Priority Brief

Cancer Immunology Research

Pretreatment Serum VEGF Is Associated with Clinical Response and Overall Survival in Advanced Melanoma Patients Treated with Ipilimumab

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Abstract

Ipilimumab, an antibody that blocks CTL antigen 4 (CTLA-4), improves overall survival (OS) for patients with metastatic melanoma. Given its role in angiogenesis and immune evasion, serum VEGF levels were evaluated for association with clinical benefit in ipilimumab-treated patients. Sera were collected from 176 patients treated at 3 ($n = 98$) or 10 mg/kg ($n = 68$). The VEGF levels before treatment and at induction completion (week 12) were analyzed using the Meso Scale Discovery kit. The association of the levels of VEGF with clinical responses and OS were assessed using the Fisher exact and Kaplan–Meier log-rank tests. VEGF as a continuous variable was associated with OS ($P = 0.002$). Using 43 pg/mL as the cutoff pretreatment VEGF value defined by maximally selected log-rank statistics, pretreatment VEGF values correlated with clinical benefit at week 24 ($P = 0.019$; 159 patients evaluable). Pretreatment VEGF $\geq$ 43 pg/mL was associated with decreased OS (median OS 6.6 vs. 12.9 months, $P = 0.006$; 7.4 vs. 14.3 months, $P = 0.037$ for 3 mg/kg; and 6.2 vs. 10.9 months, $P = 0.048$ for 10 mg/kg). There was no correlation between VEGF changes and clinical outcome. Serum VEGF may be a predictive biomarker for ipilimumab treatment and is worthy of prospective investigation with various forms of immunologic checkpoint blockade. *Cancer Immunol Res; 2(2); 127–32.* ©*2014 AACR.*

Introduction

CTL antigen 4 (CTLA-4) is a coinhibitory molecule expressed on activated T cells and regulatory T cells. CTLA-4 is of primary importance in maintaining immune homeostasis by downregulating T-cell signaling to inhibit the CD28-B7 costimulatory pathway (1, 2). Blockade of CTLA-4 is thought to prevent inhibition of T cells and potentiate immune responses to tumors. Therapeutic strategies that block CTLA-4 have demonstrated increased immunologic responses and enhanced antitumor immunity (3–5). Two phase III clinical trials have shown improvement in overall survival (OS) for advanced melanoma patients treated with ipilimumab in the first- or second-line setting, leading to the U.S. Food and Drug Administration approval of ipilimumab in 2011 (6, 7).

Despite the durable clinical efficacy, only a subset of patients benefit from ipilimumab treatment. Patients may also experience mechanism-based inflammatory toxicities, so-called immune-related adverse events (IRAE) or adverse events of special interest (AEOSI; ref. 8). Therefore, there remains a critical need for identifying predictive biomarkers for ipilimumab. Serum lactate dehydrogenase (LDH) level indicates necrosis in fast-growing tumors or high tumor cell turnover, and is an independent prognostic marker for patients with melanoma. Several biomarkers, including absolute lymphocyte count (ALC; refs. 9–11), sustained induction of inducible costimulator (ICOS) on $CD4^+$ T cells (12, 13), integrated NY-ESO-1 antigen-specific responses (14, 15), and myeloid-derived suppressor cells (MDSC; ref. 16) have been reported to correlate with clinical response. Biomarker development is important to our understanding of the mechanisms involved in effective outcomes and patient care.

The immunosuppressive tumor microenvironment may restrict the effectiveness of cancer therapy, with the abnormal tumor vasculature promoting such an effect (17). VEGF is a potent angiogenic factor that regulates angiogenesis while increasing the proliferation, migration, and metastasis of melanoma. In addition, VEGF is a potent inhibitor of dendritic cell maturation and T-cell responses (18, 19), contributing to

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the suppression of antitumor immune responses. Serum VEGF levels have been shown to correlate with melanoma stage and high circulating serum VEGF predicts poor prognosis in patients with melanoma (20–23). Prior investigations focused on predictive biomarkers for the response to high-dose interleukin-2 (IL-2) have identified serum VEGF level as an informative marker (24). The prognostic value of VEGF in melanoma patients treated with ipilimumab is unknown. We analyzed the VEGF levels in patients with melanoma before and after ipilimumab therapy to assess its potential role as a prognostic biomarker.

