Bevacizumab plus Ipilimumab in Patients with Metastatic Melanoma


Abstract

Ipilimumab improves survival in advanced melanoma and can induce immune-mediated tumor vasculopathy. Besides promoting angiogenesis, vascular endothelial growth factor (VEGF) suppresses dendritic cell maturation and modulates lymphocyte endothelial trafficking. This study investigated the combination of CTLA4 blockade with ipilimumab and VEGF inhibition with bevacizumab. Patients with metastatic melanoma were treated in four dosing cohorts of ipilimumab (3 or 10 mg/kg) with four doses at 3-week intervals and then every 12 weeks, and bevacizumab (7.5 or 15 mg/kg) every 3 weeks. Forty-six patients were treated. Inflammatory events included giant cell arteritis (n = 1), hepatitis (n = 2), and uveitis (n = 2). On-treatment tumor biopsies revealed activated vessel endothelium with extensive CD8+ and macrophage cell infiltration. Peripheral blood analyses demonstrated increases in CCR7+/CD45RO+ cells and anti-galectin antibodies. Best overall response included 8 partial responses, 22 instances of stable disease, and a disease-control rate of 67.4%. Median survival was 25.1 months. Bevacizumab influences changes in tumor vasculature and immune responses with ipilimumab administration. The combination of bevacizumab and ipilimumab can be safely administered and reveals VEGF-A blockade influences on inflammation, lymphocyte trafficking, and immune regulation. These findings provide a basis for further investigating the dual roles of angiogenic factors in blood vessel formation and immune regulation, as well as future combinations of antiangiogenesis agents and immune checkpoint blockade. Cancer Immunol Res 2(7); 632–42. ©2014 AACR.

Introduction

CTLA4 blockade with ipilimumab improves survival in patients with metastatic melanoma when compared with a gp100 peptide vaccine (1) and in combination with dacarbazine chemotherapy when compared with dacarbazine alone (2). Efforts to further enhance the efficacy of immune checkpoint blockade through rational treatment combinations are needed.

In pursuit of predictive markers, pretreatment levels of vascular endothelial growth factor (VEGF-A) influence clinical outcomes to ipilimumab therapy (3). Therefore, determinants that may limit ipilimumab efficacy include immunosuppressive angiogenic factors such as VEGF. VEGF has profound effects on immune regulatory cell function, specifically inhibiting dendritic cell maturation and antigen presentation (4, 5). Furthermore, there is increasing evidence for the role angiogenic factors play in influencing lymphocyte trafficking across endothelia into tumor deposits (6). Previous studies have demonstrated the effects of ipilimumab on vessels feeding tumor deposits, resulting in an immune-mediated vasculopathy (7). As a result of CTLA4 blockade, granulocytes and lymphocytes infiltrate the endothelia, resulting in its destruction and tumor necrosis. The clinical efficacy of targeting VEGF-A and its effects on pathologic angiogenesis have been extensively studied with the use of bevacizumab (8–14), and these findings suggest a role in counteracting the immunosuppressive actions of VEGF. Given the effects on tumor vasculature witnessed in patients with melanoma being treated with ipilimumab and the known activity of bevacizumab, we conducted a phase I study to investigate the potential synergies of this combination in patients with metastatic melanoma.

Materials and Methods

Study design and treatment

The protocol (Supplementary Appendix A) was approved by the Dana-Farber/Harvard Cancer Center institutional review board.
board, and all patients provided signed informed consent. Patient eligibility included measurable unresectable stage III or stage IV melanoma, Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, adequate end-organ function. Exclusion criteria included central nervous system (CNS) metastases, prior treatment with ipilimumab or bevacizumab, history of autoimmune disease, melanoma involvement in the gastrointestinal tract, ulcerated skin lesions, or ongoing treatment with full-dose warfarin, heparin equivalent, nonsteroidal anti-inflammatory drugs, aspirin within 10 days of enrollment, or any medication that inhibits platelet function within 2 weeks before enrollment. Four cohorts of patients each received four doses of ipilimumab at 3-week intervals and then every 3 months, plus bevacizumab every 3 weeks (continuous): cohort 1, ipilimumab at 3-week intervals and then every 3 months, cohort 2, ipilimumab 10 mg/kg + bevacizumab 7.5 mg/kg; cohort 3, ipilimumab 3 mg/kg + bevacizumab 7.5 mg/kg; cohort 4, ipilimumab 3 mg/kg + bevacizumab 15 mg/kg (Supplementary Fig. S1). Cohorts that included 3 mg/kg were added following the approval of ipilimumab at this dose to gain safety data and experience.

