 1). Three cohorts were evaluated: cohort A included patients with mismatch repair-deficient colorectal adenocarcinomas, cohort B included patients with mismatch repair-proficient colorectal adenocarcinomas, and cohort C included patients with mismatch repair-deficient cancers of types other than colorectal.

Study Oversight

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The [protocol](#), available with the full text of this article at NEJM.org, was approved by the institutional review board at each site, and the study was conducted in accordance with the provisions of the Declaration of Helsinki and the International Conference on Harmonisation Good Clinical Practice guidelines. All the patients provided written informed consent before study entry. The first author (the principal investigator) and the last author (the Investigational New Drug sponsor) were responsible for oversight of the study. Merck donated the study drug and reviewed the final drafts of the protocol and of this manuscript before submission; they did not participate in the analysis of the data.

Demographic and
Baseline
Characteristics of the
Patients.

Study Design

This phase 2 trial was conducted with the use of a Green–Dahlberg two-stage design and included the three parallel cohorts described above. The study agent, pembrolizumab, was administered intravenously at a dose of 10 mg per kilogram of body weight every 14 days (Fig. S1 in [Supplementary Appendix 1](#), available at NEJM.org). Pembrolizumab is a humanized monoclonal anti-PD-1 antibody of the IgG4 kappa isotype that blocks the interaction between PD-1 and its ligands, PD-L1 and PD-L2 (Fig. S1 in [Supplementary Appendix 1](#)).

Safety assessments were performed before each treatment. At the start of each treatment cycle, the total tumor burden was assessed by means of measurement of serum biomarkers. Radiographic assessments were performed at 12 weeks and every 8 weeks thereafter. Further details concerning the clinical [protocol](#) are available at NEJM.org.

Analysis of Mismatch-Repair Status

Tumors with genetic defects in mismatch-repair pathways are known to harbor hundreds to thousands of somatic mutations, especially in regions of repetitive DNA known as microsatellites. The accumulation of mutations in these regions of the genome is termed microsatellite instability.^{26–28} Mismatch-repair status was assessed in tumors with the use of the MSI Analysis System (Promega), through the evaluation of selected microsatellite sequences that are particularly prone to copying errors when mismatch repair is compromised.^{26–28} Additional details are provided in [Supplementary Appendix 1](#).

Genomic and Bioinformatic Analyses

Primary tumor samples and matched normal peripheral-blood specimens were obtained from a subgroup of patients with mismatch repair–deficient carcinomas and a subgroup with mismatch repair–proficient carcinomas, for whom sufficient tumor tissue was available for exome sequencing³⁰ and HLA haplotyping. To assess the potential for mutant peptide binding, somatic exome data combined with each individual patient's major histocompatibility complex (MHC) class I HLA haplotype were applied to an epitope prediction algorithm.^{31,32} This algorithm provided an estimate of the total number of mutation-associated neoantigens in each tumor. Additional details are provided in [Supplementary Appendix 1](#).

Statistical Analysis

The primary end points for cohorts A and B were the immune-related objective response rate and the 20-week immune-related progression-free survival rate, assessed with the use of immune-related response criteria.³³ The primary end point for cohort C was the immune-related progression-free survival rate at 20 weeks (Fig. S1 in [Supplementary Appendix 1](#)). Immune-related criteria (i.e., one of the types of criteria used to evaluate immune-based therapies) are based on radiographic responses, and unlike Response Evaluation Criteria in Solid Tumors (RECIST), they capture newly developed lesions detected on radiography in the measurement of tumor burden; these criteria are defined and compared with RECIST, version 1.1, in Table S1 in [Supplementary Appendix 1](#). The response rate and 20-week progression-free survival rate were evaluated and reported in this study with the use of both RECIST, version 1.1, and immune-related response criteria. Progression-free survival and overall survival were summarized by means of the Kaplan–Meier method. Details of the hypothesis, the decision rules for the rejection of the null hypotheses, decision rules for early discontinuation of the study in a cohort because of efficacy or futility, and statistical methods are provided in [Supplementary Appendix 1](#).

RESULTS

Patients

A total of 41 consecutive patients were enrolled in the study and treated during the period from September 2013 through January 2015 (Table 1). Recruitment included patients in pursuit of a clinical trial option who were known to have tumors with mismatch-repair defects or who had tumors of unknown status who were then tested. One patient in the cohort with mismatch repair–deficient colorectal cancer was enrolled under an institutional review board eligibility waiver allowing a grade 3 bilirubin level (i.e., higher than the cutoff specified in the inclusion criteria). A total of 32 patients with colorectal cancer were enrolled in cohorts A and B. All patients with colorectal cancer had received two or more previous chemotherapy regimens (a median of four regimens), except for 1 patient with mismatch repair–proficient cancer who had received one chemotherapeutic and one (non–PD1-based) immunotherapeutic regimen.

Nine patients with mismatch repair–deficient solid tumors other than colorectal cancer were enrolled in cohort C. All patients in cohort C had received one or more previous therapeutic regimens (a median of two regimens).