Materials and Methods

Patient eligibility and selection

Patients were treated and biospecimens were collected at Memorial Sloan-Kettering Cancer Center (MSKCC; New York, NY) and Dana-Farber/Harvard Cancer Center (Boston, MA) on Institutional Review Board (IRB)–approved protocols. All subjects in this study were treated for metastatic melanoma with ipilimumab (Bristol Myers-Squibb trials CA184-008, -025, -045, and -087). Patients were $\geq$16 years of age, had normal hematologic and organ function, and had an Eastern Cooperative Oncology Group status of 0 or 1. Exclusion criteria included any other prior invasive malignancy, autoimmune disease, active infection, or pregnancy or lactation in women. Patients received ipilimumab at 10 mg/kg ($n = 78$) or 3 mg/kg ($n = 98$) every 3 weeks for four treatments. Clinical responses were defined at week 24 by Response Evaluation Criteria in Solid Tumors (RECIST) as well as the recently proposed immune-related response criteria (irRC; ref. 25). Those patients originally receiving ipilimumab at 10 mg/kg without dose-limiting toxicity and with evidence of clinical benefit, defined as an objective response or stable disease at week 24, could continue receiving ipilimumab at 10 mg/kg every 12 weeks until progressive disease, death, toxicity, or withdrawal of consent.

Measurement of serum VEGF

Sera were collected from whole blood by centrifugation at 800 $\times$ *g* for 10 minutes. The sera were aliquoted and stored at -20°C until analysis. Circulating VEGF measurements were performed on an MSD SECTOR Imager 2400 instrument (Meso Scale Discovery, Inc.) with a VEGF singleplex array plate according to the manufacturer's instructions. Briefly, 25 μL of sera along with the appropriate standard were added to each well of the 96-well plates that had been pretreated with 25 μL of human serum cytokine assay diluent. The plate was then incubated at room temperature for 2 hours with vigorous shaking. The well contents were then discarded and the plate was washed three times with 150 μL PBS + 0.05% Tween-20 before 25 μL of $\times$1 Detection Antibody solution was added to each well. The plate was again incubated at room temperature with vigorous shaking for 2 hours. After rewashing with PBS–Tween, 150 μL of $\times$2 Read Buffer was added to each well and the plate was read on the instrument.

Statistical analyses

Patient characteristics are presented by the median and range for continuous variables and by frequency and percentages for categorical variables. The association between OS and VEGF as a continuous variable was analyzed. Continuous VEGF value was dichotomized for ease of clinical utility. A minimum *P* value approach was used to perform a cutoff point analysis. In the minimum *P* value approach, selected values of VEGF are examined as candidates for the cutoff point. The value is chosen that best separates patient outcomes according to a maximum relative risk and minimum *P* value as opposed to an arbitrary selection at the median value. The *P* value is adjusted to account for the problem of multiple testing. It was verified that the relationship between survival and the prognostic factor remained significant when the variable was dichotomized.

Comparison between the VEGF cutoff groups and clinical benefit was assessed using the Fisher exact test. The Kaplan–Meier log-rank test and Cox proportional hazards regression were used to evaluate the association between VEGF (high vs. low) and OS. OS is defined from time of first ipilimumab dose to date of death or last follow-up. Patients alive at last follow-up are censored. Univariate Cox proportional hazards regression was performed on baseline and week 12 VEGF, pretreatment ALC, and pretreatment LDH variables. Variables significant in the univariate setting were then assessed in a multivariate model. The association between VEGF at week 12 and OS was also assessed. Patients who died or were lost to follow-up before week 12 were excluded. $P < 0.05$ was considered statistically significant. Analysis was performed using R v. 3.0.0 (http://www.r-project.org/).

Results

Patient demographics

A total of 176 patients with advanced melanoma at MSKCC and Dana-Farber Cancer Institute were analyzed for serum VEGF concentration before and after treatment (78 patients with melanoma were treated with 10 mg/kg and 98 patients with 3 mg/kg dose of ipilimumab). Patient characteristics are described in Supplementary Table S1. The median age was 62 years (range, 16–91), 123 of 176 (70%) patients were male, and 172 of 176 (98.7%) patients had stage IV disease. The median number of prior systemic therapies was two. The majority [102 (58%)] had received prior cytotoxic chemotherapy, 10 patients (5.7%) had received prior interferon therapy in the adjuvant setting, and 24 patients (13.6%) had received high-dose IL-2 for metastatic disease (Supplementary Table S1).