Patients were first enrolled in cohorts of five with 10 mg/kg of ipilimumab. The dose-limiting toxicity (DLT) period was 12 weeks. If ≥3 of 5 patients in cohort 1 did not experience a DLT, then the study was permitted to proceed to the next cohort. If ≥3 patients in cohort 3 experienced a DLT, the study was designed to stop. To ensure that toxicity at the maximum tolerated dose (MTD) was acceptable and to gain additional experience with this combination, an additional 12 patients were accrued at MTD. There was a 94% probability of dose escalation if the true rate of DLT was no more than 20%. If the true rate of DLT exceeded 50%, the probability of escalation was less than 50%.

Statistical analysis
Best overall response (BORR) was defined as the proportion of patients with complete or partial response at any time while on study. The disease-control rate (DCR) was the proportion of patients with complete response, partial response, or stable disease. Differences in response rates or DCR by cohort were assessed using the Fisher exact test. Time to progression (TTP), overall survival (OS), and duration of response were assessed using the method of Kaplan–Meier, with pointwise, 95% confidence intervals (CI) estimated using log[−log(survival)] methodology. Equality of survival curves by cohort was assessed using the log-rank test. Comparisons of the incidences of adverse events by cohort according to CTCAE 3.0 System Class were conducted using the Fisher exact test. All P values were two-sided, with statistical significance defined as P < 0.05. There were no corrections for multiple comparisons.

Flow cytometry
Peripheral blood samples from patients receiving ipilimumab alone on an expanded access study were obtained on Dana-Farber/Harvard Cancer Center review board–approved protocols and used for comparison with samples from the current trial. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque (GE Biosciences) density gradient centrifuge. Cells were stained with fluorescent conjugated antibodies, and analyzed on a FC500 FACS analyzer (Beckman Coulter). A total of 1 × 10^7 events were collected. FACS analysis results are expressed as percentages (CD4-PE Cy7, CD8-PE Cy7, CD28-PerCP, and CD45RO-ECD antibodies were from Beckman Coulter, CCR7-FITC from R&D Systems, and CD57-PE from Abcam). Percentage increase (cutoff ≥5%) between pretreatment and posttreatment by 50% was considered a significant increase. Differences in the proportions increased between ipilimumab and ipilimumab plus bevacizumab groups were determined by the Fisher exact test.

Immunohistochemistry
Metastatic melanoma samples from patients that received ipilimumab plus bevacizumab (n = 10) and ipilimumab alone (n = 6) on an expanded access study were obtained before and after treatment initiation. Ipilimumab-alone samples were obtained from patients being treated on approved clinical trials. Posttreatment samples from both the current ipilimumab–bevacizumab trial and ipilimumab alone were obtained at the same time from the initiation of therapy, immediately following the completion of ipilimumab induction at approximately week 12. Biopsies of end-organs suspected of inflammatory events related to treatment were obtained whenever possible. Formalin-fixed paraffin-embedded tissue sections were stained for biomarkers that included CD3 (Dako; A0452; 1:250), CD8 (Dako; M7103; 1:100), CD20 (Dako; N1502; undiluted), Foxp3 (Dako; M7003; 1:100), CD120 (Dako; N1502; undiluted), Foxp3 (BioLegend; 320102; 1:50), and CD163 (Vector Laboratories; VP-C374; 1:500) to characterize immune infiltrates. To assess vessel morphology and activation, CD31 (Dako; N1596; undiluted) and E-selectin (Neuronics; M020039; 1:50) antibodies were used.