Primary End Point

The immune-related objective response rate in cohort A was 40% (4 of 10 patients; 95% confidence interval [CI], 12 to 74), and the immune-related progression-free survival rate at 20 weeks was 78% (7 of 9 patients; 95% CI, 40 to 97) (Table S2 in [Supplementary Appendix 1](#)); the corresponding rates in cohort C were 71% (5 of 7 patients; 95% CI, 29 to 96) and 67% (4 of 6 patients; 95% CI, 22 to 96). In cohort B, which included patients with mismatch repair–proficient colorectal cancers, the immune-related objective response rate was 0% (95% CI, 0 to 20), and the immune-related progression-free survival rate at 20 weeks was 11% (2 of 18 patients; 95% CI, 1 to 35). Both cohorts with mismatch repair–deficient cancers (cohorts A and C) reached the prespecified point at which

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the protocol indicated that the study reached its primary efficacy end point when 4 patients were free from disease progression at 20 weeks and objective responses on the basis of immune-related response criteria were observed in 4 patients (Table S2 and the Methods section in [Supplementary Appendix 1](#)).

The median follow-up was 36 weeks (range, 5 to 55) for patients with mismatch repair–deficient colorectal cancer (cohort A), 20 weeks (range, 4 to 52) for patients with mismatch repair–proficient colorectal cancer (cohort B), and 21 weeks (range, 0.1 to 49) for patients with mismatch repair–deficient noncolorectal cancer (cohort C). All patients for whom the 20-week immune-related progression-free survival rate could be evaluated were followed for at least 20 weeks.

Radiographic Evaluation

Of the 10 patients with mismatch repair–deficient colorectal cancer (cohort A) who could be evaluated for RECIST, 4 (40%; 95% CI, 12 to 74) had objective responses according to these criteria (Table 2 and Figure 1, and Fig. S2 in [Supplementary Appendix 1](#)). Patients were considered not to have been evaluated unless they underwent a radiographic scan at 12 weeks. The rate of disease control, which was defined as the percentage of patients who had an objective response or whose disease was stable, was 90% in cohort A (9 of 10 patients; 95% CI, 55 to 100). Of the 7 patients in cohort C who could be evaluated, 5 (71%; 95% CI, 29 to 96) had objective responses as defined by RECIST (Table 2 and Figure 1, and Fig. S2 in [Supplementary Appendix 1](#)), and the rate of disease control was 71% (5 of 7 patients; 95% CI, 29 to 96).

Patients in cohort C had faster responses than did patients in cohort A (median time to response according to RECIST, 12 weeks vs. 28 weeks; $P=0.03$). Furthermore, all 6 patients (100%) with mismatch repair–deficient tumors that were not associated with the Lynch syndrome had an objective response, whereas only 3 of 11 patients (27%) with tumors associated with the Lynch syndrome had a response (Table S3 in [Supplementary Appendix 2](#)) ($P=0.009$). No other baseline characteristics had a significant association with objective responses.

Among the 18 patients with mismatch repair–proficient colorectal cancers in cohort B, no objective responses as defined by RECIST were observed (Table 2 and Figure 1, and Fig. S2 in [Supplementary Appendix 1](#)). In this group, the rate of disease control was 11% (2 of 18 patients; 95% CI, 1 to 35).

All the patients who had a response as defined by RECIST (Table 2) also had a response according to immune-related response criteria (Table S2 in [Supplementary Appendix 1](#)).

Survival

In the cohort of patients with mismatch repair–deficient colorectal cancer (cohort A), the median progression-free survival and median overall survival were not reached (Figure 2). In contrast, among the patients with mismatch repair–proficient cancers (cohort B), the median progression-free survival was only 2.2 months (95% CI, 1.4 to 2.8), and the median overall survival was 5.0 months (95% CI, 3.0 to not estimable). In cohort C (patients with mismatch repair–deficient noncolorectal cancer), the median progression-free survival was 5.4 months (95% CI, 3 to not estimable), and the median overall survival was not reached. A post hoc comparison of the cohorts with mismatch repair–deficient and mismatch repair–proficient colorectal cancers showed hazard ratios for disease progression or death (0.10; 95% CI, 0.03 to 0.37; $P<0.001$) and for death (0.22; 95% CI, 0.05 to 1.00; $P=0.05$) that favored patients with mismatch repair–deficient colorectal cancer (Figure 2).

To evaluate whether the difference in survival might be due to prognostic differences, we measured the time since the diagnosis of metastatic disease and the clinical performance of the regimen that patients had received before enrollment. We found that there was no significant difference between patients with mismatch repair–deficient colorectal cancer and patients with mismatch repair–proficient colorectal cancer with respect to the duration of metastatic disease ($P=0.77$ by the log-rank test) or the median progression-free survival while receiving their previous regimens ($P=0.60$ by the log-rank test) (Fig. S3 in [Supplementary Appendix 1](#)). We also performed an additional multivariate analysis of progression-free and overall survival to examine the difference in outcomes between mismatch repair–deficient colorectal cancer and mismatch repair–proficient colorectal cancer, adjusting for elapsed time since the initial diagnosis. The magnitude of the hazard ratios for disease progression or death (hazard ratio, 0.04; 95% CI 0.01 to 0.21; $P<0.001$) and for death (hazard ratio, 0.18; 95% CI, 0.03 to 1.01; $P=0.05$), representing the differing effects of pembrolizumab between mismatch repair–deficient tumors and mismatch repair–proficient tumors, was maintained after adjustment for this potential difference.

