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Research Article

Cancer Immunology Research

Phase I/II Study of Metastatic Melanoma Patients Treated with Nivolumab Who Had Progressed after Ipilimumab

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Abstract

The checkpoint inhibitor nivolumab is active in patients with metastatic melanoma who have failed ipilimumab. In this phase I/II study, we assessed nivolumab's safety in 92 ipilimumab-refractory patients with unresectable stage III or IV melanoma, including those who experienced grade 3–4 drug-related toxicity to ipilimumab. We report long-term survival, response duration, and biomarkers in these patients after nivolumab treatment (3 mg/kg) every 2 weeks for 24 weeks, then every 12 weeks for up to 2 years, with or without a multipeptide vaccine. The response rate for ipilimumab-refractory patients was 30% (95% CI, 21%–41%). The median duration of response was 14.6 months, median progression-free survival was 5.3 months, and median overall survival was 20.6 months, when patients were followed up for a median of 16 months. One- and 2-year survival rates were 68.4% and 31.2%, respectively. Ipilimumab-naïve and ipilimumab-refractory patients showed no significant difference in survival. The 21 patients with prior grade 3–4 toxicity to ipilimumab that was managed with steroids tolerated nivolumab well, with 62% (95% CI, 38%–82%) having complete or partial responses or stabilized disease at 24 weeks. High numbers of myeloid-derived suppressor cells (MDSC) were associated with poor survival. Thus, survival and long-term safety were excellent in ipilimumab-refractory patients treated with nivolumab. Prior grade 3–4 immune-related adverse effects from ipilimumab were not indicative of nivolumab toxicities, and patients had a high overall rate of remission or stability at 24 weeks. Prospectively evaluating MDSC numbers before treatment could help assess the expected benefit of nivolumab. *Cancer Immunol Res; 4(4); 1–9. ©2016 AACR.*

Introduction

Nivolumab, an IgG4 human antibody that blocks the programmed death-1 (PD-1) receptor on T and B cells, has significant clinical activity in previously treated patients with melanoma who are ipilimumab (anti–CTLA-4) naïve (1, 2). In a phase III trial, anti–PD-1 had a higher objective response rate (ORR) and superior toxicity profile when compared with investigator-chosen chemotherapy in patients who had progressed after treatment with ipilimumab alone or with a BRAF inhibitor (3). Those data supported the FDA approval of nivolumab in ipilimumab-refractory melanoma in late 2014, but follow-up was short, and overall survival (OS) data were not mature at the time of publication.

A trial in treatment-naïve melanoma patients showed that nivolumab had superior survival compared with dacarbazine as first-line therapy, albeit with a median duration of follow-up of less than a year (4). Long-term follow-up studies in a phase I cohort of nivolumab-naïve, treatment-refractory melanoma patients who had not been exposed to ipilimumab showed that the 3-year OS rate was 41%, with a median survival of 22 months (5, 6). These data established the utility of nivolumab in ipilimumab-naïve and ipilimumab-refractory melanoma patients, but long-term toxicity, response duration, and OS have not been described in ipilimumab-refractory patients treated with nivolumab. It is still not clear how patients who had severe or dose-limiting (grades 3–4) immune-related adverse events (irAE) from ipilimumab would respond to nivolumab, because those patients have generally been excluded from trials of the PD-1 antibodies, such as nivolumab and pembrolizumab (7, 8). Predictive markers for the utility of nivolumab and other PD-1/PD-L1 blocking antibodies have not been well defined, although in multiple studies in melanoma and other tumor histologies, programmed-death ligand-1 (PD-L1) staining of the tumor, stroma, or combinations of those two tissues appear to be associated with improved OS after treatment with PD-1 antibodies (9–12).

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Note: Supplementary data for this article are available at Cancer Immunology Research Online (http://cancerimmunolres.aacrjournals.org/).

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In this article, we expand upon a previous report on the treatment of 90 patients with ipilimumab-naïve or ipilimumab-refractory melanoma with nivolumab with or without a peptide vaccine (13) and provide long-term duration of response and survival data from a cohort of 92 patients treated with nivolumab whose disease had progressed after treatment with ipilimumab. We demonstrate that patients who finished a two-and-a-half-year regimen of nivolumab with a complete response (CR), partial response (PR), or stable disease (SD) do not progress after stopping therapy, and that patients who had grade 3 or 4 irAEs from ipilimumab and did not receive infliximab did not recapitulate the same toxicity when treated with nivolumab. The regimen of the current cohort included treatment with nivolumab at 3 mg/kg every other week for 24 weeks, but patients then received drug every 12 weeks for the next 96 weeks, a schedule that differs from those used in other nivolumab phase II/III trials in which the drug was given every other week for at least 96 weeks, or until progression (3–6). Analysis of the pretreatment peripheral blood in the current trial showed that higher levels of myeloid-derived suppressor cells (MDSC) were associated with lower response rate, higher rates of progression of disease, and shorter survival.

