Nivolumab in Resected and Unresectable Metastatic Melanoma: Characteristics of Immune-Related Adverse Events and Association with Outcomes

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Abstract

Purpose: Retrospective analysis of irAEs in melanoma patients treated with nivolumab.

Experimental Design: Data were pooled from 148 patients (33 resected, 115 unresectable) treated with nivolumab plus peptide vaccine or nivolumab alone every 2 weeks for 12 weeks. Patients with stable disease or regression received an additional 12-week cycle, then nivolumab alone every 12 weeks for up to 2 additional years. Frequency, grade, and characteristics of immune-related adverse events (irAE) were analyzed. A 12-week landmark survival analysis using a multivariate time-dependent Cox proportional hazard model assessed difference in overall survival (OS) in the presence or absence of irAEs.

Results: IrAEs of any grade were observed in 68.2% of patients (101 of 148). Grade III/IV irAEs were infrequent: 3 (2%) had grade III rash, 2 (1.35%) had asymptomatic grade III elevation in amylase/lipase, and 2 (1.35%) had grade III colitis. A statistically significant OS difference was noted among patients with any grade of irAE versus those without ($P \leq 0.001$), and OS benefit was noted in patients who reported three or more irAE events ($P \leq 0.001$). Subset analyses showed statistically significant OS differences with rash [$P = 0.001$; HR, 0.423; 95% confidence interval (CI), 0.243–0.735] and vitiligo ($P = 0.012$; HR, 0.184; 95% CI, 0.036–0.94). Rash and vitiligo also correlated with statistically significant OS differences in patients with metastatic disease ($P = 0.004$ and $P = 0.028$, respectively). No significant survival differences were seen with other irAEs (endocrinopathies, colitis, or pneumonitis).

Conclusions: Cutaneous irAEs are associated with improved survival in melanoma patients treated with nivolumab, and clinical benefit should be validated in larger prospective analyses. *Clin Cancer Res; 22(4); 886–94. ©2015 AACR.*

Introduction

In the last decade, immune checkpoint inhibition and adoptive cell therapy have led to major therapeutic advances in both hematologic and solid tumor oncology. Immune checkpoint targets of clinical importance are cytotoxic T-lymphocyte antigen-4 (CTLA-4), an inhibitory receptor expressed by activated effector T cells and regulatory T cells (Treg) that appears to be important for immune priming in the peripheral lymphoid tissue, and programmed death receptor 1 (PD-1), expressed by antigen-experienced, "exhausted" T cells, which plays an important role in the tumor microenvironment (1). PD-1 is bound by programmed death receptor ligand-1 (PD-L1) expressed by a variety of tumors and antigen-presenting cells in the tumor microenvironment, and can mediate a signal though SHP-2 phosphatase that blocks T-cell–mediated effector function (2). Recent studies of checkpoint protein inhibition in advanced melanoma have demonstrated improved overall survival (OS) and higher objective response rates (ORR) in randomized phase III trials compared with chemotherapy, both with anti–CTLA-4 antibody therapy (ipilimumab; refs. 3, 4) and anti–PD-1 antibodies [pembrolizumab (5, 6) and nivolumab (7, 8)]. Immune checkpoint inhibition results in a unique side effect profile, commonly described as immune-related adverse events (irAE), and includes rash, vitiligo, colitis, pneumonitis, hepatitis, thyroiditis, nephritis, and hypophysitis (9). These irAEs range in severity, many require prompt recognition and intervention, and may evolve to dose-limiting toxicities. In contrast with CTLA-4 inhibitors, PD-1–blocking antibodies seem to demonstrate less immune-related toxicity (10, 11) while demonstrating a high level of clinical benefit that appears to be superior to CTLA-4 blockade (6). Recent trials of concurrent anti–CTLA-4 and anti–PD-1 therapies showed an impressive ORR of over 50% with long duration of response, and superior progression-free survival (PFS) to ipilimumab alone, but grade III/IV irAEs occurred in over 50% of patients in the concurrent-regimen group (12, 13).

Clinical experience with the anti–PD-1 antibody nivolumab (formerly BMS-93658/MDX-1106) was first gained in patients with treatment-refractory metastatic solid tumors (melanoma,

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

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Translational Relevance

To date, immunotherapy for cancer has generated significant response rates and prolonged survival, particularly in melanoma, where sustained clinical responses can be seen even after cessation of therapy. Current treatments rely on inhibiting critical immune checkpoints, but also carry the risk of immune-related adverse events (irAE), which have been well described in the literature. There is growing interest in balancing the potential for benefit and risk with immunotherapy, thereby identifying patients who would gain the most from treatment. Because depth of immune activation may correlate with the likelihood of immune-related toxicity, an association between irAEs and disease outcomes might also exist. In this article, we describe our single institutional experience of treating 148 patients with resected and unresectable metastatic melanoma with the anti–PD-1 antibody nivolumab, and describe the irAE toxicity profile (including onset, resolution, and need for supportive therapy) and the potential association of those irAEs with survival.