Serum VEGF in melanoma patients treated with ipilimumab

We performed VEGF analyses on serum samples collected from patients with melanoma at baseline and 12 weeks after beginning treatment with 3 or 10 mg/kg of ipilimumab. The range of baseline VEGF was from 0.1 to 894.4 pg/mL. The baseline VEGF value (102.13 $\pm$ 132.30 pg/kg; mean $\pm$ SD) in patients treated with the 3-mg/kg dose ipilimumab was higher than in patients who received 10 mg/kg (59.78 $\pm$ 110.72 pg/kg; mean $\pm$ SD). Following ipilimumab induction, patients experienced both increases and decreases in serum VEGF levels at week 12 following four doses of ipilimumab (Fig. 1).

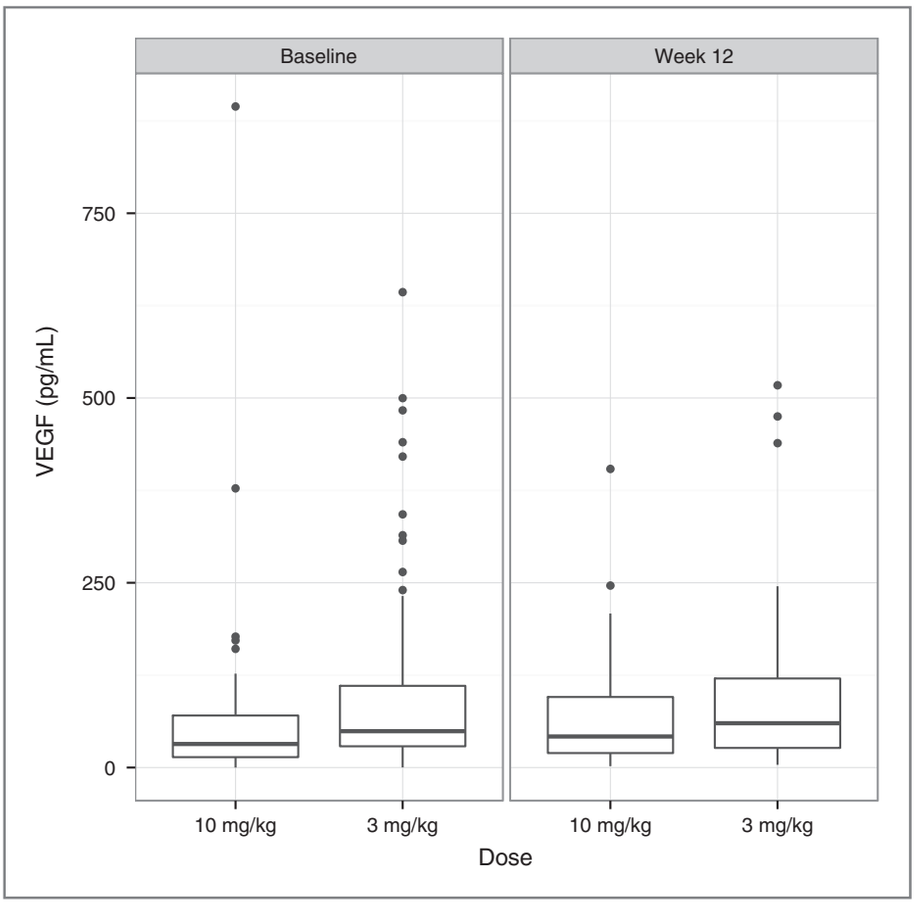

Figure 1. VEGF in serum samples collected from patients with melanoma treated with 3 or 10 mg/kg of ipilimumab at baseline and week 12 after treatment. The baseline VEGF values (mean ± SD) in patients treated with the 3- or 10-mg/kg dose of ipilimumab were 102.13 ± 132.30 and 59.78 ± 110.72 pg/mL, respectively. Changes were observed in some patients with melanoma relative to treatment, but these changes were not correlated with clinical outcomes.