Materials and Methods

Patients

One hundred and twenty-six patients were enrolled at Moffitt Cancer Center onto a trial approved by the University of South Florida Institutional Review Board (ClinicalTrials.gov identifier: NCT01176461), of which 92 had progressed after ipilimumab without response or SD and were deemed ipilimumab refractory; they are the principal subject of this report. Inclusion criteria included written informed consent; age 16 years or older; histologic diagnosis of unresectable stage III or IV melanoma with measurable disease by modified World Health Organization (mWHO) criteria; progressive disease after at least one previous systemic treatment; positive tumor staining in at least 10% of tumor cells for gp100, NY-ESO-1, and/or MART-1; Eastern Cooperative Oncology Group performance status of 0 or 1; and adequate hepatic, renal, and hematologic function. Patients were prescreened for HLA-A*0201 by allele-specific PCR for cohorts 1–5, whose patients also received a multi-peptide vaccine (Supplementary Table S1). Patients with treated brain metastases were allowed if they were radiographically stable 8 weeks after treatment; patients with untreated brain metastases were allowed in cohort 6. Patients in cohorts 4–6 were required to start nivolumab 8 or more weeks after prior ipilimumab. Any number of prior therapies was allowed; treatment with prior anti–PD-1 or anti–PD-L1 was not allowed. Patients in cohort 5 had grade 3 or 4 irAEs with ipilimumab but could not have had grade 4 colitis, nor could they have received infliximab. The 92 patients in cohorts 4–6 (ipilimumab refractory) all had progressive disease without responding to prior ipilimumab. Cohorts 4 and 5 were consecutively accrued and received nivolumab with peptide vaccine, and cohort 6 received nivolumab alone and accrued concurrently with cohorts 4–5; in cohorts 4–6, one patient withdrew consent but was included in the analysis for safety and efficacy and was replaced per protocol. No patients were ineligible or lost to follow-up. The assignment of patients by cohort is shown in Supplementary Table S1.

Study design and treatment

Nivolumab was provided by Bristol-Myers Squibb. The gp100209-217 [210M; National Service Center (NSC) No. 683472] and MART- 126-35 (27L; NSC No. 709401) peptides were provided by the Cancer Therapy Evaluation Program of the National Cancer Institute. The good manufacturing practice grade gp100280-288 (288V; NSC No. 683473) and NY-ESO-1157-165 (165V; NSC No. 717388) peptides were produced by Clinalfa. All peptides were emulsified in Montanide ISA 51 VG (Seppic) and were included to assess the effects of PD-1 blockade on antigen-specific T-cell reactivity. The protocol was conducted under Investigational New Drug number BB 13704 with the Food and Drug Administration (13). Primary endpoints were toxicity and tolerability, and secondary endpoints were ORR, duration of response, progression-free survival (PFS) and OS, as well as correlative immune assays.

Assessment of response and adverse effects

Tumor assessments included chest, abdomen, and pelvis CT and brain MRI with contrast every 12 weeks. Objective response (CR and PR) was evaluated using mWHO and immune-related response criteria (14); immune-related response criteria were used to only determine whether patients should remain on treatment in case of a mixed response. Patients were assessed with history and physical examinations every 2 weeks for up to 24 weeks and then every 12 weeks thereafter. Leukapheresis was performed before treatment, at week 12, and at week 24 in cohorts 4 and 5, and 80 mL of peripheral blood was collected at the same time points from patients in cohort 6. Patients were discontinued from treatment for disease progression, dose-limiting nivolumab-, or vaccine-related adverse events as defined in the Supplementary Materials and Methods, or upon withdrawal of consent.

Flow cytometry analysis for MDSC

Peripheral blood mononuclear cells were collected by leukapheresis as previously described and purified using Lymphoprep (Stemcell Technologies) gradient, then frozen in liquid nitrogen prior to thawing and analysis (13). Phenotypic markers of MDSCs were evaluated by flow cytometry with a lineage-marker–negative population gated to exclude CD3-, CD19-, or CD56-expressing cells using antibodies to CD11b, CD14, HLA-DR, and CD33 from BD Biosciences, except where indicated. The lineage-marker–positive cells ($CD3^+$, $CD19^+$, and $CD56^+$) highly expressed HLA-DR, which was used as a reference to set the $HLA\text{-}DR^{low}$ gate, which included the cells below the bottom edge of the clearly positive expression of that molecule from the lineage-positive cells. Peripheral blood mononuclear cells were stained with Live/Dead violet dye (Invitrogen) to gate on live cells. Data were acquired on an LSR II flow cytometer (BD Biosciences) and analyzed with FlowJo software (TreeStar). Data were analyzed by the principal investigator, J. Weber, and biostatisticians X. Zhao and Y.A. Chen.

Statistical analysis

The primary objective of this study for cohorts 4–6 was to assess the safety and tolerability of nivolumab with or without a peptide vaccine in ipilimumab-refractory patients. The secondary objectives were to evaluate the ORR, PFS, OS, and changes in immunity. Toxicity rate was calculated by using the number of patients with grade 3 or greater toxicity divided by all patients. The ORR was estimated using the number of CRs