renal cell carcinoma, colorectal cancer, and non–small cell lung cancer) (14). In that phase I trial, receptor occupancy by nivolumab was prolonged well beyond the measured serum half-life of 12 to 20 days, indicating high biologic durability (14). A follow-up trial of repeated biweekly nivolumab showed durable complete or partial tumor regressions in approximately one third of patients with advanced melanoma (14), with grade I or II irAEs including fatigue (56.3%), diarrhea, and pruritus (18.8% each). Of note is that fatigue, reported elsewhere as an immune-related side effect of checkpoint inhibition (15), occurs cumulatively with therapy even in the absence of endocrinopathy and can be improved with steroids. No grade III–IV irAEs occurred in the 28-day period following the first dose (14). Durable clinical responses were observed in a cohort of 107 metastatic melanoma patients given nivolumab every 2 weeks for up to 96 weeks, with a median OS of 16.8 months across doses from 0.1 to 10 mg/kg, and 62%, 44%, and 40% of patients were alive at 1, 2, and 3 years, respectively (16). Among 33 patients in that phase I trial with objective tumor regressions (31%), the Kaplan–Meier-estimated median response duration was 2 years (17), and irAEs of any grade were seen in 58 of 107 patients with melanoma (54%) including skin disorders (36%), gastrointestinal (GI) events (18%), and endocrinopathies (13%). In a recent study of 90 previously treated patients that received nivolumab with or without a vaccine and were either naïve to, or refractory to, ipilimumab, irAEs of any grade including rash was observed in 42%, diarrhea in 26%, endocrinopathy in 13%, and pneumonitis in 5.5%, with a 5.5% rate of all grade III–IV irAEs (18).

It is possible that irAEs may be associated with durable response and clinical benefit and that association has previously been studied with anti–CTLA-4 therapy (19), although contradictory reports exist (20). Investigation is currently under way to develop predictive indicators of successful anti–PD-1 therapy, including tumor PD-L1 expression and tumor immune infiltrate (21–23) and other potential biomarkers (24); however, there has been no published investigation of whether the irAE profile of anti–PD-1 therapy is associated with disease outcomes. Here, we describe the irAE toxicity profile seen in 148 patients with both resected and unresectable metastatic melanomas treated with nivolumab at our institution and investigate the potential association of those irAEs with OS.

Materials and Methods

Patient characteristics

Two phase I, open-label clinical trials utilizing nivolumab were conducted at our institution (Moffitt Cancer Center), with accrual beginning January 2010 and ending July 2014: (1) NCI P-7997, nivolumab in combination with a peptide vaccine in resected stage IIIC/IV melanoma (NCT01176474; ref. 25) and (2) NCI P-8316, nivolumab in combination with a peptide vaccine in unresectable stage III/IV melanoma (NCT01176461; ref. 26). The peptide vaccine included the gp100$_{280-288}$ (288V; NSC# 683473) and NY-ESO-1$_{157-165}$ (165V; NSC# 717388) peptides produced by Clinalfa, S.A., Zurich, Switzerland, and provided by the investigators, and the gp100$_{209-217}$ (210M; NSC# 683472) and Melan-A$_{26-35}$ (27L; NSC# 709401) peptides provided by the Cancer Therapy Evaluation Program of the NCI.

P-8316 trial for resected stage IIIC/IV melanoma

Thirty-three patients were enrolled in this trial, with 2 (6%) having stage IIIC disease and 31 (94%) having resected stage IV disease. Detailed patient characteristics are in Supplementary Table S1. The primary objective of this adjuvant study was to assess the safety and tolerability of nivolumab in combination with a peptide vaccine in HLA-A*0201–positive subjects, and overall safety and clinical results have been previously reported in this elsewhere (27).

P-7997 trial for unresectable stage III/IV melanoma

One hundred twenty-six patients were enrolled in this trial, of which 115 (92%) had week 24 response data at the time of this analysis; 109 (94.7%) had stage IV disease, 20 (17.3%) had previously treated central nervous system metastases, 8 (7%) had uveal melanoma, and 6 (5.2%) had unresectable stage IIIC disease. Detailed patient characteristics can be seen in Supplementary Table S1. The primary objectives of this study were to assess the safety and tolerability of nivolumab in combination with or without a peptide vaccine in HLA-A*0201–positive subjects, and overall safety and clinical results have been previously reported elsewhere (28).

Laboratory and safety assessments

Blood samples for measurement of immune safety parameters [thyroid-stimulating hormone (TSH), free T4 level, adrenocorticotropic hormone (ACTH)] were drawn at screening, week 7, week 12, week 19, week 24, and every 12 weeks thereafter for up to 24 months of follow-up. Additional laboratory investigation, prompted by patient symptoms, was at the investigators' discretion. Patients underwent physical examination and assessment for immune-related toxicities every 2 weeks for up to 24 weeks while on treatment, and every 12 weeks thereafter for up to 24 months of follow-up.

Response evaluation in P-7997 trial for unresectable stage III/IV melanoma

Tumor response was evaluated using modified World Health Organization (mWHO) and immune-related response criteria (irRC). At week 24, subjects with an overall response of immune-related complete response (irCR), immune-related

partial response (irPR), immune-related stable disease (irSD), or unconfirmed immune-related progressive disease (irPD) were eligible to receive nivolumab treatment every 12 weeks for up to 2 additional years. Subjects who relapsed at the disease evaluations at weeks 12, 24, or later did not receive further treatment.