Cutoff point of VEGF by using maximally selected log-rank statistic

We first analyzed the relationship between OS and VEGF as a continuous variable and found VEGF to be associated with OS ($P = 0.002$ for all 176 patients; $P = 0.019$ for patients treated with 3 mg/kg; $P = 0.046$ for patients treated with 10 mg/kg). To determine the clinical consequence of baseline serum concentrations of VEGF, we divided patients into two categories relative to the VEGF value, according to the cutoff determined by maximally selected log-rank statistics. The cutoff for baseline VEGF value was 39 pg/mL for the 98 patients with melanoma treated with the 3-mg/kg dose of ipilimumab and 43 pg/mL for the 78 patients treated with the 10-mg/kg dose of ipilimumab. When both groups were combined, the cutoff baseline value for all 176 patients was 43 pg/mL (Fig. 2). Therefore, $VEGF^{hi}$ patients were defined as baseline VEGF $\geq$ 43 pg/mL, whereas $VEGF^{low}$ patients had VEGF value < 43 pg/mL.

Correlation of baseline VEGF with clinical benefit

On the basis of the definition of $VEGF^{hi}$ and $VEGF^{low}$, we next asked whether there was an association between baseline VEGF and patient clinical response at week 24. We obtained the status of clinical response at week 24 from 159 of 176 patients with melanoma. Of note, 53 of 159 (33%) patients experienced clinical benefit at week 24, including 3 complete responders, 13 partial responders, and 37 patients with stable disease as per investigator assessments. For the 106 patients without clinical benefit, 54 had progressive disease and 52 died by week 24. Thirty-seven of 90 (41.1%) $VEGF^{low}$ patients experienced clinical benefit, whereas 16 of 69 (23.2%) $VEGF^{hi}$ patients had clinical benefit. There was a significant correlation between the baseline VEGF levels and the likelihood of clinical benefit ($P = 0.019$; Table 1). In addition to irRCs, there was an association between baseline VEGF and RECIST responses ($n = 155$; $P = 0.023$).

Correlation of baseline VEGF with OS

In addition to demonstrating the importance of baseline VEGF levels relative to disease control with ipilimumab, we next asked whether there was a relationship between baseline VEGF levels and OS. Using the Kaplan–Meier method, low serum baseline VEGF was correlated with improved OS for patients treated at the 3-mg/kg dose of ipilimumab (median OS 14.33 vs. 7.44 months; $P = 0.0367$), as well as those treated at 10 mg/kg (median OS 10.85 vs. 6.16 months; $P = 0.0477$). The median OS for all $VEGF^{low}$ patients was 12.87 months compared with 6.56 months for $VEGF^{hi}$ patients ($P = 0.006$). Kaplan–Meier survival curves are shown for the 3 mg/kg, the 10 mg/kg, and the combined 176 patients treated with ipilimumab in Fig. 3. We also analyzed the association between VEGF levels at week 12 and patient OS by using the same 43 pg/mL cutoff. There

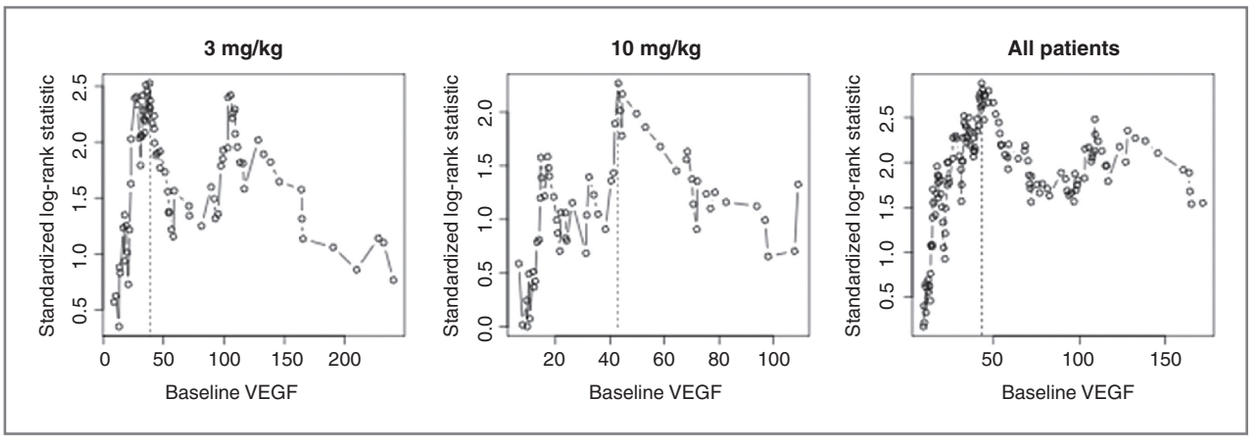

Figure 2. Cutoff point of baseline VEGF defined by using maximally selected log-rank statistics. To determine the cutoff level of pretreatment VEGF value, maximally selected log-rank statistics was used to define the cutoff point of baseline VEGF for patients treated with 3- or 10-mg/kg dose of ipilimumab. The estimated cutoffs of baseline VEGF for 3 mg/kg, 10 mg/kg, or all patients were 39, 43, and 43 pg/mL, respectively.