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and PRs at 24 weeks divided by the total number of patients treated. Patients were required to be observed for at least 24 weeks to be declared a confirmed PR, CR, or SD. The SD rate was estimated using the number of patients with SD for at least 24 weeks divided by the total number of patients. Progression-free survival rate was calculated as the sum of the ORR and SD rate. Duration of response is defined from first response to progression or last follow-up for continuous responders. To visualize the response overall time, we plotted the response from on-treatment time in swimmer's plots. A Wilcoxon rank sum test was performed to determine whether the number of MDSCs before treatment (baseline) differed between those with a response and SD (responder + stable, R+S), and those who did not respond (nonresponder, NR), with all groups evaluated at 24 weeks. The Kaplan–Meier product-limit analysis and log-rank test were performed to investigate the association between OS and amounts of pretreatment monocytic–MDSC (M-MDSC). Proportions of M-MDSCs were dichotomized using the median as the cut point. Tumor change was calculated as sum of maximum tumor shrinkage and minimum tumor increase in size from baseline for each target lesion. Waterfall plots were used to visualize the maximum tumor reduction or minimum increase in size. An alpha level of 0.05 was used to declare statistical significance. The binomial CIs were calculated using the exact Clopper–Pearson method. We used SAS 9.2 (SAS Institute) and Matlab 2015 (MathWorks) for the statistical analyses.

Results

Baseline patient characteristics

Between August 2010 and December 2013, 152 patients were screened for all 6 cohorts, and 126 patients were enrolled. Ninety-two patients were enrolled in cohorts 4–6 and all had progressed without response after receiving ipilimumab. All 92 patients were evaluated for toxicity and for response. Thirty-four patients in cohorts 1–3 have been previously described (13). In cohorts 4 and 5, 15 patients received nivolumab (3 mg/kg) with peptide vaccine; an additional 16 patients in cohort 5, and 61 patients in cohort 6 received nivolumab (3 mg/kg) alone. Three patients dropped out of cohort 6 for early progression of disease, and were replaced, but were evaluable for survival and toxicity. The median age was 60 years. Sixty-five percent of patients were male, and 86% (80/92) had American Joint Commission on Cancer M1c disease. Sixty patients (60/92 = 65%) received two or more prior therapies for metastatic disease. Eighty-five patients had primary cutaneous melanoma. Three patients had ocular melanoma, and 4 patients had an unknown primary. *BRAF* mutational status was known for 69 tumors, and 20 tumors (20/69 = 28%) were *BRAF* mutated. Four patients had experienced progression after a BRAF-targeted therapy before enrollment. Ten patients had radiated brain metastases, and 6 additional patients in cohort 6 had untreated brain metastases. Patient characteristics at trial entry are listed in Table 1.

Table 1. Demographics: ipilimumab-refractory cohorts 4–6

	Number	Percentage	Median
Gender			
Male	60	65	
Female	32	35	
Age			60
Prior regimens			2
Chemotherapy	47	51	
Immunotherapy (not ipilimumab)	65	70	
IL2	26	28	
Targeted	25	27	
BRAF status			
+	20		
−	49		
Unknown	23		
Subtype			
Cutaneous		85	
Ocular		3	
Unknown		4	
Stage			
IIIC		4	
IVa		4	
IVb		4	
IVc		80	
Brain metastases	15	16	—

Safety

Treatment-related adverse events are listed in Table 2 by cohort. The most common adverse events were rash and pruritis, fatigue, arthralgias, and diarrhea across all cohorts. Most events were mild to moderate in severity and easily managed by supportive treatment. Dose-limiting grade 3–4 colitis was not seen in this trial. In the 92 patients in ipilimumab-refractory cohorts 4–6, one dose-limiting toxicity (grade 3 rash) was observed in cohort 5 in a patient who had previously had grade 3 colitis with ipilimumab that resolved completely with a 6-week prednisone taper from 60 mg. One episode of grade 3 pneumonitis was observed in a cohort 5 patient who had prior grade 4 transaminase elevation with ipilimumab, requiring prednisone tapers from 120 mg lasting 3 to 4 months for complete resolution, but which occurred after the dose-limiting toxicity (DLT) period of 12 weeks (at week 14). Both patients fully recovered to baseline without sequelae. One patient in cohort 5 had late-onset grade 3 arthralgias that caused him to stop treatment at week 96, and one additional patient had late grade 3 rash after week 48 that did not require treatment discontinuation. An additional patient who had grade 4 necrotizing fasciitis after ipilimumab that required amputation had grade 2 rash with nivolumab. No other treatment-related grade 3 irAEs were seen in cohort 5. More grade 1 or 2 infusion reactions were observed in cohorts 4–6 (13 of 92 patients, 14%) than in cohorts 1–3 (1 of 34 patients, 3%), although this was not statistically significant ($P = 0.08$). The overall toxicity profile for all 92 ipilimumab-refractory patients in cohorts 4–6 by grades 1–2 and 3–4 for all three cohorts is shown in Table 2. Among the 92 ipilimumab-refractory patients, no patients discontinued nivolumab, and no treatment-related deaths were observed.