Endpoints

PFS in P-7997 was defined as time from start of treatment to documented disease progression or death due to any cause, and OS in both P-7997 and P-8316 was defined as time from start of treatment to death due to any cause. Adverse events (AE) were recorded continually throughout treatment up to 30 days after treatment (90 days for serious AEs). All AEs were graded according to the NCI-CTCAE, version 4.0. Investigators specified whether an AE was considered to be treatment related and/or immune related. All analyses were performed using a data cutoff date of April 1, 2015, with only 3 patients lost in the OS analysis with a 12-week landmark ($n = 112$).

Statistical analysis

Kaplan–Meier curves were plotted for time to event, and the log-rank test was used to test difference of survival distributions for clinical variables, including age, gender, and substage. Univariate time-dependent Cox proportional regression models assessed hazard risk ratio for each irAE (yes/no) as a time-varying variable and each clinical variable. Multivariate time-dependent Cox proportional hazard models with specific irAEs then assessed differences in survival, hazard risk of death, and disease progression with respect to the presence or absence of irAEs. All multivariate analyses were adjusted for age and gender. Statistical analyses were performed using R 3.1.0 and SAS 9.4. In addition, a 12-week landmark survival analysis was performed, including only patients alive at follow-up starting at 12 weeks after the first treatment ($n = 143$). The OS analysis was based on this landmark assessment of irAEs that developed within the first 12 weeks (i.e., a patient alive and had rash by week 12 was compared with a patient without rash by week 12). Survival curves between these two groups were compared, using data starting at 12 weeks after treatment. All analyses were performed using a data cutoff date of April 1, 2015, with only 3 patients lost in the 12-week landmark OS analysis of patients with unresectable IIIC/IV disease ($n = 112$) and 2 patients lost in the resected population ($n = 31$). The median follow-up of patients in the resected melanoma cohort was 138 weeks, and median follow-up of the unresectable cohort was 140 weeks.

Results: Characteristics of irAEs

General irAE profile

In a pooled analysis of 148 patients (33 resected, 115 unresectable) treated with nivolumab once every 2 weeks for 12 weeks, irAEs of any grade were observed in 68.2% of patients (101 of 148). "irAEs" were defined as AEs with a potential immunologic basis that required more frequent monitoring and potential intervention with immune suppression or endocrine therapy. These included cutaneous, gastrointestinal, endocrine, and pulmonary irAEs (no neurologic or renal toxicities were seen). Grade III/IV irAEs were infrequently observed, with 3 patients (2%) experiencing grade III rash, 2 patients (1.35%) having asymptomatic grade III elevation in amylase/lipase, and 2 patients (1.35%) with grade III colitis. No significant difference was seen in the frequency of irAE in peptide-vaccinated versus nonvacci-

nated patients, and there was no observed increased frequency of irAEs as the doses of nivolumab were escalated from 1 mg/kg to 3 mg/kg and 10 mg/kg (data not shown). The grade and frequency distribution of irAEs in both patient cohorts (resected and unresectable) can be seen in Table 1. Within the unresectable patient population, patients in cohort 5 with prior grade III/IV ipilimumab toxicity had a slightly higher incidence of grade I mucositis than those without prior ipilimumab toxicity (9% vs. 3%), but otherwise did not demonstrate differences in toxicity profiles. Elevated amylase and lipase generally occurred early, after one dose, followed by diarrhea and colitis after 2 or 3 doses of nivolumab. Cutaneous irAEs were seen next, after 3 or 4 doses, with hyper- and hypothyroidism as well as pneumonitis subsequently at dose 5 or later, and hypophysitis coming after 10 doses. These data are shown in Table 2. At the time of analysis, all potentially immune-mediated AEs had completely resolved except vitiligo and endocrine irAEs, as the latter had resolution of symptoms with replacement hormone therapy.

Cutaneous irAEs

Forty-six (40%) patients with unresectable and 18 (54.6%) patients with resected melanoma experienced a diffuse, predominantly maculopapular rash. The median time to onset was 5.6 weeks. One case of grade III rash was seen, with the onset of diffuse rash on the neck, upper chest, back, arms, and legs within 1 week of treatment. The rash improved within a week of initiating hydroxyzine and a brief oral methylprednisolone taper. Vitiligo was seen in 8 (24.2%) resected patients and 11 (9.6%) unresectable metastatic patients, with median time to onset of 5.4 weeks following initiation of study drug. Of the 19 patients in whom vitiligo was seen, only 3 had been previously treated with IL2, and none of these three had vitiligo prior to study enrollment. In nearly all cases, vitiligo continued beyond completion of therapy, varied in distribution, and became coalescent over time.

Gastrointestinal irAEs

Mucositis was observed in 3 (2.6%) unresectable patients and 6 (18.2%) resected patients, and in all cases was grade I/II.