Humoral immune responses detected following treatment
Antibodies presented in posttreatment sera were screened using ProtoArray Human Protein Microarray v5 (Invitrogen) according to the manufacturer’s instructions. Antibody targets were identified by a Z factor of ≥0.4. The presence of galectin antibodies in the sera were confirmed by immunoblot analysis using recombinant human galectins-1, -3, and -9 (R&D Systems). To compare antibody levels as a function of treatment, galectin-1, -3, and -9 immunoblots (blocking 5% BSA) were incubated with pretreatment and posttreatment sera (1:2,000 dilution in PBS with 2% BSA) overnight, followed by horseradish peroxidase–conjugated goat anti-human IgG antibody (Invitrogen), and visualized with enhanced chemiluminescence. Densities of protein bands and backgrounds were quantified using NIH ImageJ software. After background subtraction, galectin antibody responses to treatment were determined by the formula: fold change = (density_post − density_pre)/density_pre. A fold change of ≥0.5 was considered a significant increase.
Figure 1. High-grade treatment-related adverse events. A, grade 4 events. ALT, alanine aminotransferase (SGPT); AST, aspartate aminotransferase (SGOT). B, grade 3 events. Eleven study patients had treatment-related, grade 3 events [23.9%, (95% exact CI, 13%–39%)]. Hematoxylin and eosin-stained, formalin-fixed, paraffin-embedded tissue sections showing temporal artery, cut in cross section, with transmural acute and chronic inflammation at \( \times 200 \) (C) and \( \times 400 \) (D) final magnification. E and F, skin with chronic inflammation intermixed with eosinophils within the mid-dermis at \( \times 400 \) (E) and \( \times 1,000 \) (F) final magnification. G and H, core of liver with acute and chronic inflammation including prominent eosinophils, at \( \times 400 \) (G) and \( \times 1,000 \) (H) final magnification.

<table>
<thead>
<tr>
<th>Toxicity (CTCAE V3.0)</th>
<th>Total</th>
<th>Cohort 1</th>
<th>Cohort 2</th>
<th>Cohort 3</th>
<th>Cohort 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>Grade 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatic-other</td>
<td>1</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Grade 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT, SGPT</td>
<td>2</td>
<td>–</td>
<td>1</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>AST, SGOT</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Abdomen, pain</td>
<td>1</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Adrenal insufficiency</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td>Allergic reaction</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Colitis</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>Endocrine-other</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>Fatigue</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Head/headache</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hemorrhage-other</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hepatic-other</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hypertension</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Hypoventrism</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lipase</td>
<td>2</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Muco/stomatitis by exam, oral cavity</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Rash/desquamation</td>
<td>2</td>
<td>–</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Thrombosis/thrombus/embolism</td>
<td>1</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Vascular-other (specify)</td>
<td>1</td>
<td>1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
Results

Patients and treatment
A total of 46 patients were treated (Fig. 1, CONSORT diagram). Five patients in cohort 1 were first treated without any DLTs. Five patients were then treated in cohort 2 without toxicities; therefore, cohort 2 (ipilimumab 10 mg/kg plus bevacizumab 15 mg/kg) was determined to be MTD. An additional 12 patients were treated at MTD. The study was amended to enroll 12 patients each to cohorts 3 and 4 following the regulatory approval of ipilimumab at 3 mg/kg. Demographics, disease status, and prior treatment according to cohort are summarized in Supplementary Table S1. Patients were predominantly male (61%) with a median age of 58 years (range, 25–80). Eighty-nine percent of the patients had an ECOG performance status of 0. Forty-one patients (89%) had stage IV melanoma and 5 (11%) had unresectable, stage III disease. Thirteen (28%) patients had prior chemotherapy and radiation, 7 (15%) had radiation and no chemotherapy, 16 (35%) had chemotherapy and no radiation, and 8 (17%) had neither radiation nor chemotherapy. The median number of sites of disease was 3 (range, 1–8): 83% had lymph node disease, 63% had lung, and 61% had soft tissue. The median follow-up was 11.8 months (range, 2.6–42.5).