Clinical activity

The confirmed ORR rate by mWHO criteria in 92 ipilimumab-refractory patients who received nivolumab was 29%, with 3% CR and 26% PR. An additional 11% of patients had confirmed SD at week 24. The waterfall plot in Fig. 1A shows a transition point at 63%. For cohort 5, as shown in Fig. 1B, the transition point was at 68%. Out of 92 patients, 10 patients progressed before week 24, and no post-treatment scans were obtained. The median of the maximum tumor change for all

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Table 2. Drug-related toxicities for ipilimumab-refractory cohorts 4–6 (includes any irAEs or >5% of total)

	Cohort 4 ($n = 10$)		Cohort 5 ($n = 21$)		Cohort 6 ($n = 61$)		Cohort s4 + 5 + 6 ($n = 92$)	
	Grades 1–2	Grades 3–4	Grades 1–2	Grades 3–4	Grades 1–2	Grades 3–4	Grades 1–2	Grades 3–4
Adrenal insufficiency			1 (5%)		2 (3%)	2 (3%)	3 (3%)	2 (2%)
Alanine aminotransferase increased			2 (10%)		4 (7%)		6 (7%)	
Alkaline phosphatase increased			4 (19%)		3 (5%)		7 (8%)	
Allergic reaction			1 (5%)		1 (2%)		2 (2%)	
Anemia	7 (70%)	1 (10%)			7 (11%)	1 (2%)	14 (15%)	2 (2%)
Anorexia	1 (10%)		1 (5%)		8 (13%)	1 (2%)	10 (11%)	1 (1%)
Arthralgia	8 (80%)		9 (43%)	1 (5%)	11 (18%)		28 (30%)	1 (1%)
Aspartate aminotransferase increased			2 (10%)		3 (5%)		5 (5%)	
Chills	1 (10%)		5 (24%)		5 (8%)		11 (12%)	
Colitis		1 (10%)						1 (1%)
Confusion					1 (2%)		1 (1%)	
Constipation	1 (10%)		2 (10%)		4 (7%)		7 (8%)	
Cough			3 (14%)		1 (2%)		4 (4%)	
Creatinine increased					3 (5%)		3 (3%)	
Dehydration		1 (10%)	1 (5%)		1 (2%)	1 (2%)	2 (2%)	2 (2%)
Diarrhea	5 (50%)		14 (67%)		20 (33%)		39 (42%)	
Dry eye					3 (5%)		3 (3%)	
Dry mouth	1 (10%)		2 (10%)		4 (7%)		7 (8%)	
Dry skin			1 (5%)		4 (7%)		5 (5%)	
Dyspnea	1 (10%)		3 (14%)		4 (7%)		8 (9%)	
Endocrine disorders—Other, specify	1 (10%)		6 (29%)		8 (13%)		15 (16%)	
Erythema multiforme					2 (3%)		2 (2%)	
Fatigue	5 (50%)		16 (76%)		32 (52%)	1 (2%)	53 (58%)	1 (1%)
Fever			5 (24%)	1 (5%)	10 (16%)		15 (16%)	1 (1%)
Flu-like symptoms			4 (19%)				4 (4%)	
Gastrointestinal disorders—Other, specify			1 (5%)		1 (2%)		2 (2%)	
Generalized muscle weakness			2 (10%)		3 (5%)		5 (5%)	
Hyperglycemia			1 (5%)	1 (5%)			1 (1%)	1 (1%)
Headache	4 (40%)		3 (14%)		8 (13%)		15 (16%)	
Hyperhidrosis	1 (10%)		1 (5%)		1 (2%)		3 (3%)	
Hyperthyroidism			3 (14%)		3 (5%)		6 (7%)	
Hyponatremia				1 (5%)	8 (13%)	1 (2%)	8 (9%)	2 (2%)
Hypothyroidism	2 (20%)		4 (19%)		5 (8%)		11 (12%)	
Immune system disorders—Other, specify	1 (10%)		5 (24%)		10 (16%)		16 (17%)	
Infusion-related reaction	3 (30%)		5 (24%)		5 (8%)		13 (14%)	
Injection-site reaction	7 (70%)		8 (38%)				15 (16%)	
Lipase elevated			1 (5%)	1 (5%)			1 (1%)	1 (1%)
Localized edema			2 (10%)		2 (3%)		4 (4%)	
Lymphocyte count decreased	7 (70%)	1 (10%)			1 (2%)	1 (2%)	8 (9%)	2 (2%)
Mucositis oral	1 (10%)		2 (10%)		1 (2%)		4 (4%)	
Myalgia			3 (14%)		1 (2%)		4 (4%)	
Nausea	1 (10%)	1 (10%)	6 (29%)		10 (16%)		17 (18%)	1 (1%)
Neutrophil count decreased	8 (80%)	2 (20%)			2 (3%)		10 (11%)	2 (2%)
Pain	1 (10%)		2 (10%)		1 (2%)		4 (4%)	
Pain in extremity			2 (10%)		1 (2%)		3 (3%)	
Pancreatitis			1 (5%)		1 (2%)		2 (2%)	
Pneumonitis			2 (10%)	1 (5%)			2 (2%)	1 (1%)
Platelet count decreased	3 (30%)	2 (20%)			1 (2%)		4 (4%)	2 (2%)
Pruritus	7 (70%)		7 (33%)		34 (56%)	1 (2%)	48 (52%)	1 (1%)
Rash acneiform					1 (2%)		1 (1%)	
Rash maculo-papular	5 (50%)		14 (67%)	1 (5%)	44 (72%)	4 (7%)	75 (82%)	6 (7%)
Skin and subcutaneous tissue disorders—Other, specify	1 (10%)	1 (10%)	7 (33%)		4 (7%)		12 (13%)	1 (1%)
Stomach pain					3 (5%)		3 (3%)	
Vomiting		1 (10%)	2 (10%)		5 (8%)		7 (8%)	1 (1%)
Weight loss			2 (10%)		3 (5%)		5 (5%)	
White blood cell decreased	1 (10%)	1 (10%)	2 (10%)		4 (7%)		19 (21%)	1 (1%)