Safety Assessment

Adverse events occurring in more than 5% of patients are listed in Table 3. Events of clinical interest included rash or pruritus (24%); thyroiditis, hypothyroidism, or hypophysitis (10%); and asymptomatic pancreatitis (15%). Although the numbers were small, thyroid-function abnormalities were limited to the cohorts with mismatch repair–deficient cancer (Table 3).

Tumor Markers

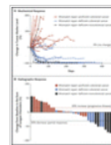
In the two cohorts with colorectal cancer, it was possible to evaluate levels of carcinoembryonic antigen (CEA) before enrollment; in 29 of 32 patients, these levels were above the upper limit of normal (3 mg per deciliter). Substantial decreases in CEA level occurred in 7 of the 10 patients with mismatch repair–deficient colorectal cancer and in none of the 19 patients with mismatch repair–proficient colorectal cancer in whom CEA could be evaluated (Figure 1, and Fig. S4 in [Supplementary Appendix 1](#)). Among

TABLE 2

Response	RECIST	Immune-Related
Objective response	4 (40%)	4 (40%)
Stable disease	6 (60%)	6 (60%)
Progressive disease	0 (0%)	0 (0%)
Not evaluable	0 (0%)	0 (0%)
Total	10 (100%)	10 (100%)

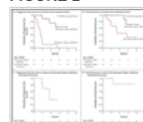
Objective Responses According to RECIST Criteria.

FIGURE 1



Clinical Responses to Pembrolizumab Treatment.

FIGURE 2



Clinical Benefit of Pembrolizumab Treatment According to Mismatch-Repair Status.

TABLE 3

Adverse Event	Number of Patients	Percentage of Patients
Rash or pruritus	8	24%
Thyroiditis	3	10%
Hypothyroidism	3	10%
Hypophysitis	3	10%
Asymptomatic pancreatitis	5	15%

Adverse Events.

patients with mismatch repair–deficient noncolorectal cancer, levels of tumor markers (CEA, CA19-9, or CA-125) were elevated above the upper limit of normal in 4 patients. Declines in CA19-9 or CA-125 of more than 70% occurred in 3 of these 4 patients. Tumor marker kinetics in all three cohorts are shown in [Figure 1](#). The degree of CEA decline after one dose (between day 14 and day 28) of pembrolizumab was predictive of both progression-free survival ($P=0.01$) and overall survival ($P=0.02$). The CEA response occurred well in advance of radiographic confirmation of disease control (range, 10 to 35 weeks). In contrast, patients who had disease progression had rapid biomarker elevation within 30 days after the initiation of therapy. Thus, changes in CEA levels significantly preceded and correlated with ultimate radiographic changes.

Genomic Analysis

The analysis of whole-exome sequences showed a mean of 1782 somatic mutations per tumor in patients with mismatch repair–deficient cancer (nine patients), as compared with 73 mutations per tumor in patients with mismatch repair–proficient cancer (six patients) ($P=0.007$ by nonparametric Wilcoxon test) (Fig. S5 in [Supplementary Appendix 1](#) and Table S3 in [Supplementary Appendix 2](#)). Most of these mutations (63%) are predicted to alter amino acids.

These mutations were then assessed for their immunogenic potential in the context of each patient's MHC haplotype. We identified a mean of 578 potential mutation-associated neoantigens from the tumors of patients with mismatch repair–deficient cancers; 21 such neoantigens were identified in tumors from patients with mismatch repair–proficient cancers (Table S3 in [Supplementary Appendix 2](#)). The percentage of potential mutation-associated neoantigens among all somatic mutations was similar in the two cohorts (a mean of 32% in patients with mismatch repair–deficient cancer and 29% in patients with mismatch repair–proficient cancer). High numbers of somatic mutations and potential mutation-associated neoantigens were associated with longer progression-free survival and with a trend toward objective response (Fig. S5 and Table S4 in [Supplementary Appendix 1](#)).

Immunohistochemical Analysis

The expression of CD8 and PD-L1 was evaluated within the tumor and at the invasive fronts of the tumor in an immunohistochemical analysis in the 30 cases in which tumor tissue was available (Fig. S6 in [Supplementary Appendix 1](#)). Tumors from patients in cohorts A and C contained a greater density of CD8-positive lymphoid cells than did tumors from patients in cohort B ($P=0.10$) (Fig. S7 in [Supplementary Appendix 1](#)), and CD8 labeling was associated with a trend toward objective response and stable disease (Fig. S8 and Table S5 in [Supplementary Appendix 1](#)). This CD8-positive lymphoid infiltrate was especially prominent at the invasive fronts of the tumors ($P=0.04$) (Fig. S7 in [Supplementary Appendix 1](#)). Membranous PD-L1 expression occurred only in patients with mismatch repair–deficient cancer and was prominent on tumor-infiltrating lymphocytes and tumor-associated macrophages located at the invasive fronts of the tumor ($P=0.04$) (Fig. S7 in [Supplementary Appendix 1](#)). The expression of CD8 and PD-L1 was not significantly associated with progression-free survival or overall survival (Table S5 in [Supplementary Appendix 1](#)).