Table 1. irAEs by cohort and grade

irAE	Cohort	Grade I–II, n (% of subgroup)	Grade III, n (% of subgroup)
Rash	Unresectable	46 (40)	2 (1.7)
	Resected	18 (54.6)	1 (3)
Diarrhea/colitis/enteritis	Unresectable	34 (22.9)	—
	Resected	14 (42.4)	2 colitis (6.1)
Vitiligo	Unresectable	11 (9.6)	—
	Resected	8 (24.2)	—
Hypothyroidism	Unresectable	10 (8.7)	—
	Resected	6 (21.2)	—
Elevated amylase/lipase	Unresectable	—	—
	Resected	5 (15.1)	2 (6.1)
Mucositis	Unresectable	3 (2.6)	—
	Resected	6 (18.2)	—
Pneumonitis	Unresectable	3 (2.6)	—
	Resected	—	—
Hyperthyroidism	Unresectable	2 (1.7)	—
	Resected	—	—
Hypophysitis	Unresectable	—	—
	Resected	1 (3)	—
Elevated ALT/AST	Unresectable	—	—
	Resected	1 (3)	—

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Table 2. Timing of onset and resolution for irAEs

irAE	n (%)	Median weeks to onset	Median weeks to resolution	Systemic steroid therapy	Median weeks of steroid therapy
Elevated amylase/lipase	7 (4.7)	2	16	—	—
Elevated ALT/AST	1 (0.7)	2	6	—	—
Diarrhea/enteritis	48 (32.4)	4.2	1.3	Yes	5
Colitis	2 (1.4)	5.3	4	Yes	5
Rash	64 (43.2)	5.6	6.4	—	—
Vitiligo	19 (12.8)	5.4	—	—	—
Hyperthyroidism[a]	2 (1.4)	9.1	11.3	—	—
Hypophysitis	1 (0.7)	20.3	13.8	—	—
Mucositis	9 (6.1)	9.7	4	—	—
Hypothyroidism[a]	16 (10.8)	10.7	17.6	—	—
Pneumonitis	3 (2.6)	10.9	14.9	Yes	4

[a]Resolution of endocrine irAEs defined as symptom resolution.

Diarrhea was observed in 34 (22.9%) unresectable patients and 14 (42.4%) resected patients. Severe diarrhea (grade III/IV, with more than six diarrheal bowel movements above baseline in 24 hours) was seen in only 2 (6.1%) resected patients. In one case of grade III diarrhea, the patient experienced multiple loose stools and 20-pound weight loss within 6 weeks of study enrollment. The patient was treated with a prednisone taper (starting dose: 80 mg) and budesonide 9 mg daily; however, the patient eventually required hospitalization for i.v. fluids and methylprednisolone. There was one case of grade II enteritis in a patient who had previously developed grade I diarrhea at week 72; treatment at week 84 resulted in brief hospitalization, with CT of the abdomen showing thickened loops of small bowel but no evidence of obstruction. This patient was treated with i.v. methylprednisolone for 3 days followed by a 45-day oral steroid taper (starting dose: 100 mg prednisone daily), but at follow-up 12 weeks had ongoing grade II diarrhea requiring budesonide and diphenoxylate/atropine. Study drug was subsequently discontinued due to toxicity concerns. Of note, infliximab was not required for treatment of diarrhea or colitis in our patient population.

In the resected population, one case of immune-related grade II hepatotoxicity was seen, with an asymptomatic increase of transaminases and bilirubin. No grade III–IV elevation of transaminases was seen. Asymptomatic grade I–II elevation in amylase and lipase was seen in 7 patients, with 2 patients (6.1%) having grade III elevation but no dose-limiting toxicity. All cases were associated with a higher dose of nivolumab (10 mg/kg), began within 2 weeks of starting study drug, and resolved 6 to 16 weeks thereafter with observation alone.

Endocrine irAEs

Immune-related hypothyroidism occurred in 16 (10.8%) patients, with a median time to onset of 10.7 weeks from the start of study drug. Two patients experienced grade II hyperthyroidism; in the case of 1 patient, the TSH was markedly diminished (0.008 uIU/mL) within 9 weeks of starting treatment, which later evolved to hypothyroidism within 6 weeks (TSH 26.470 uIU/mL). The patient was treated with levothyroxine, with normalization of TSH levels within 6 weeks. One case of grade II hypophysitis was seen in the resected trial (P-8316), when the patient presented 4 months after initiation of treatment with generalized fatigue and headaches. Laboratory workup revealed an undetectable ACTH (<5 pg/mL) and cortisol (<0.1 µg/mL). The patient was started on hydrocortisone twice daily, with improvement in fatigue and headache symptoms within 13 weeks of steroid replacement.

Pulmonary irAEs

Grade II pneumonitis was seen in 3 (2.6%) patients in the group of patients with unresectable melanoma, with a median time to onset of 11 weeks after initiation on trial. One trial participant developed severe shortness of breath, had a chest x-ray suggestive of pneumonia, and did not improve after being placed on antibiotics. At week 12 follow-up, restaging CT of the chest showed bilateral pulmonary infiltrates, and although the patient complained of fatigue, fevers, and dyspnea, no hypoxia was noted at rest or with exertion. After a diagnosis of grade II pneumonitis attributed to study drug was made, treatment was held, and the patient was started on a steroid taper (60 mg of prednisone daily, tapering down 10 mg every 5 days for 30 days) with eventual recovery to baseline after 6 months. The patient received no further nivolumab.