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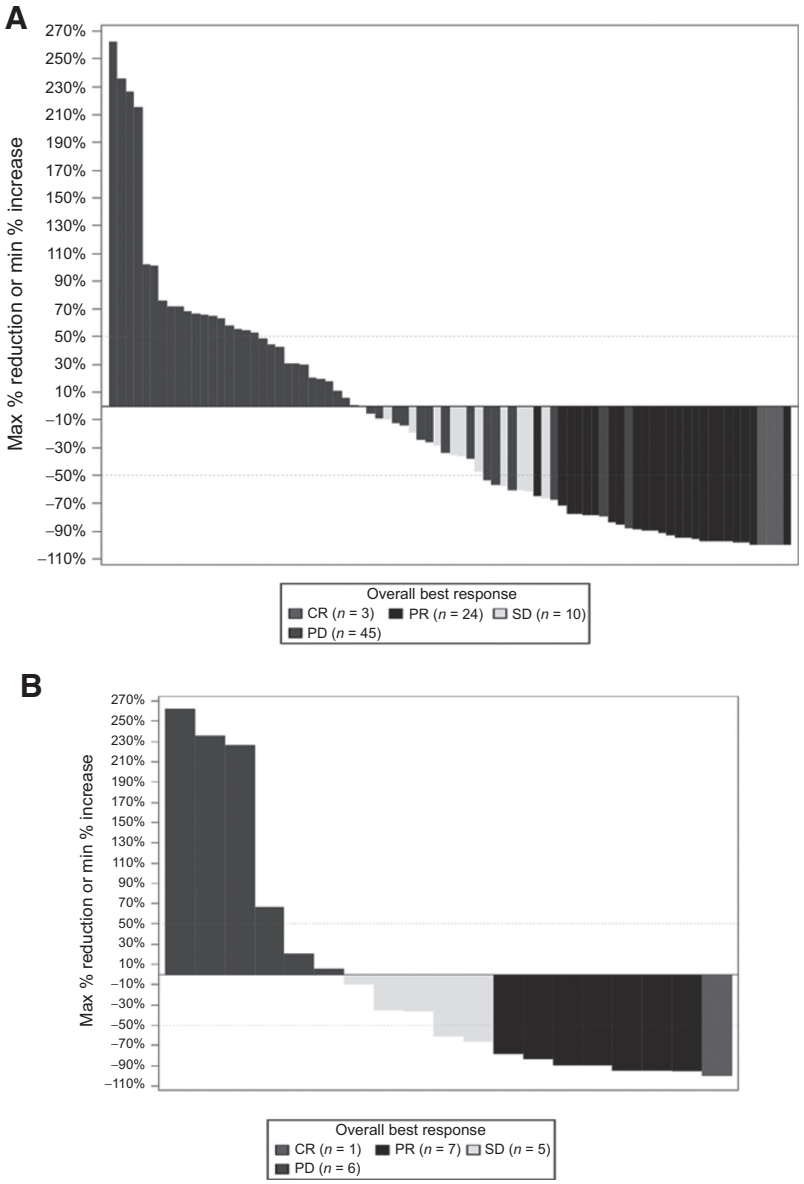

Figure 1.
Waterfall plots in 82 ipilimumab-refractory patients receiving nivolumab (3 mg/kg) with ($n = 14$) or without ($n = 82$) a peptide vaccine. Ten of the 92 patients in cohorts 4–6 and 2 of those in cohort 5 progressed before week 12, which precluded collection of their post-treatment data. A, patients in cohorts 4–6 who were refractory to ipilimumab and received nivolumab. The transition point is indicated by the arrow at 63%. B, cohort 5's 19 patients who were refractory to ipilimumab, and who experienced grade 3–4 irAEs after treatment with ipilimumab.

82 patients (with pre- and post-scans) was shrinkage of 36%. The swimmer's plot in Fig. 2A shows that with a median follow-up of 16 months, 37 of 92 (40%) patients had a CR, PR, or SD, of which 28 of 37 responses were ongoing as of the database lock on May 1, 2015, indicated by the arrow at the end of each line in ongoing patients. The median duration of response or stability (PR+CR+SD) in cohorts 4–6 was 14.3 months, while the median duration of response (CR+PR) was 14.6 months (95% CI, 2.8–31.9). The median duration of SD was 12.0 months. Forty-nine patients in the entire group of 126 treated ipilimumab-naïve or ipilimumab-refractory patients had SD, CR, or PR confirmed at week 24, and 15 of 49 (30.6%) either discontinued nivolumab due to toxicity or continued on treatment and reached their final treatment date at 120 weeks; of those 15 patients, none have progressed to date. Nine of those patients were in the ipilimumab-refractory cohorts 4–6. In the cohort of 21 patients in cohort 5 who had grade 3 or 4 toxicity to prior ipilimumab, there were 8 confirmed responders (1 CR and 7 PRs) and 5 with SD, all confirmed at 24 weeks for a disease control rate of 62%. Only 2 of the 13 patients with disease control in cohort 5 have progressed. In addition to the 13 patients, three additional progressors are also alive. For the 16 patients who are still alive, the minimum, median, and maximum follow-up times are 11 months, 20 months, and 38.9 months, respectively. The waterfall plot of those cohort 5 patients is shown in Fig. 1B, and the swimmer's plot is shown in Fig. 2B. Six patients with at least one previously untreated brain metastasis were treated on this trial in cohort 6, and there was 1 confirmed PR, 1 confirmed patient with SD, and 4 who progressed. For all 92 ipilimumab-refractory patients in cohorts 4–6, with a median follow-up of 16 months, the median PFS was 5.3 months, and the median OS was 20.6 months, both shown in the Kaplan–Meyer plot for PFS in Fig. 3A, and for OS in Fig. 3B,