DISCUSSION

The data from this small phase 2 trial of pembrolizumab for the treatment of tumors with and without mismatch-repair deficiency support the hypothesis that mismatch repair–deficient tumors are more responsive to PD-1 blockade than are mismatch repair–proficient tumors. Mismatch-repair deficiency occurs in many cancers, including those of the colorectum, uterus, stomach, biliary tract, pancreas, ovary, prostate, and small intestine.^{18,34-42} It is possible that patients with mismatch repair–deficient tumors of these types may also benefit from anti-PD-1 therapy, as may patients whose tumors contain other DNA repair deficiencies, such as those with mutations in *POLD*, *POLE*, or *MYH*.^{18,43,44}

The hypothesis that mismatch repair–deficient tumors stimulate the immune system is not a new idea⁴⁵; it has been supported by observations of the dense immune infiltration and Th1-associated cytokine-rich environment in mismatch repair–deficient tumors.^{19-22,46} A recent study refined these classic observations by showing that the mismatch repair–deficient tumor microenvironment strongly expressed several immune checkpoint ligands, including PD-1, PD-L1, CTLA-4, LAG-3, and IDO, which indicates that their active immune microenvironment is counterbalanced by immune inhibitory signals that resist tumor elimination.⁴⁷ The most likely explanation for both the old and new findings was that the immune infiltrate associated with mismatch repair–deficient carcinomas was directed at neoantigens. The correlation of a higher mutational load and a higher rate of response to anti-CTLA-4 in melanoma⁴¹ and anti-PD-1 in lung cancer⁴⁸ provides further support for the idea that mutation-associated neoantigen recognition is an important component of the endogenous antitumor immune response.

On the basis of the results of the current and previous studies, we suggest that the greatly increased number of mutation-associated neoantigens resulting from mismatch-repair deficiency (more than 20 times higher than in tumors without this deficiency) (Table S4 in [Supplementary Appendix 1](#)) is the basis for the enhanced anti-PD-1 responsiveness of this genetically defined subset of cancers. Although our estimates of the number of mutation-associated neoantigens in tumors are based only on in silico predictions of binding affinity, this suggestion is consistent with the observation that mismatch repair–proficient tumors have far less infiltration of lymphocytes than do mismatch repair–deficient tumors (Fig. S6 and S7 and Table S5 in [Supplementary Appendix 1](#)). Recent studies^{49,50} have shown that only a tiny proportion of predicted neo-epitopes are actually presented on the cell surface with MHC and are targets of endogenous T cell responses. It seems likely, however, that the number of predicted mutation-associated neoantigens is proportionate to the number of actual mutation-associated neoantigens, and tumors with a high number of actual mutation-associated neoantigens are more likely to stimulate the immune system to react against the tumor.

Alternative mechanisms underlying the difference in anti-PD-1 responsiveness between mismatch repair–deficient tumors and mismatch repair–proficient tumors should also be considered. For example, different signaling pathways activated in the two types of tumors may result in differences in the secretion of soluble factors that could result in differential activation of the PD-1 pathway within the tumor microenvironment.²⁶⁻²⁸ Genetic differences could affect epigenetic differences that alter the expression of tumor-associated self-antigens; in turn, these could alter the antigenicity of

alter the expression of tumor-associated self-antigens; in turn, these could alter the antigenicity of the tumor. Experimental analyses of antigen-specific immune responses and of changes in immune microenvironments should help to define the relative contribution of these factors to the striking responsiveness of mismatch repair-deficient tumors to PD-1 antibodies.

Several other notable observations were made during the course of this study. First, changes in serum levels of protein biomarkers, such as CEA, corresponded with clinical benefit after a single dose of therapy. Declines in CEA levels preceded objective radiographic evidence of treatment benefit by several months; perhaps other biomarkers, such as circulating tumor DNA, would also be beneficial as surrogate markers of early response.⁵¹⁻⁵³ Second, our results suggest that the evaluation of tumor genomes can help guide immunotherapy. They support the view that the number and type of alterations may prove to be valuable for judging the potential usefulness of immune checkpoint inhibitors, even in mismatch repair-proficient cancers.^{41,48,54} Most importantly, our results show an approach for the treatment of a specific class of tumors that is based solely on genetic status — that is, without regard to the underlying tumor type.

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[Disclosure forms](#) provided by the authors are available with the full text of this article at NEJM.org.

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ORIGINAL ARTICLE

Combined Nivolumab and Ipilimumab or Monotherapy in Untreated Melanoma

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Considerable progress in the treatment of metastatic melanoma has been made in the past 5 years, with the approval of immune checkpoint–blocking antibodies and, in parallel, agents targeting aberrant signaling in the 40 to 50% of melanomas with *BRAF* mutations.^{1–6} Ipilimumab, an anti–cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) antibody, acts to up-regulate antitumor immunity and was the first agent to be associated with an improvement in overall survival in a phase 3 study involving patients with metastatic melanoma.^{5,6} Ipilimumab was associated with responses in 10% and 15% of patients^{5,6}; approximately 20% of treated patients had long-term survival.^{7,8}