Results: Association of irAEs with Survival

OS and PFS were evaluated in 112 patients with unresectable stage IIIC/IV disease. OS was also evaluated in the combined patient cohorts ($n = 143$). No significant differences in OS or PFS were seen in vaccinated versus nonvaccinated patients ($P = 0.987$ and 0.359, respectively), those with or without lactate dehydrogenase (LDH) elevation ($P = 0.334$ and 0.742, respectively) or with regard to dose of nivolumab ($P = 0.73$ and 0.933, respectively). A statistically significant OS difference was noted in all patients (143) in the combined cohorts who experienced any irAE versus those who did not ($P \leq 0.001$), with greater OS benefit noted in patients who reported 3 or more irAE events ($P \leq 0.001$) compared with those with none or only 1 irAE event, as seen in Fig. 1.

At a 12-week landmark survival analysis of individual irAEs, a statistically significant improvement in OS was associated with rash of any grade in 112 patients with metastatic unresectable disease [$P = 0.004$; HR, 0.45; 95% confidence interval (CI), 0.251–0.766]. Vitiligo was also associated with statistically significant OS benefit in those patients ($P = 0.028$; HR, 0.22; 95% CI, 0.025–0.806). Survival curves for these patients can be seen in Fig. 2. Rash and vitiligo were also associated with statistically significant OS differences in the pooled population of patients with both resected and unresectable metastatic disease ($P = 0.001$ and $P = 0.012$, respectively); survival curves for the combined patient population (143 patients) can be seen in Fig. 3. No statistically significant OS differences were seen in patients with any grade of hypothyroidism ($P = 0.117$), hyperthyroidism ($P = 0.489$), diarrhea ($P = 0.132$), or pneumonitis ($P = 0.493$). Hypophysitis was excluded from analysis due to small sample size.
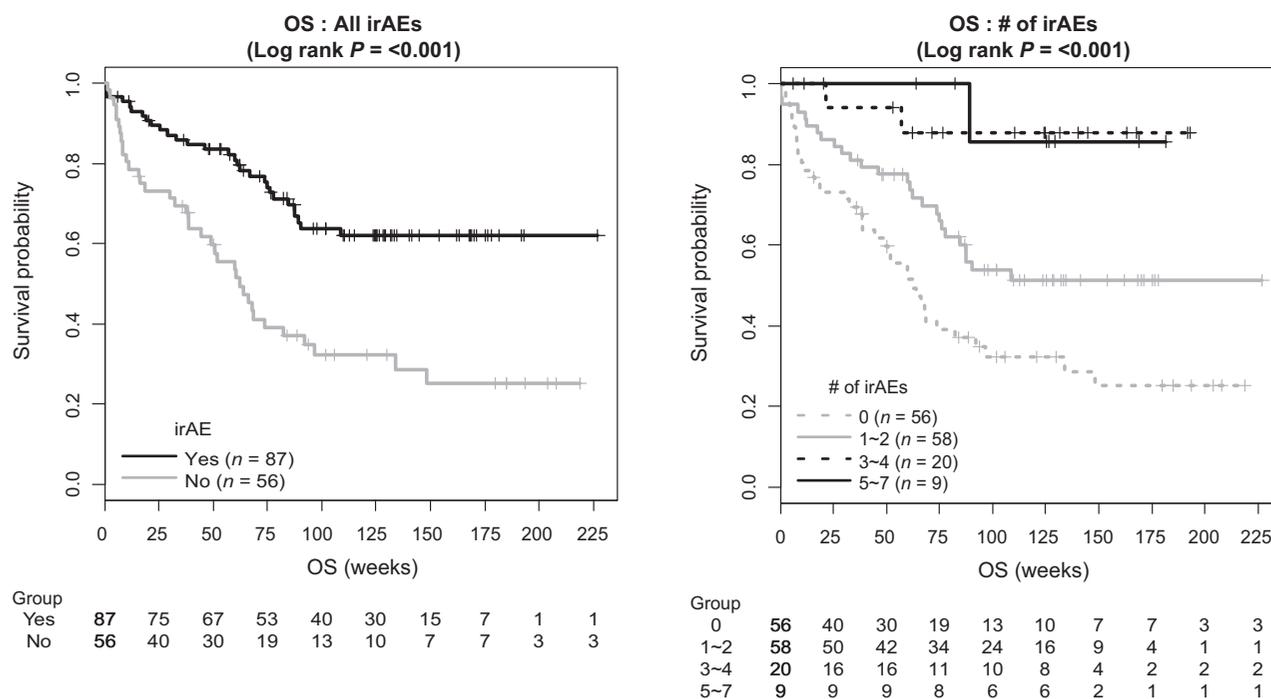
OS : All irAEs
(Log rank *P* = <0.001)

Group										
Yes	87	75	67	53	40	30	15	7	1	1
No	56	40	30	19	13	10	7	7	3	3

OS : # of irAEs
(Log rank *P* = <0.001)

Group										
0	56	40	30	19	13	10	7	7	3	3
1~2	58	50	42	34	24	16	9	4	1	1
3~4	20	16	16	11	10	8	4	2	2	2
5~7	9	9	9	8	6	6	2	1	1	1

Figure 1.
OS among patients who experienced an irAE. A statistically significant OS difference was noted among those experiencing any irAE versus those who did not ($P \leq 0.001$), with greater OS benefit in patients reporting 3 or more events ($P \leq 0.001$).