was no correlation between changes in VEGF levels following treatment and clinical outcome (Supplementary Table S2). Overall, 143 of 176 patients with 99 deaths were available for this analysis. The association of VEGF with OS was maintained when week 12 VEGF levels were used (median OS for $VEGF^{low}$ 14.6 vs. 7.17 months for $VEGF^{hi}$ group, $P = 0.023$).

LDH and ALC are potential biomarkers for ipilimumab-treated patients with melanoma. We next analyzed the correlation between VEGF, LDH, and ALC. There were no strong correlations between VEGF and ALC (correlation = 0.17) or between VEGF and LDH (correlation = 0.19; Supplementary Fig. S1). Baseline and week 7 ALC were analyzed for all patients where data were available. There was a significant correlation between baseline ALC and patient OS ($n = 176$; $P = 0.013$). There was also a significant correlation between ALC at week 7 and patient OS ($n = 125$; $P < 0.001$). We next performed univariate and multivariate analyses including LDH and ALC for all 176 patients with melanoma. Patients with a baseline VEGF $\geq$ 43 pg/mL were 1.6 times more likely to die from the disease during this period than were patients with a baseline VEGF < 43 pg/mL (HR, 1.621; $P = 0.007$). When adjusted for LDH and ALC, the HR for baseline VEGF was 1.279. When adjusted for LDH and ALC, this association was no longer statistically significant ($P = 0.184$; Supplementary Table S3).

Discussion

We analyzed the serum concentrations of VEGF in patients with advanced melanoma treated with ipilimumab and found that the baseline VEGF level is associated with clinical response to ipilimumab therapy. Patients with baseline serum VEGF value greater than 43 pg/mL treated with either the 3- or the 10-mg/kg dose of ipilimumab were less likely to have clinical benefit. In addition, higher serum baseline VEGF levels were correlated with a significantly poorer OS. There was no association between serum VEGF level changes and the likelihood of clinical response. Therefore, the baseline serum VEGF level may serve as a predictive biomarker for ipilimumab in patients with advanced melanoma and indicate that prospective studies are warranted.

Table 1. Association of baseline VEGF with clinical response at week 24 for 157 patients treated with ipilimumab

Response	# Patients status at week 24 (%)	# Patients (VEGF < 43 pg/mL)	# Patients (VEGF $\geq$ 43 pg/mL)
Complete response	3	2	1
Partial response	13	8	5
Stable disease	37	27	10
Clinical benefit	**53 (33%)**	**37 (41.1%)**	**16 (23.2%)**
Progressive disease	54	30	24
Disease of death	52	23	29
No clinical benefit	**106 (67%)**	**53 (58.9%)**	**53 (76.8%)**
Total	159	90	69

NOTE: Fisher exact *t* test; $P = 0.019$.