Two anti–programmed death 1 (PD-1) antibodies, nivolumab and pembrolizumab, were approved by the Food and Drug Administration in 2014 for the treatment of metastatic melanoma after progression during ipilimumab treatment and, in patients with *BRAF*-mutated melanoma, after progression during treatment with a *BRAF* inhibitor. These antibodies were associated with objective responses in 30 to 40% of patients, with the majority of responses being durable. Two phase 3 trials have shown superior efficacy of nivolumab, as compared with chemotherapy, in previously untreated patients with wild-type *BRAF* tumors⁹ or in patients with either mutant or wild-type *BRAF* tumors after progression during ipilimumab therapy and, in patients with tumors positive for *BRAF* mutation, after progression during treatment with a *BRAF* inhibitor.¹⁰ Similar results were observed in a phase 2 trial of pembrolizumab versus chemotherapy.¹¹ Recently, pembrolizumab was associated with longer progression-free survival and overall survival and higher response rates than those associated with ipilimumab in a phase 3 trial involving patients with advanced melanoma.¹²

The results of a phase 2 study that compared combined nivolumab and ipilimumab with ipilimumab alone in patients with *BRAF* wild-type melanoma showed objective response rates of 61% with the combination therapy and 11% with the monotherapy, with complete responses in 22% and 0% of patients, respectively.¹³ Treatment-related adverse events of grade 3 or 4 were reported in 54% of the patients in the combination group and in 24% of those in the ipilimumab group. Expression of the PD-1 ligand, PD-L1, has been reported to result in greater benefit with anti–PD-1 monotherapy^{9,10} but not with the combination of anti–PD-1 and anti–CTLA-4 therapy.^{13,14} However, effective cutoff points for defining PD-L1 expression and clinical usefulness have not yet been established.

To confirm and extend these findings, we report one of the coprimary end points (progression-free survival) of a randomized, double-blind, multicenter, phase 3 trial (CheckMate 067) that was conducted to evaluate the safety and efficacy of nivolumab alone or nivolumab combined with ipilimumab in comparison with ipilimumab alone in patients with previously untreated metastatic melanoma. Data on overall survival are insufficiently mature to present.

METHODS

Patients

Eligible patients had histologically confirmed stage III (unresectable) or stage IV melanoma and had received no prior systemic treatment for advanced disease. Other eligibility criteria included an age of at least 18 years; an Eastern Cooperative Oncology Group (ECOG) performance-status score of 0, indicating no symptoms, or 1, indicating mild symptoms (on a scale from 0 to 5, with higher scores indicating greater disability); measurable disease as assessed by means of computed tomography or magnetic resonance imaging according to Response Evaluation Criteria in Solid

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tomography or magnetic resonance imaging according to Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1¹⁵; availability of tissue collected from metastatic or unresectable tumors (archival or recently biopsied samples) for the assessment of PD-L1 status; and known *BRAF* V600 mutation status. Key exclusion criteria were an ECOG performance-status score of 2 (indicating moderate symptoms, with the patient being ambulatory and capable of all self-care but unable to carry out any work activities) or higher and the presence of active brain metastases, ocular melanoma, or autoimmune disease.

Study Design and Treatment

In this double-blind, phase 3 study, enrolled patients were randomly assigned in a 1:1:1 ratio to receive one of the following regimens: 3 mg of nivolumab per kilogram of body weight every 2 weeks (plus ipilimumab-matched placebo); 1 mg of nivolumab per kilogram every 3 weeks plus 3 mg of ipilimumab per kilogram every 3 weeks for 4 doses, followed by 3 mg of nivolumab per kilogram every 2 weeks for cycle 3 and beyond; or 3 mg of ipilimumab per kilogram every 3 weeks for 4 doses (plus nivolumab-matched placebo). Both nivolumab and ipilimumab were administered by means of intravenous infusion.

Randomization was stratified according to tumor PD-L1 status (positive vs. negative or indeterminate), *BRAF* mutation status (V600 mutation–positive vs. wild-type), and American Joint Committee on Cancer metastasis stage (M0, M1a, or M1b vs. M1c). Treatment continued until disease progression (as defined by RECIST, version 1.1), development of unacceptable toxic events, or withdrawal of consent. Patients could be treated after progression, provided that they had a clinical benefit and did not have substantial adverse effects, as assessed by the investigator (further details are provided in the study protocol, available with the full text of this article at NEJM.org).

Progression-free survival and overall survival were coprimary end points; results regarding progression-free survival are presented here. Secondary and other end points included objective response rate, tumor PD-L1 expression as a predictive biomarker for efficacy outcomes, and safety.

Assessments

Patients were assessed for tumor response, according to RECIST, version 1.1,¹⁵ at 12 weeks after randomization, then every 6 weeks for 49 weeks, and then every 12 weeks until progression or treatment discontinuation, whichever occurred later. Progression-free survival was defined as the time between the date of randomization and the date of documented progression or death, whichever occurred first. Patients treated after progression were considered to have had progressive disease at the time of the initial progression event, as assessed by the investigator, regardless of subsequent tumor responses.

Expression of PD-L1 on the surface of the tumor cells was assessed in a central laboratory by means of immunohistochemical testing in formalin-fixed, paraffin-embedded tumor specimens with the use of a rabbit monoclonal antihuman PD-L1 antibody (clone 28–8) and an analytically validated automated assay developed by Dako. PD-L1 positivity was defined as at least 5% of tumor cells showing PD-L1 staining of any intensity on the cell surface in a section containing at least 100 tumor cells that could be evaluated.