Adverse events
Toxicities (regardless of attribution) for the first 12 weeks representing the dose-limiting window are presented in Supplementary Table S2. Two patients experienced DLTs, 1 from cohort 3 and 1 from cohort 4. No DLTs occurred in cohort 1 or 2. There were no treatment-related deaths. All 46 patients reported adverse events. The most commonly reported adverse events (any grade) were fatigue (n = 14), rash/desquamation (n = 15), headache (n = 16), and cough (n = 17). Supplementary Table S3 presents the grade 3 and grade 4 events for each dose level for all events and those events reported adverse events (any grade) were fatigue (46 patients reported adverse events. The most commonly representing the dose-limiting window are presented in Supplementary Table S1. Patients were predominantly male (61%) with a median age of 58 years (range, 25–80). Eighty-nine percent of the patients had an ECOG performance status of 0. Forty-one patients (89%) had stage IV melanoma and 5 (11%) had unresectable, stage III disease. Thirteen (28%) patients had prior chemotherapy and radiation, 7 (15%) had radiation and no chemotherapy, 16 (35%) had chemotherapy and no radiation, and 8 (17%) had neither radiation nor chemotherapy. The median number of sites of disease was 3 (range, 1–8): 83% had lymph node disease, 63% had lung, and 61% had soft tissue. The median follow-up based on patients alive at the time of data retrieval was 11.8 months (range, 2.6–42.5).

### Table 1. Number of treatment-related adverse events per patient

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Median</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>13</td>
<td>1.4</td>
<td>0.7</td>
<td>1.0</td>
<td>1.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Cohort</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>2.0</td>
<td>—</td>
<td>2.0</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>2 (MTD)</td>
<td>6</td>
<td>1.2</td>
<td>0.4</td>
<td>1.0</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>1.7</td>
<td>1.2</td>
<td>1.0</td>
<td>1.0</td>
<td>3.0</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>1.3</td>
<td>0.6</td>
<td>1.0</td>
<td>1.0</td>
<td>2.0</td>
</tr>
</tbody>
</table>

NOTE: Worst grade of reported adverse event/toxicity per patient. Thirteen patients had treatment-related, grade 3 or 4 events [28.3%, (95% exact CI: 16%–43%)]. For patients with treatment-related, grade 3 or 4 events, the table summarizes the number per patient. Adverse events classified as "not related" are included. Grades: 1, mild; 2, moderate; 3, severe; 4, life-threatening; 5, death.

Study outcomes
Treatment with ipilimumab plus bevacizumab resulted in morphologic changes in intratumoral endothelia with rounded and columnar CD31⁺ cells compared with pretreatment or posttreatment samples from patients receiving ipilimumab alone (Fig. 2A and Supplementary Fig. S2). To illustrate variations in pathologic responses observed, examples of intermediate and no response are provided in Supplementary Fig. S3. Increased expression of E-selectin as a function of therapy was observed with combined treatment relative to ipilimumab alone, revealing further biochemical evidence for endothelial activation by the addition of bevacizumab. Concentrated CD31 staining was observed at the interendothelial junctions (Supplementary Fig. S2). Pathology reviewer further revealed that these endothelial changes were associated with extensive immune cell infiltration of tumors. Vessel density was not affected significantly by bevacizumab therapy.

With pathologic examination of immune infiltrates associated with treatment, significant trafficking of CD8⁺ T cells and CD163⁺ dendritic macrophages (Fig. 3) across the tumor vasculature was witnessed in ipilimumab plus bevacizumab posttreatment biopsies that was qualitatively increased in comparison with that elicited by ipilimumab alone. There was minimal change in FoxP3⁺ cellular composition.