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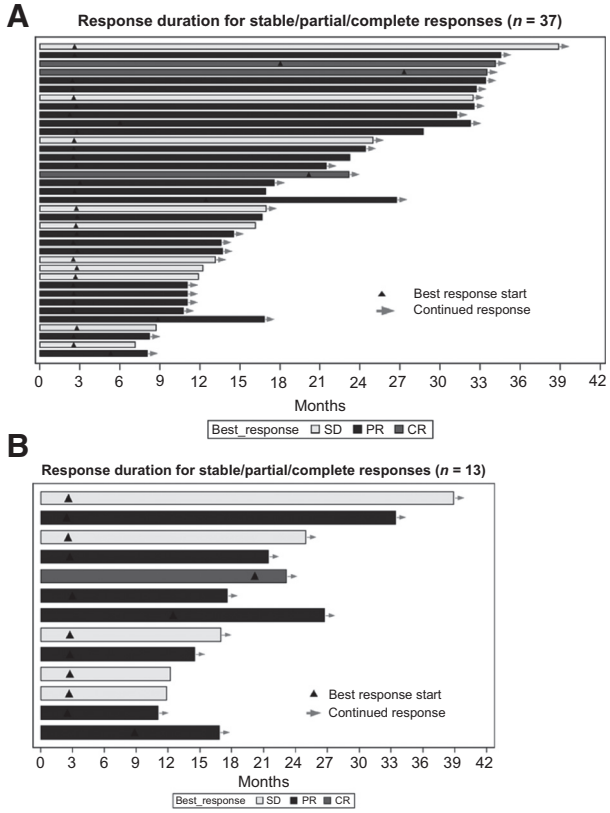

Figure 2.
Swimmer's plots for ipilimumab-refractory patients receiving nivolumab. Bar length indicates duration of stability or response. Triangles show time point when SD, PR, or CR was achieved. Arrowheads indicate patients whose SD or response was ongoing at the time of data analysis. A, patients ($n = 37$) in cohorts 4–6 who had SD, PR, or CR at week 24. B, patients ($n = 13$) in cohort 5 who had SD, PR, or CR; 11 of these responses were sustained at week 24.

respectively. One- and 2-year survival rates were 68.4% and 31.2%, respectively.

Immune biomarkers

MDSCs have been described as immature, myeloid-derived cells that have immunoregulatory properties (15). In the cancer-bearing host, MDSCs are diverted from normal differentiation pathways to become potent suppressors of innate and adaptive immunity. They are broadly grouped into granulocytic and monocytic categories. Monocytic MDSCs were measured in frozen peripheral blood mononuclear cells (PBMC) that were thawed and then rested briefly, and subjected to flow cytometry analysis for a lineage-negative $CD11b^{+}/CD14^{+}/HLA\ DR^{low}$ population. MDSCs were measured as a proportion of total live cells within the total PBMCs. The gating strategy for MDSCs is shown in Supplementary Fig. S1. Figure 4A shows the association between ipilimumab-refractory patients in cohorts 4–6 who received nivolumab and had a CR, PR, or SD, at week 24 (responder + stable, R+S), and the proportion of MDSCs in peripheral blood, compared with those who did not respond (nonresponders, NR). The results indicate a statistically significant association between response and SD (R+S) and fewer pretreatment MDSCs ($P = 0.003$). The association between survival and the proportion of MDSCs measured in the peripheral blood before treatment (Fig. 4B) shows that for 88 patients with available PBMCs in cohorts 4–6, the proportion of pretreatment M-MDSCs and OS was significantly inversely associated ($P = 0.0007$), with the proportion of MDSCs separated at the median value of 12.6%. There was also a significant association between the proportion of MDSCs in peripheral blood before treatment and median and PFS ($P = 0.002$, data not shown). T-cell function could be suppressed by M-MDSCs (Supplementary Fig. S2).