Any patient who received at least one dose of study drug was included in the assessment of safety. The severity of adverse events was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.¹⁶ Safety assessments were made continuously during the treatment phase and up to 100 days after the last dose of study drug. Guidelines for the management of adverse events were provided by the sponsor and have been published previously.^{9,10,13}

Study Oversight

The study protocol and all amendments were approved by the institutional review board at each participating study site and are available (along with the statistical analysis plan) at NEJM.org. The study was conducted in accordance with the provisions of the Declaration of Helsinki and with Good Clinical Practice guidelines as defined by the International Conference on Harmonisation. All the patients (or their legal representatives) provided written informed consent before enrollment. The trial was designed as a collaboration between the senior academic authors and the sponsor, Bristol-Myers Squibb. Data were collected by the sponsor and analyzed in collaboration with all the authors.

All the authors vouch for the accuracy and completeness of the data and analyses reported and for the fidelity of the study to the protocol. The first draft of the manuscript was prepared by the first and last authors. All the authors contributed to subsequent drafts and provided final approval to submit the manuscript for publication. Medical-writing support, funded by the sponsor, was provided by StemScientific.

A data and safety monitoring committee was established to provide oversight of safety and efficacy considerations, in order to assess the benefit–risk profile of nivolumab combined with ipilimumab. As per the statistical analysis plan, the results of the coprimary end point of progression-free-survival were released by the monitoring committee on March 17, 2015, after a positive statistical outcome. The study remains blinded with respect to overall survival because follow-up of the patients is planned to continue until the specified number of events have occurred.

Statistical Analysis

A study sample of approximately 915 patients was planned. For the comparison of progression-free survival, we estimated that the number of events that was projected to be observed at a follow-up of at least 9 months would give the study approximately 83% power to detect an average hazard ratio of 0.71 at a type I error rate of 0.005 (two-sided) for all comparisons. Progression-free survival was compared between the nivolumab group or the nivolumab-plus-ipilimumab group and the ipilimumab group, with the use of a two-sided log-rank test with stratification as described above. The study was not designed for a formal statistical comparison between the nivolumab group and the nivolumab-plus-ipilimumab group.

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Hazard ratios and corresponding two-sided 99.5% confidence intervals were estimated with the use of a Cox proportional-hazards model, with treatment group as a single covariate, stratified according to the factors listed above. Progression-free survival curves, medians with 95% confidence intervals, and progression-free survival rates at 6, 12, and 18 months with 95% confidence intervals were estimated with the use of the Kaplan–Meier method.

RESULTS

Patients and Treatment

From July 2013 through March 2014, a total of 1296 patients were enrolled at 137 centers in Australia, Europe, Israel, New Zealand, and North America. A total of 945 patients underwent randomization: 316 patients were assigned to the nivolumab group, 314 to the nivolumab-plus-ipilimumab group, and 315 to the ipilimumab group (Fig. S1 in the [Supplementary Appendix](#), available at NEJM.org). Baseline characteristics were balanced across the three groups. A total of 58.0% of the patients had stage M1c disease, 36.1% had an elevated lactate dehydrogenase level, 31.5% had a *BRAF* mutation, and 23.6% had positive PD-L1 status (Table 1).

The database lock occurred on February 17, 2015. At a median follow-up ranging from 12.2 to 12.5 months across the three groups, 117 of 313 patients (37.4%) in the nivolumab group, 93 of 313 (29.7%) in the nivolumab-plus-ipilimumab group, and 50 of 311 (16.1%) in the ipilimumab group were continuing study treatment (Table S1 in the [Supplementary Appendix](#)). The most frequent reason for discontinuation was disease progression in the nivolumab and ipilimumab monotherapy groups (154 of 313 patients [49.2%] and 202 of 311 [65.0%], respectively) and toxic effects of the study drug in the nivolumab-plus-ipilimumab group (120 of 313 [38.3%]).

The median number of doses was 15 (range, 1 to 38) in the patients who received nivolumab alone and 4 (range, 1 to 4) in those who received ipilimumab alone. In the nivolumab-plus-ipilimumab group, the median number of doses was 4 (range, 1 to 39) of nivolumab and 4 (range, 1 to 4) of ipilimumab; 147 of 313 patients (47.0%) received more than 4 doses of nivolumab monotherapy.

Efficacy

The median progression-free survival was 6.9 months (95% confidence interval [CI], 4.3 to 9.5) in the nivolumab group, 11.5 months (95% CI, 8.9 to 16.7) in the nivolumab-plus-ipilimumab group, and 2.9 months (95% CI, 2.8 to 3.4) in the ipilimumab group. Significantly longer progression-free survival was observed in the nivolumab-plus-ipilimumab group than in the ipilimumab group (hazard ratio for death or disease progression, 0.42; 99.5% CI, 0.31 to 0.57; $P < 0.001$) and in the nivolumab group than in the ipilimumab group (hazard ratio, 0.57; 99.5% CI, 0.43 to 0.76; $P < 0.001$) (Figure 1A). The hazard ratio for the comparison between the nivolumab-plus-ipilimumab group and the nivolumab group was 0.74 (95% CI, 0.60 to 0.92).