We next sought to identify altered immune responses resulting from bevacizumab plus ipilimumab combination therapy. To pursue functional changes, flow cytometry detecting T-cell phenotypes in the peripheral blood demonstrated enhancement in CCR7⁺/CD45RO⁺ populations for CD4⁺ (Fig. 4A) and CD8⁺ (Fig. 4B) T cells (individual responses Supplementary Fig. S4). Bevacizumab plus ipilimumab significantly increased circulating memory cell phenotypes compared with...
ipilimumab alone (Fig. 4C). Furthermore, analyses with post-treatment sera using protein arrays identified galectin-1 and galectin-3 in 2 of 4, and 3 of 4 patients, respectively (Z-factor = 0.49 and 0.7 for galectin-1, and 0.58, 0.83, and 0.87 for galectin-3). Galectin-9 was not included in the protein array. Given its biologic significance in immune regulation, galectin-9 was included in subsequent analyses. The presence of anti-galectin antibodies in sera was further confirmed by immunoblot using recombinant proteins (Fig. 4D). Patients who received ipilimumab plus bevacizumab had a significantly higher number of responses to galectins-1, -3, and -9 than patients who received ipilimumab alone (Fisher exact P = 0.02; Fig. 4E).

**Efficacy**

The median follow-up at the time of this analysis was 17.3 months (95% CI, 11.1–30.2 months). Thirty-nine (85%) patients stopped treatment, and 7 patients remained on study. Nine patients (23%) discontinued treatment due to toxicity, 29 (74%) due to progressive disease, and 1 (3%) due to withdrawal of consent. The median number of cycles patients received was 7 (range, 2–37).

The 12-week response for the entire treated patient population was 10.9% (95% exact CI, 4%–24%; highest cohort 2, 17.6%). Clinical activity is shown in Fig. 5. BORR was 19.6% [95% exact CI, 9%–34%; highest cohort 2, 29.4% (95% exact CI, 10%–56%); 1 complete response; Supplementary Table S4]. In addition to examples of pseudoprogression (Fig. 6), delayed best responses (Supplementary Fig. S5) were observed. Response kinetics for all patients are presented in Supplementary Fig. S6A and for cohort 2 (MTD) in Supplementary Fig. S6B. DCR for all patients was 67.4% [95% exact CI, 52%–81%; cohort 2, 76.5% (95% exact CI, 50%–93%)]. There were no statistical differences in the response or DCR between cohorts. Most durable responses achieved best response after months of therapy. One patient in cohort 2 (MTD) experienced 7 months of stable disease before a partial response and subsequently had a complete response beginning 17 months following the initiation of therapy. Eleven patients in cohorts 1 and 2 are alive, months after discontinuation of therapy. The median TTP was 9.0 months [95% CI, 5.5–14.5 months; longest cohort 2, 14.5 months (95% CI, 3.8–¥ months)]; Supplementary Fig. S7]. There were no differences in progression-free survival (PFS) by cohort (log-rank P = 0.32).

Radiographic evidence for early metabolic antitumor responses without significant anatomic response was exemplified with PET/CT imaging (Fig. 6A–D). Transient increases in lesion size with decreased density before gradual decrease in size with subsequent imaging (Fig. 6E–H) were observed in a number of patients, consistent with pseudoprogression.

Thirty (65%) patients were alive at the time of data retrieval. Kaplan–Meier estimates for TTP and OS are shown in Supplementary Fig. S7. Median OS was 25.1 months [95% CI, 12.7–¥ months; cohort 2, 25.1 months (95% CI, 9.6–¥ months)]; Supplementary Fig. S7]. There were no differences in OS by cohort (log-rank P = 0.98). The Kaplan–Meier estimate of 1-year OS was 79% (95% CI, 62%–89%), and
6-month TTP was 63% (95% CI, 47%–75%). The lower bounds of both confidence intervals exceed those of Korn and colleagues (15).