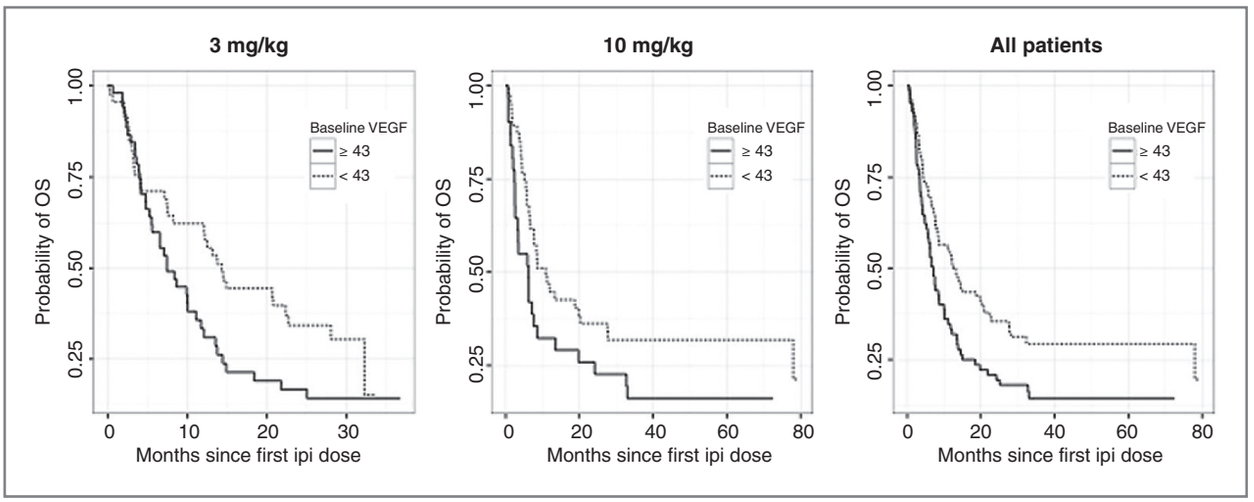

Figure 3. Baseline VEGF value correlated with patient OS. Kaplan–Meier curves demonstrating the difference in OS for patients with $VEGF^{low}$ and $VEGF^{hi}$ are statistically significant: 3 mg/kg dose of ipilimumab (ipi; median OS 14.33 vs. 7.44 months; $P = 0.0367$); 10 mg/kg dose of ipilimumab (median OS 10.85 vs. 6.16 months; $P = 0.0477$); all patients (median OS 12.87 vs. 6.56 months; $P = 0.006$).

LDH is one of the independent prognostic serum markers for patients with melanoma. We did not note the correlation between LDH and VEGF in this study, likely due to differences in tissue distribution, metabolism, and function. Although the baseline VEGF level was significantly associated with OS in the univariate analysis, in the multivariate analyses when corrected for LDH and ALC, this association was not statistically significant. This may be due to the variation in the patient population or the lack of statistical power for multivariate analysis or linked to disease burden.

VEGF has been investigated as a predictive biomarker for other immunotherapies. It serves as a predictive marker for patient responses to high-dose IL-2 in melanoma and renal cell carcinoma (24). The current study confirms a generalizable mechanism to immunotherapy resistance via angiogenic cytokines such as VEGF. However, we did not note the OS benefit for patients with elevated VEGF levels in this study. The median OS for patients with elevated VEGF levels was 6.6 months, which was close to the 6.4 months of OS for patients on the gp100 peptide control arm in the ipilimumab phase III registrational trial (6). Ipilimumab monotherapy may be unable to overcome high immunosuppressive mechanisms in patients with melanoma with high serum VEGF levels. The implications of VEGF as a predictive biomarker (before treatment initiation) versus changes in VEGF levels as a result of treatment may reflect differing biology in the relationship of tumor angiogenesis to immune checkpoint blockade. The predictive nature of VEGF levels and the correlative biomarker reflecting changes during treatment will need further exploration prospectively. These results further emphasize the potential role for VEGF in immune modulation and the possibilities for synergies involving combinations of immuno- and antiangiogenic therapies.

The abnormal vascular structure causes spatially and temporally heterogeneous blood perfusion in tumors, limiting the access of drugs to the poorly perfused regions, and consequently hypoxia induces the upregulation of VEGF and other angiogenic cytokines. Hypoxia confers resistance to a variety of treatments, including radio- and immunotherapies. High circulating level of VEGF correlates with the level of hypoxia in tumors and influences other parameters in the tumor microenvironment. Therefore, understanding the interplay between angiogenesis and immune regulation will provide insights into biomarkers and combinatorial approaches to cancer therapeutics.

Disclosure of Potential Conflicts of Interest

J. Naidoo, D.B. Page, and J.D. Wolchok have received commercial research support from Bristol-Myers Squibb and have served as consultants/advisory board members for the same. F.S. Hodi has received commercial research support from Bristol-Myers Squibb through his academic institution and has served as a consultant/advisory board member for Bristol-Myers Squibb. No potential conflicts of interest were disclosed by the other authors.

Disclaimer

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J. Yuan, J. Zhou, X. Wu, F.S. Hodi
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J. Yuan, S. Tandon, D. Kuk, K.S. Panageas, J. Naidoo, D.B. Page, J.D. Wolchok, F.S. Hodi
Writing, review, and/or revision of the manuscript: J. Yuan, D. Kuk, K.S. Panageas, P. Wong, X. Wu, J. Naidoo, D.B. Page, J.D. Wolchok, F.S. Hodi
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): Z. Dong, S. Tandon, F.S. Hodi
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