Analyses of progression-free survival in prespecified subgroups showed consistently longer progression-free survival with nivolumab or with nivolumab plus ipilimumab than with ipilimumab, including in subgroups defined according to PD-L1 status, *BRAF* mutation status, and metastasis stage (Figure 1B and 1C, and Fig. S2 in the [Supplementary Appendix](#)). In the nivolumab-plus-ipilimumab group, the median progression-free survival was 11.7 months (95% CI, 8.0 to not reached) among patients with a *BRAF* mutation and 11.2 months (95% CI, 8.3 to not reached) among patients with wild-type *BRAF*.

Among patients with a positive PD-L1 tumor status, the median progression-free survival was 14.0 months (95% CI, 9.1 to not reached) in the nivolumab group, 14.0 months (95% CI, 9.7 to not reached) in the nivolumab-plus-ipilimumab group, and 3.9 months (95% CI, 2.8 to 4.2) in the ipilimumab group (Figure 1B). Among patients with a negative PD-L1 tumor status, the median progression-free survival was 5.3 months (95% CI, 2.8 to 7.1), 11.2 months (95% CI, 8.0 to not reached), and 2.8 months (95% CI, 2.8 to 3.1), respectively (Figure 1C).

The rates of investigator-assessed objective response were 43.7% (95% CI, 38.1 to 49.3) in the nivolumab group, 57.6% (95% CI, 52.0 to 63.2) in the nivolumab-plus-ipilimumab group, and 19.0% (95% CI, 14.9 to 23.8) in the ipilimumab group (Table 2). The percentage of patients with a complete response was higher in the nivolumab-plus-ipilimumab group (11.5%) than in either the nivolumab group (8.9%) or the ipilimumab group (2.2%) (Table 2). The time to an objective response was similar in the three groups (Table 2), and the median duration of response was not reached in any group.

The tumor-burden change was assessed as the change from baseline in the sum of the longest diameters of the target tumor lesions. The median change was –34.5% (interquartile range, –75.4 to 15.4) in the nivolumab group, –51.9% (interquartile range, –75.8 to –10.2) in the nivolumab-plus-ipilimumab group, and 5.9% (interquartile range, –28.0 to 33.3) in the ipilimumab group (Figure 2).

Among patients with PD-L1–positive tumors, the objective response rates were 57.5% (95% CI, 45.9 to 68.5) in the nivolumab group, 72.1% (95% CI, 59.9 to 82.3) in the nivolumab-plus-ipilimumab group, and 21.3% (95% CI, 12.7 to 32.3) in the ipilimumab group. Among patients with PD-L1–negative tumors, the objective response rates were 41.3% (95% CI, 34.6 to 48.4), 54.8% (95% CI, 47.8 to 61.6), and 17.8% (95% CI, 12.8 to 23.8), respectively (Table S2 in the [Supplementary Appendix](#)).

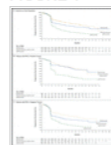
Adverse Events

Treatment-related adverse events of any grade occurred in 82.1% of the patients in the nivolumab group, 95.5% of those in the nivolumab-plus-ipilimumab group, and 86.2%

TABLE 1

Characteristics of the Patients at Baseline.

FIGURE 1

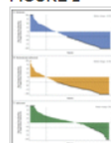


Progression-free Survival.

TABLE 2

Response to Treatment.

FIGURE 2



Tumor-Burden Change in Target Lesions.

or those in the ipilimumab group (Table 3). The most common adverse events in the nivolumab-plus-ipilimumab group were diarrhea (in 44.1% of patients), fatigue (in 35.1%), and pruritus (in 33.2%). The incidence of treatment-related adverse events of grade 3 or 4 was also higher in the nivolumab-plus-ipilimumab group (55.0%) than in the nivolumab group (16.3%) or the ipilimumab group (27.3%).

Treatment-related adverse events of any grade that led to discontinuation of the study drug occurred in 7.7% of the patients in the nivolumab group, 36.4% of those in the nivolumab-plus-ipilimumab group, and 14.8% of those in the ipilimumab group, with the most common events being diarrhea (in 1.9%, 8.3%, and 4.5%, respectively) and colitis (in 0.6%, 8.3%, and 7.7%, respectively). One death due to toxic effects of the study drug was reported in the nivolumab group (neutropenia) and one in the ipilimumab group (cardiac arrest), but none were reported in the nivolumab-plus-ipilimumab group.

Select adverse events — defined as those with a potential immunologic cause — were analyzed according to organ category, as in previous studies.^{9,10} The most frequent treatment-related select adverse events of grade 3 or 4 were diarrhea (in 2.2% of patients in the nivolumab group, 9.3% of those in the nivolumab-plus-ipilimumab group, and 6.1% of those in the ipilimumab group), colitis (in 0.6%, 7.7%, and 8.7%, respectively), and increased alanine aminotransferase level (in 1.3%, 8.3%, and 1.6%, respectively) (Table S3 in the [Supplementary Appendix](#)).