Discussion

The influences on immunologic effects witnessed with the addition of bevacizumab to ipilimumab reveal novel mechanisms of action for bevacizumab in patients. First, morphologic and biochemical alterations in the tumor vasculature resulted in endothelial activation associated with qualitative increases in lymphocyte and myeloid/monocyte cell trafficking into tumor deposits. The monocytes had extensive dendritic processes. These patterns of immune infiltrates for some patients were associated with transient increases in lesion size with decreased density before gradual decrease in size with subsequent imaging. Detailed characterization of these infiltrates and cellular functions are areas for future investigation. Vessel changes were similar to those observed in high endothelial venules (HEV) found in secondary lymphoid organs and associated with lymphocyte extravasation (16). Such endothelial appearances correlated with the ability of lymphocytes to migrate into tissues. E-selectin expression induced by bevacizumab facilitates lymphocyte adhesion and rolling (17). In addition, CD31 influences adhesive and signaling functions for vascular cellular extravasation (18, 19). Specifically, the concentration of CD31 staining at the interendothelial junctions of bevacizumab plus ipilimumab–treated specimens indicates vessels adapted for efficient lymphocyte trafficking (20). These results are consistent with previous observations of anti-VEGF treatment increasing lymphocyte tumor infiltrates in adoptive therapy models (21, 22).

Further evidence for immunologic changes by the combination was demonstrated in the peripheral blood through increasing circulating memory T cells resulting from the addition of bevacizumab. This provides a definitive role for bevacizumab in effecting broad changes in the circulating immune composition. Furthermore, the coordinated T- and B-cell responses witnessed previously with checkpoint blockade (23, 24) are confirmed by the increased antibody recognition of the galectin family members. This finding suggests that the concerted effects of combination therapy...
Cellular and humoral immune responses in the peripheral blood are altered by the addition of bevacizumab to ipilimumab. All samples were obtained pretreatment and at week 12 at the completion of ipilimumab or ipilimumab–bevacizumab induction. A, example of changes as a function of treatment in CD4^+CCR7^+CD45RO^+ and CD4^+CCR7^−CD45RO^+ T-cell populations to ipilimumab plus bevacizumab treatment, compared with changes with ipilimumab treatment alone. B, example of changes as a function of treatment in CD8^+CCR7^+CD45RO^+ and CD8^+CCR7^−CD45RO^+ T-cell populations to ipilimumab plus bevacizumab treatment, compared with the responses to ipilimumab treatment alone. C, number of patients with melanoma who have at least 50% enhancement in CD4^+/CD8^+CCR7^+CD45RO^+ and CD4^+/CD8^+CCR7^−CD45RO^+ T-cell populations following treatment with ipilimumab (3 mg/kg), or ipilimumab (3 mg/kg) plus bevacizumab, or ipilimumab (10 mg/kg) plus bevacizumab. *, P < 0.05 between ipilimumab and ipilimumab plus bevacizumab; **, P < 0.01. D, representative immunoblots of antibody responses in 4 bevacizumab plus ipilimumab–treated patients to a total of zero, one, two, or three galectins. Arrows, increased antibody levels in posttreatment sera samples. Galectin-1, -3, and -9 proteins were mixed together and equally loaded and separated by gel electrophoresis. Following transfer onto a membrane, strips were incubated with equally diluted pretreatment and posttreatment sera. Density analysis using NIH ImageJ software confirmed the increases in densities of the indicated bands after subtracting background taken from nearby areas of each band. As in most of the cases, density change was seen in only one or two of the three galectins; protein(s) without density change served as loading controls. E, antibody responses to galectins in patients treated with bevacizumab plus ipilimumab and ipilimumab alone. Anti-galectin-1, anti-galectin-3, and anti-galectin-9 antibodies were detected in pretreatment and posttreatment patient sera by immunoblot analyses. Percentages of patients with increased levels of antibodies to a total of zero, one, two, or three galectins in bevacizumab plus ipilimumab–treated patients (n = 45) and ipilimumab-alone patients (n = 18). Density of each band was measured using NIH ImageJ software and the antibody fold change was calculated using the formula: fold change = (density_{Post} − density_{Pre})/density_{Pre}. Antibody levels were considered as increased when the fold change $\geq$ 0.5.
can result in additional immune recognition that extends implications for both immune regulation and angiogenesis. As the galectin family members are involved in tumor-cell invasion, metastases, and angiogenesis as well as immune regulation, it will be important to define in future studies the functions of these antibodies and their potential therapeutic roles.