When those patients who received systemic steroids (9 total) were assessed for OS compared with those who did not (134), there was a statistically significant difference in favor of the steroid group with $P = 0.026$. If OS was assessed only for patients who did not receive steroids, there was still a significant benefit for those with any grade of irAE, with $P = 0.001$,
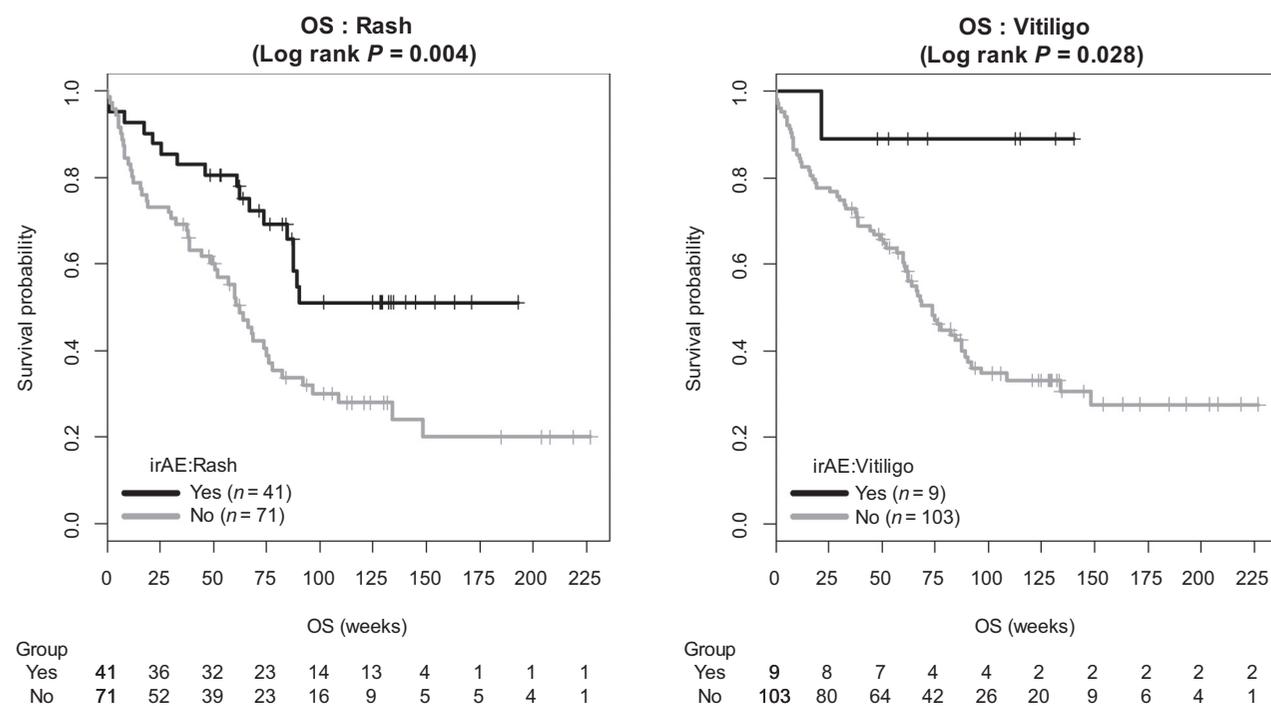
OS : Rash
(Log rank *P* = 0.004)

Group										
Yes	41	36	32	23	14	13	4	1	1	1
No	71	52	39	23	16	9	5	5	4	1

OS : Vitiligo
(Log rank *P* = 0.028)