Immune modulatory agents, including topical agents, to manage adverse events were used in 47.0% of the patients in the nivolumab group, 83.4% of those in the nivolumab-plus-ipilimumab group, and 55.9% of those in the ipilimumab group, with secondary immunosuppressive agents (e.g., infliximab) used in 0.6%, 6.1%, and 5.1% of the patients, respectively. Resolution rates for select adverse events of grade 3 or 4 were between 85 and 100% in the nivolumab-plus-ipilimumab group for most organ categories (Table S4 in the [Supplementary Appendix](#)). As in prior studies, most endocrine events did not resolve.

DISCUSSION

In this randomized, double-blind, phase 3 study involving patients with previously untreated advanced melanoma, treatment with nivolumab alone or with the combination of nivolumab and ipilimumab resulted in significantly longer progression-free survival and higher objective response rates than did treatment with ipilimumab alone. In the two nivolumab-containing groups, as compared with ipilimumab, these results were observed independently of PD-L1 tumor status, *BRAF* mutation status, or metastasis stage. The characteristics of the study participants at baseline were typical of patients with advanced melanoma, although the proportion of patients with *BRAF* mutation (31.5%) was lower than the 40 to 50% that is generally reported for patients with advanced disease.¹⁷ Although the study was not designed for a formal statistical comparison between the nivolumab group and the nivolumab-plus-ipilimumab group, the combination of nivolumab and ipilimumab resulted in numerically longer progression-free survival and a higher rate of response than did nivolumab alone in the overall study population.

The median progression-free survival that was observed with the combination of nivolumab and ipilimumab in this study (11.7 months in patients with *BRAF* mutation) is similar to that recently reported with combined *BRAF* and MEK inhibition in patients with *BRAF*-mutated metastatic melanoma (9.9 months with vemurafenib and cobimetinib² and 9.3 to 11.4 months with dabrafenib and trametinib^{3,4}). However, it remains unclear how the duration of response with nivolumab and ipilimumab compares with that observed with combined *BRAF* and MEK inhibition. The rate of objective response in the nivolumab-plus-ipilimumab group (57.6%) is numerically higher than that observed with PD-1 blockade alone in patients with advanced melanoma (40% with nivolumab in previously untreated patients with wild-type *BRAF*⁹ or 33% with pembrolizumab in previously treated patients and previously untreated patients¹²). Given the results of a phase 1 trial in which nivolumab plus ipilimumab was associated with a survival rate of 79% at 2 years,¹⁸ as well as the long-term survival data reported for ipilimumab,^{7,8} it will be interesting to determine whether the efficacy results reported here will be reflected in an overall survival benefit.

The results of subgroup analyses suggest that the greatest benefit with the combination of nivolumab and ipilimumab versus nivolumab alone may occur in the context of negative PD-L1 tumor expression. In the subgroup of patients with PD-L1–positive tumors, both nivolumab alone and nivolumab plus ipilimumab resulted in a similar prolongation of progression-free survival as compared with ipilimumab alone, although objective response rates were numerically higher in the combination group than in either monotherapy group. Thus, the use of PD-L1 as a biomarker may allow clinicians to make more informed decisions about the benefit–risk ratio of combination therapy versus monotherapy. However, caution is warranted in interpreting these data because the effects on overall survival are not yet known and because the most effective method and cutoff point for assaying PD-L1 expression remain to be determined. Nonetheless, the observation of at least additive activity of the combination of ipilimumab and nivolumab in the context of negative PD-L1 expression is of interest in melanoma as well as in other tumor types in which PD-1 checkpoint inhibitors are under evaluation.

The incidence of adverse events in this study was, in general, lowest in the nivolumab group and highest in the combination group. The overall incidence of treatment-related adverse events of grade 3 or 4 was higher in the combination group than in either monotherapy group, as a result of a higher incidence of most adverse events — in particular, hepatic toxic events, for which the rates of grade 3 or 4 elevations of the aminotransferase levels were 6.1% (for aspartate aminotransferase) and 8.3% (for alanine aminotransferase) in the combination group and approximately 1 to 2% in each of the monotherapy groups. In general, the safety profile of the combination therapy was consistent with previous experience with nivolumab or ipilimumab alone.^{5,9,10} No new safety signals were identified, and there were no drug-related deaths in the combination group. Adverse events were manageable with established treatment guidelines, and most select adverse events resolved with the use of immune-modulatory agents.

In conclusion, among patients with previously untreated advanced melanoma, we found longer

TABLE 3

Adverse Event	Nivolumab (n=264)	Nivolumab + Ipilimumab (n=264)	Ipilimumab (n=264)
Diarrhea	44.1%	44.1%	33.2%
Fatigue	35.1%	35.1%	33.2%
Pruritus	33.2%	33.2%	33.2%
Colitis	0.6%	8.3%	7.7%
Increased alanine aminotransferase level	1.3%	8.3%	1.6%
Grade 3 or 4 adverse events	16.3%	55.0%	27.3%

Adverse Events.

progression-free survival and higher rates of objective response with nivolumab alone and with the combination of nivolumab and ipilimumab than with ipilimumab alone. The management of adverse events with the combination therapy suggests that it can be used safely in a broad range of clinical settings.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

Drs. Hodi and Wolchok contributed equally to this article.

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