In preclinical animal models and in humans, VEGF has been associated with altered antitumor immune responses, including the suppression of dendritic-cell maturation (4, 5), proliferation of regulatory T cells, inhibition of T-cell responses (25), and accumulation of myeloid-derived suppressor cells (26-28). In patients with colorectal cancer, bevacizumab improved the antigen-presenting capacity of circulating dendritic cells (29). The increase in dendritic-cell infiltrates associated with the current study suggests a role for VEGF blockade in influencing the association of antigen-presenting cell with tumor cells and their maturation. This reveals an additional mechanism for bevacizumab on immune function in the context of checkpoint blockade.

Inflammatory toxicities were generally higher than expected with ipilimumab alone, but remained manageable. These included rare vasculitic events suggesting immune recognition of unique sets of antigens (30) and eosinophil-driven processes. Importantly, there did not seem to be an increased incidence of dermatologic or gastrointestinal side effects such as colitis, which are most concerning for ipilimumab. As such, the combination of blocking CTLA4 and VEGF-A may broaden the recognized antigen repertoire.

Recent clinical trials reporting survival outcomes for patients with metastatic melanoma have led to regulatory approval for ipilimumab and vemurafenib. Phase II trials of ipilimumab revealed 2-year survival rates of 24.2% to 32.8% (31, 32). In patients who had received at least one prior therapy, ipilimumab, when compared with a gp100 peptide vaccine, improved the median OS from 6.4 to 10.1 months (1). OS in previously treated patients with melanoma receiving vemurafenib was 15.9 months (33). In the current phase I trial combining bevacizumab and ipilimumab, the clinical activity was favorable compared with ipilimumab alone. As all but one partial response occurred in cohorts 1 and 2 (10 mg/kg ipilimumab), the ipilimumab dose may influence the efficacy when ipilimumab is combined with bevaciuzumab. The median survival of the entire 46-patient cohort was greater than 2 years with significant antitumor activity witnessed at MTD. With the lower bounds of both confidence intervals exceeding those of Korn and colleagues (15), this combination is worthy of further pursuit. Importantly, these results establish a mechanistic foundation for combining antiangiogenesis with immune checkpoint blockade for cancer treatment.

Antiangiogenic therapy for cancer has traditionally provided a means to limit the blood supply and improve delivery of antineoplastic agents to tumor sites. When combined with chemotherapy or interferon, bevacizumab has proved efficacious at improving the outcomes of patients with colorectal cancer, renal cell carcinoma, non–small cell lung cancer, breast cancer, ovarian cancer, and glioblastoma multiforme (8-12, 34, 35).

For many tumors, hypoxia creates a microenvironment of inhibitory inflammatory cells (27, 36). The role of angiogenic factors in suppressing inflammation to promote vessel growth has increasingly been recognized in the context of tumor immunology (36). The ability to counteract the dual function
of angiogenic factors in promoting vessel growth and suppressing immune responses is provided in the current study by the parallel effects on host immunity and tumor vasculature. Mechanisms defining the roles for bevacizumab in the context of immune checkpoint blockade were uncovered. The combination provoked inflammatory events in patients, promoted memory cells circulating in the peripheral blood, enhanced intratumoral trafficking of effector cells, and improved endogenous humoral immune responses to galectins. Further investigation is needed to evaluate the mechanistic basis of bevacizumab activity and the full impact of clinical activity. Continued development of immune checkpoint and antiangiogenic combination therapies are warranted for the treatment of melanoma and other cancers.