Group										
Yes	9	8	7	4	4	2	2	2	2	2
No	103	80	64	42	26	20	9	6	4	1

Figure 2.
Twelve-week landmark survival analysis of individual irAEs in the metastatic melanoma population ($n = 112$). A statistically significant OS improvement was associated with rash ($P = 0.004$) and vitiligo ($P = 0.028$).
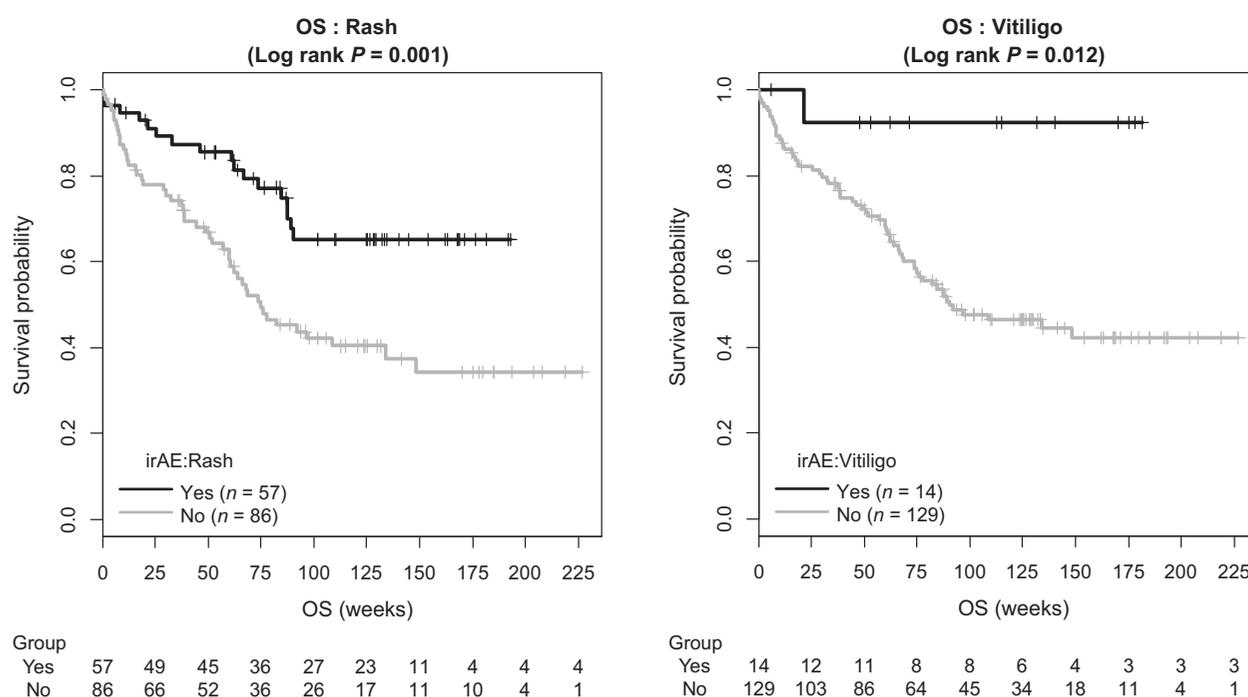
Figure 3.
Twelve-week landmark survival analysis of individual irAEs in the combined patient population ($n = 143$). Rash and vitiligo were also associated with statistically significant OS differences ($P = 0.001$ and $P = 0.012$, respectively).

suggesting that the group of patients that received steroids did not account alone for the benefit in OS.

Analysis of irAE related to PFS was studied in the metastatic unresectable patient population. Although 8- and 10-week landmark survival analyses indicated that both rash and vitiligo were associated with significantly improved PFS (not shown), at the 12-week landmark analysis, a large number of patients were excluded ($n = 40$) due to disease progression prior to the week 12 scans, rendering the analysis difficult to interpret. When the relationship of ORR (PR + CR vs. SD + PD) to development of vitiligo and rash was assessed, there was a statistically significant relationship between ORR and rash ($P = 0.03$) or vitiligo ($P = 0.009$) seen in Supplementary Table S2.

The effects of irAE development on survival are summarized as an HR using time-dependent Cox regression analyses in Table 3. When irAEs were examined individually, statistically significant improvement in OS was associated with any grade of rash ($P = 0.002$; HR, 0.423; 95% CI, 0.243–0.735) and vitiligo ($P = 0.042$; HR, 0.184; 95% CI, 0.036–0.94) in both univariate and multivariate analyses.

Discussion

PD-1 blockade to enhance antitumor immunity has led to durable clinical responses in patients with advanced melanoma, other solid tumors (29, 30), and Hodgkin lymphoma (31) and appears to have a favorable immune toxicity profile. Survival outcomes and durable clinical activity even long after cessation of therapy have been seen with nivolumab in several prior studies (32). Given the growing interest in immune checkpoint inhibition and establishing predictors of clinical outcome, we profiled irAEs associated with nivolumab and investigated potential associations with clinical benefit.

The overall irAE profile in all treated unresectable and resected patients was favorable, consistent with prior studies of nivolumab. Grade III/IV irAEs were infrequently observed (4.7% of all patients) and responded well to conventional steroid and/or supportive therapy. No significant difference was seen in the frequency of irAEs in patients with normal or elevated LDH, and there was no increased frequency of irAEs at higher doses of nivolumab escalating from 1 mg/kg to 3 mg/kg and 10 mg/kg. It is also important to note the excellent toxicity

Table 3. Effect of irAE development on survival, using time-dependent Cox regression analyses

	Univariate				Multivariate			
	HR	LB	UB	P	HR	LB	UB	P
Diarrhea/colitis	0.616	0.343	1.108	0.106	0.632	0.348	1.149	0.132
Hyperthyroidism	2.439	0.682	8.729	0.17	1.604	0.42	6.118	0.489
Hypothyroidism	0.37	0.104	1.325	0.127	0.36	0.1	1.291	0.117
Mucositis	0.09	0.005	1.49	0.093	0.087	0.005	1.448	0.089
Myalgias	0.313	0.019	5.192	0.418	0.377	0.022	6.477	0.502
Pneumonitis	0.346	0.021	5.729	0.459	0.371	0.022	6.313	0.493
Rash	**0.427**	0.246	0.74	**0.002**	**0.423**	0.243	0.735	**0.002**
Vitiligo	**0.178**	0.035	0.912	**0.038**	**0.184**	0.036	0.94	**0.042**

profile in patients previously treated with ipilimumab, even with dose-limiting toxicity to that drug, indicating that sequential CTLA-4/PD-1 blockade may be well tolerated (33). This may represent an important therapeutic option for patients who either did not respond to, or could not tolerate, anti–CTLA-4 therapy, or who might not tolerate concurrent nivolumab and ipilimumab. In the Checkmate-069 trial, combination therapy yielded a higher ORR versus ipilimumab alone (61% vs. 11%, P < 0.001), but this carried a higher rate of grade 3–4 irAEs (54% vs. 24%, respectively; ref. 13). These data were confirmed in the Checkmate-067 trial, demonstrating an ORR of 43.7% with nivolumab, 57.6% in the nivolumab plus ipilimumab group, and 19% with ipilimumab alone. The rate of treatment-related grade 3–4 AEs was higher with combination therapy (55.0%) versus single-agent nivolumab (16.3%) or ipilimumab (27.3%; ref. 34).