Discussion

These data provide toxicity and survival information with the longest follow-up in nivolumab-treated patients that have progressed after prior ipilimumab. The results of this trial make a number of points important for patients with metastatic

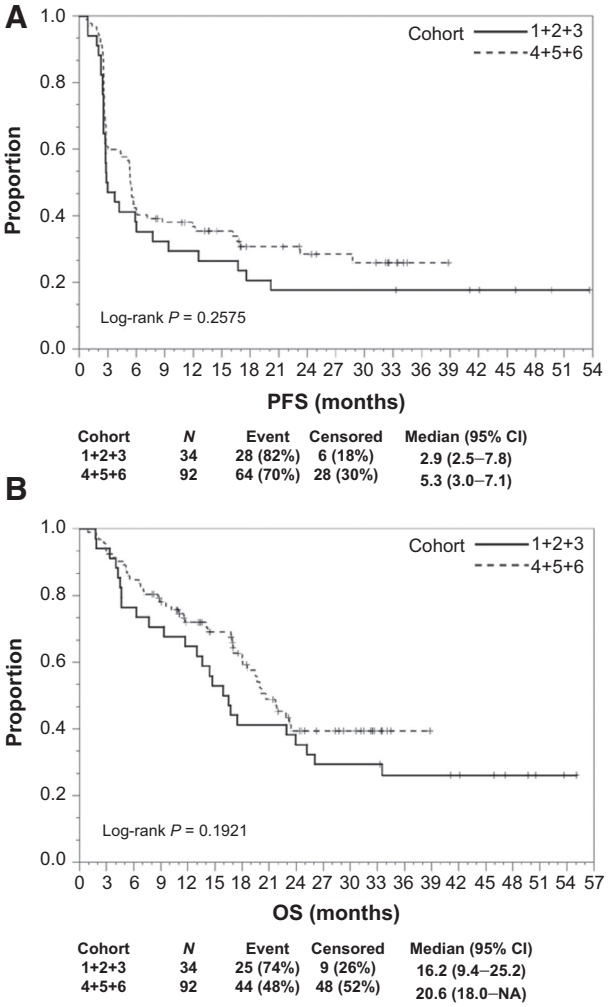

Figure 3.
Kaplan–Meyer plot comparison of cohorts 1–3 to cohorts 4–6 of the 92 ipilimumab-refractory patients receiving nivolumab. A, months of PFS; B, length of OS. *P* values were determined by the log-rank test. Data for each cohort are displayed beneath each plot.

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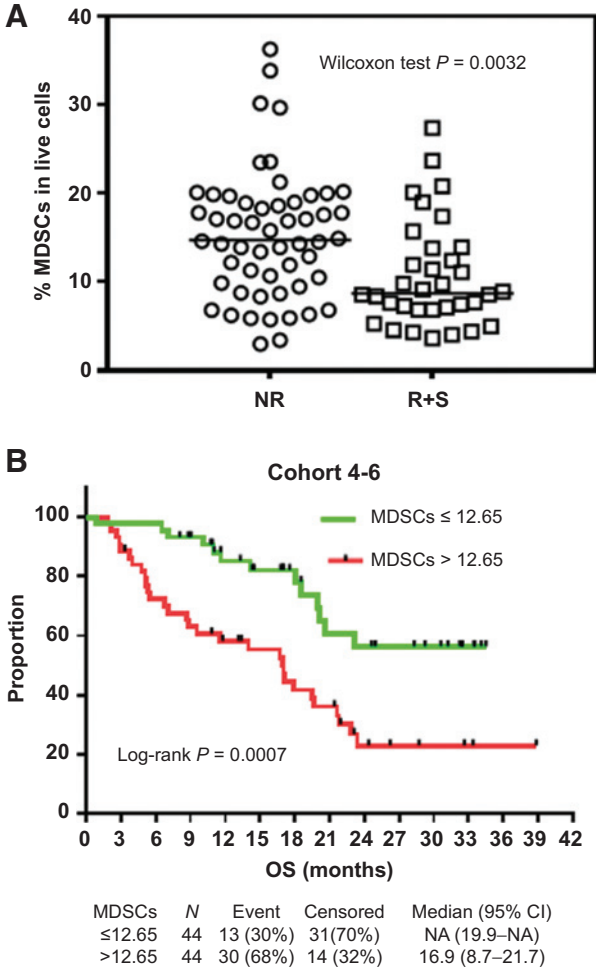

Figure 4.

MDSCs in ipilimumab-refractory and ipilimumab-naïve patients receiving nivolumab. A, proportion of $CD14^+/CD11b^+/HLA\ DR^{low}$ MDSCs present before nivolumab treatment, as a percentage of total live cells, in patients grouped as NR (nonresponders) and responders + stable patients (R+S). B, Kaplan–Meyer plot of the relation of OS to proportion of $CD14^+/CD11b^+/HLA\ DR^{low}$ MDSCs before treatment. Cutoff point was at the median (12.65 months). The red curve represents survival for patients below the median, and the green curve shows survival for those patients above the median. Data for groups are displayed beneath plot.

melanoma receiving immunotherapy. The PD-1 antibody nivolumab was well tolerated in ipilimumab-naïve or ipilimumab-refractory patients and was also well tolerated in those who had prior dose-limiting toxicities to ipilimumab and did not require secondary immune suppression with infliximab. The duration of response for patients with confirmed PR, CR, or SD in this trial was highly clinically meaningful, with a median duration of 14.6 months. The median PFS was 5.4 months in this group of 92 patients in cohorts 4–6, with median OS of 20.6 months. These data suggest that even after failing prior immunotherapy with another checkpoint protein inhibitor, responses of long duration may be seen with nivolumab, as has been observed in treatment-naïve melanoma patients and in patients with other histologies. Fifteen patients in the overall trial cohort of 126 ipilimumab-naïve or ipilimumab-refractory patients completed two-and-a-half years of therapy, or stopped treatment due to toxicity, and had SD, PR, or CR. None of those 15 patients has progressed to this date, including 9 in the ipilimumab-refractory group in cohorts 4–6. Minimum, median, and maximum follow-up time is 31.2, 33.5, and 53.7 months, respectively. All of the patients are still alive without progression.