Figure 6. Example of pretreatment (A and B) and 8-week posttreatment (C and D) PET CT images. An early metabolic response is noted in a dominant left hepatic metastasis. E, axial contrast-enhanced CT image obtained at baseline demonstrates a solitary heterogeneous hypodense lesion in segment II of the liver, consistent with a metastatic deposit. F, axial contrast-enhanced CT obtained 4 months after treatment demonstrates slight increase in the lesions with interval decrease in density (from 40 HU to 19 HU, approximately 50%), consistent with treatment effect. The increase in size represents pseudoprogression. G and H, follow-up CT scans obtained 6 and 36 months after the start of treatment demonstrate gradual decrease in size of the metastatic deposit.

Disclosure of Potential Conflicts of Interest
F.S. Hodi has received research support from Bristol-Myers Squibb and Genentech, has an ownership interest (including patents) in IP licensed to Bristol-Myers Squibb (as per institutional IP policy), and is a consultant/advisory board member for Bristol-Myers Squibb and Genentech. M.B. Atkins is a consultant/advisory board member for Bristol-Myers Squibb and Genentech. E.F. Velazquez and M.C. Mihm serve as directors for SKADA, Inc., and are consultant/advisory board members for Melasciences and Caliber ID. D. McDermott is a consultant/advisory board member for Bristol-Myers Squibb. No potential conflicts of interest were disclosed by the other authors.

Authors’ Contributions
Conception and design: F.S. Hodi, A. Giobbie-Hurder
Development of methodology: F.S. Hodi, W. Zeng, G.F. Murphy, J.T. Yap
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): F.S. Hodi, D. Lawrence, C. Lezcano, X. Wu, J. Zhou, T. Sasada, W. Zeng, M.B. Atkins, N. Ibrahim, P. Friedlander, K.T. Flaherty.


Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): F.S. Hodi, J. Zhou, W. Zeng, A.D. Van den Abbeele

Study supervision: F.S. Hodi

References


Grant Support

This study was funded by NIH CA143832 (to F.S. Hodi) and 1R01CA158487 (to F.S. Hodi and G.F. Murphy), the Melanoma Research Alliance (to F.S. Hodi), the Sharon Crowley Martin Memorial Fund for Melanoma Research (to F.S. Hodi), and the Malcolm and Emily Mac Naught Fund for Melanoma Research (to F.S. Hodi) at the Dana-Farber Cancer Institute, Genentech/Roche, and Bristol-Myers Squibb.

Received March 26, 2014; revised March 28, 2014; accepted April 7, 2014; published OnlineFirst April 21, 2014.


Correction: Bevacizumab plus Ipilimumab in Patients with Metastatic Melanoma

In this article (Cancer Immunol Res 2014;2:632–42), which appeared in the July 2014 issue of Cancer Immunology Research (1), the panels of Fig. 1 are labeled incorrectly. The grade 4 toxicities should be labeled as A, and the grade 3 toxicities as B; B to G should be labeled C to H. The authors regret this error.

Reference


Published OnlineFirst August 5, 2014.
doi: 10.1158/2326-6066.CIR-14-0141
©2014 American Association for Cancer Research.
Cancer Immunology Research

Bevacizumab plus Ipilimumab in Patients with Metastatic Melanoma

F. Stephen Hodi, Donald Lawrence, Cecilia Lezcano, et al.


Updated version
Access the most recent version of this article at:
doi:10.1158/2326-6066.CIR-14-0053

Supplementary Material
Access the most recent supplemental material at:
http://cancerimmunolres.aacrjournals.org/content/suppl/2014/04/21/2326-6066.CIR-14-0053.DC1

Cited articles
This article cites 36 articles, 13 of which you can access for free at:
http://cancerimmunolres.aacrjournals.org/content/2/7/632.full#ref-list-1

Citing articles
This article has been cited by 26 HighWire-hosted articles. Access the articles at:
http://cancerimmunolres.aacrjournals.org/content/2/7/632.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.