Two of the most common irAEs seen in patients with resected and unresectable metastatic melanoma were rash and vitiligo. Rash has been previously reported in clinical trials utilizing PD-1 blockade, but the mechanism is not well understood. One explanation may be gained from a recent study of Tregs in mice with graft-versus-host-disease (GVHD), which demonstrated that Tregs mediate immune suppression *in vivo* through modulation of the PD-1 pathway (35). PD-1 blockade has been associated with typical cutaneous changes similar to those associated with GVHD, including rash and alopecia, and may be related to CD8+ T-cell expansion, as seen in the murine model of acute and chronic GVHD (36). Although the statistically significant association between rash and survival outcomes in our patient population is impressive, it would need to be validated in larger patient samples.

Vitiligo also appeared to confer a survival benefit in our patients. This dermatologic phenomenon is associated with pigment loss due to reduction in epidermal melanocytes accompanied by T-cell infiltration of the skin (37), with both cellular and humoral immune responses felt to play a role in patients with melanoma (38). In murine models, lymphatic endothelial cells (LEC) have been shown to directly engage cytotoxic T cells by presenting an epitope derived from tyrosinase, a melanocyte differentiation protein (39). Tyrosinase-related protein-2 (TRP-2), highly expressed in cutaneous melanoma and melanocytes, has been shown to induce antibody responses, and high levels of anti–TRP-2 IgG titers have been found in patients with vitiligo. In one study of a polyvalent melanoma cell vaccine in patients with melanoma, those with high TRP-2 antibody titers after treatment had improved survival compared with nonresponders (40). In the murine model, vitiligo-affected hosts maintain gp100- and TRP2-specific memory CD8+ T cells at 10× higher frequencies compared with unaffected hosts (41). Immunotherapies for melanoma have been shown to induce vitiligo, which has previously been associated with a favorable outcome with high-dose adjuvant interferon (42) and high-dose IL2 for unresectable melanoma (43). Gogas and colleagues (44) reported increased OS in stage IIB, IIC, and III resected melanoma patients receiving adjuvant interferon who developed multiple manifestations of autoimmunity, including vitiligo; although the interpretation of that analysis was complicated by a time delay bias (45), a subsequent 1-year landmark analysis of resected stage III patients showed a trend toward survival benefit associated with induction of autoimmunity (46).

Vitiligo has also been previously reported with both anti–CTLA-4 and anti–PD-1 therapies (47); as the PD-L1:PD-1 pathway likely mediates peripheral tolerance of melanosomal proteins (including tyrosinase and TRP-2), interference with PD-1 signaling may induce autoimmune vitiligo (48). This provides a plausible explanation for the onset and persistence of depigmentation in patients treated with immunotherapy. Given the statistically significant improvement in OS associated with vitiligo in our patient population, enhancing immune recognition of melanocyte-associated proteins in patients with completely resected or advanced unresectable melanoma may be a surrogate for clinical benefit. Further evidence can be found in a recently published meta-analysis of immunotherapy in melanoma, where vitiligo was significantly associated with both PFS and OS, and patients with vitiligo had 2- to 4-times lower risk of disease progression and death, respectively, compared with patients without vitiligo (49). Further work will examine the relationship between nivolumab-treated patients developing vitiligo, the impact on numbers and function of Tregs, and development of T cells that recognize TRP-1 and TRP-2.

In this work, we performed a 12-week landmark analysis to minimize the effects of a possible time delay bias, and included 104 of 115 patients in the analysis for survival. Although the likelihood of detecting an autoimmune manifestation like vitiligo may increase with duration of immune therapy, the use of a time-dependent Cox proportional hazard model and the landmark analysis would minimize the potential source of bias.

In conclusion, our data support an association between clinical benefit (measured by OS) and the induction of cutaneous irAEs, emphasizing a close relationship between self-tolerance and tolerance to melanoma antigens. Further research will be needed to clarify the mechanisms underlying the induction of nivolumab-mediated cutaneous irAEs and tumor regression. Overall, these results suggest that cutaneous irAEs may be associated with durable clinical benefit with nivolumab. These data should be validated prospectively in subsequent analyses of larger patient cohorts with unresectable and resected melanoma.

Disclosure of Potential Conflicts of Interest

G. Gibney is a consultant/advisory board member for Bristol-Myers Squibb. J. Weber is a consultant/advisory board member for Astra Zeneca, Bristol-Myers Squibb, Genentech, and Merck. No potential conflicts of interest were disclosed by the other authors.

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Conception and design: J.S. Weber
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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M. Freeman-Keller, Y. Kim, J.S. Weber
Writing, review, and/or revision of the manuscript: M. Freeman-Keller, Y. Kim, J.S. Weber
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): H. Cronin, A. Richards, J.S. Weber
Study supervision: H. Cronin, J.S. Weber

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