Long-term toxicity data from this trial suggest that grade 2 toxicities like fatigue and arthralgias may linger in patients treated with nivolumab for over 2 years, and that some patients may develop persistent, cumulative dose-limiting toxicities that are not of grade 3 or higher but may be debilitating and result in discontinuing therapy. Three patients in the current study stopped therapy due to unacceptable grade 2 fatigue or arthralgias. Prospective studies of the quality of life in future trials of nivolumab given alone or in combination will shed more light on this issue.

The encouraging short- and long-term toxicity results from a cohort of 21 patients treated with nivolumab that had prior dose-limiting grade 3 and 4 irAEs, other than grade 4 colitis, with ipilimumab confirm that toxicities seen with one drug are not recapitulated with the other, and that the overall side-effect profile with nivolumab is not worsened in such patients. Although no patients who received infliximab were treated on this trial, we are currently treating patients who had been administered prior infliximab for severe colitis in an additional expansion cohort. The response in 8 of 21 patients (38%) in cohort 5 with 3 additional patients who had SD at week 24 provides intriguing preliminary data on the potential for increased benefit with nivolumab in those who have had irAEs with prior ipilimumab; we hope to further test this finding by treating additional patients in that cohort. These data are consistent with results of toxicity analyses in nivolumab-treated patients, which suggested that clinical benefit from nivolumab may be associated with incidence of irAEs (16).

The regimen used in the current trial utilized every-2-week nivolumab dosing for only 24 weeks, at which time the drug was then administered every 12 weeks for an additional 2 years and then discontinued. In contrast, many other trials of nivolumab or pembrolizumab used continuous dosing every 2 or 3 weeks until progression of disease, which are the recommended schedules in the package inserts for those two drugs (16–20). Nonetheless, the median survival in our cohort of 92 ipilimumab-refractory patients was equivalent to the data from a recent trial of second- or later-line nivolumab in similar populations treated up to 96 weeks or unacceptable toxicity (6). Our encouraging data also demonstrate that no patient has progressed after stopping treatment, and/or completing the two-and-a-half-year regimen with SD, PR, or CR. These findings raise the issue of how long to treat patients with PD-1–blocking antibodies once stability or response is achieved, and whether one may be able to shift to a maintenance regimen of drug every 12 weeks after an intensive induction regimen. These issues can only be resolved by the conduct of a randomized trial.

Limitations of this work include the fact that PD-1 inhibitors are increasingly being utilized as first-line treatment for metastatic melanoma due to a more favorable response rate and side-effect profile compared with ipilimumab, so the future of ipilimumab as first-line treatment may be limited.

The biomarker data from peripheral blood samples in this trial show that plentiful M-MDSCs ($CD14^+$, $CD11b^+$, and

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HLA DR^{low}) before treatment were associated with a lower likelihood of PR, CR, or SD, and poorer PFS and OS, particularly for the 92 patients who were ipilimumab refractory in cohorts 4–6. M-MDSCs have a variety of mechanisms by which they can alter T-cell responses in cancer, which may limit the clinical utility of PD-1 blockade (21). They can deplete nutrients, generate reactive oxygen species, interfere with lymphocyte trafficking and viability, and promote the function of regulatory T cells. The presence of MDSCs is associated with worse survival in melanoma (22–24). They may be prognostic in melanoma or a potential predictive marker for treatment with ipilimumab (25–28). Data from this work indicate that M-MDSCs are associated with a poorer outcome with nivolumab. The number of M-MDSCs before treatment is also inversely associated with objective response to nivolumab (Fig. 4A) and express S100A9, phosphorylated STAT3, and arginase (22, 29). Treatment with nivolumab does not affect MDSC suppressive function at week 12 (data not shown), but the ability to modulate MDSC function might be of use in patients treated with nivolumab.

Taken together, these data suggest that in previously treated, ipilimumab-refractory patients, nivolumab demonstrated an excellent safety profile, a high response rate with excellent duration of response, and median survival similar to that seen in previously treated ipilimumab-naïve patients. These data raise a number of questions, including whether checkpoint protein inhibitors that block PD-1 and CTLA-4 can be given sequentially to achieve a high rate of durable responses, or whether it is necessary to administer them concurrently, which has been shown in several trials to result in high response rates and excellent 1- and 2-year survival, albeit with high rates of toxicity and irAEs (30–33). The results of this trial also call into question whether continuous treatment with nivolumab, given every other week until progression, is necessary to achieve long-lasting clinical benefit and raise the issue of whether regimens with shorter or more intermittent exposure of drug are worthy of being tested in a prospective fashion.

Disclosure of Potential Conflicts of Interest

J.S. Weber and G. Gibney served as consultant/advisory board members for Bristol-Myers Squibb. R. Kudchadkar served as a consult/advisory board member for Bristol-Myers Squibb and Genentech.

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Phase I/II Study of Metastatic Melanoma Patients Treated with Nivolumab Who Had Progressed after Ipilimumab

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