Published in final edited form as:

Clin Cancer Res. 2021 February 15; 27(4): 1048–1057. doi:10.1158/1078-0432.CCR-20-2500.

Randomized Phase II and Biomarker Study of Pembrolizumab plus Bevacizumab versus Pembrolizumab Alone for Patients with Recurrent Glioblastoma

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Abstract

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

Prior presentation: 2018 American Society of Clinical Oncology Annual Meeting.

Purpose: VEGF is upregulated in glioblastoma and may contribute to immunosuppression. We performed a phase II study of pembrolizumab alone or with bevacizumab in recurrent glioblastoma.

Patients and Methods: Eighty bevacizumab-naïve patients with recurrent glioblastoma were randomized to pembrolizumab with bevacizumab (cohort A, $n = 50$) or pembrolizumab monotherapy (cohort B, $n = 30$). The primary endpoint was 6-month progression-free survival (PFS-6). Assessed biomarkers included evaluation of tumor programmed death-ligand 1 expression, tumor-infiltrating lymphocyte density, immune activation gene expression signature, and plasma cytokines. The neurologic assessment in neuro-oncology (NANO) scale was used to prospectively assess neurologic function.

Results: Pembrolizumab alone or with bevacizumab was well tolerated but of limited benefit. For cohort A, PFS-6 was 26.0% [95% confidence interval (CI), 16.3–41.5], median overall survival (OS) was 8.8 months (95% CI, 7.7–14.2), objective response rate (ORR) was 20%, and median duration of response was 48 weeks. For cohort B, PFS-6 was 6.7% (95% CI, 1.7–25.4), median OS was 10.3 months (95% CI, 8.5–12.5), and ORR was 0%. Tumor immune markers were not associated with OS, but worsened OS correlated with baseline dexamethasone use and increased posttherapy plasma VEGF (cohort A) and mutant *IDH1*, unmethylated *MGMT*, and increased baseline PlGF and sVEGFR1 levels (cohort B). The NANO scale contributed to overall outcome assessment.

Conclusions: Pembrolizumab was ineffective as monotherapy and with bevacizumab for recurrent glioblastoma. The infrequent radiographic responses to combinatorial therapy were durable. Tumor immune biomarkers did not predict outcome. Baseline dexamethasone use and tumor MGMT warrant further study as potential biomarkers in glioblastoma immunotherapy trials.

Introduction

Immunotherapeutic agents have transformed treatment for several cancers, although in most settings only a subset of patients benefit. In contrast, phase III studies of immunotherapies including vaccines (1) and inhibitors of immune checkpoint molecules (2), have not improved survival for glioblastoma, the most common primary adult malignant brain tumor. Explanations for these disappointing results remain unclear, but multiple complementary, immunosuppressive factors in the tumor microenvironment likely contribute (3).

Glioblastoma is a highly angiogenic cancer characterized histopathologically by vascular proliferation induced by high VEGF production by tumor cells. VEGF inhibition with bevacizumab, a humanized VEGF blocking antibody, significantly prolongs progression-free survival (PFS) but not overall survival (OS) for recurrent (4) and newly diagnosed patients (5, 6). VEGF may also contribute to tumor immunosuppression through several mechanisms (7) and preclinical studies (8–11) as well as a growing number of clinical reports (12–18) show that VEGF inhibition can enhance immunotherapy benefit for a variety of cancers.

Pembrolizumab, a high-affinity, humanized IgG4-κ inhibitory mAb of the immunomodulatory receptor programmed-death 1 (PD-1), is currently approved for the treatment of multiple malignancies based on its safety profile and efficacy. Potential biomarkers of therapeutic benefit with PD-1 blockade in other tumor types include tumor

programmed death-ligand 1 (PD-L1) expression, tumor-infiltrating lymphocyte (TIL) density, and immune activation gene expression profile (GEP; ref. 19). Although a retrospective study reported modestly prolonged PFS in a minority of patients with recurrent high-grade glioma (20), the activity of pembrolizumab for glioblastoma has not been prospectively assessed. In addition, a heavily pretreated, multifocal patient with recurrent glioblastoma with a hypermutated tumor due to a germline POLE mutation achieved a dramatic response (21).

Our investigator-initiated, multicenter, randomized phase II study evaluated the hypothesis that the addition of VEGF blockade could enhance the antitumor activity of anti-PD-1 therapy for recurrent glioblastoma, and we also evaluated the single-agent activity of pembrolizumab for this indication. The study was not designed to directly compare the arms but rather to evaluate the efficacy of each arm independently. Because bevacizumab is known to prolong PFS in recurrent glioblastoma, the addition of bevacizumab in our study offered the possibility to extend pembrolizumab exposure to enhance its potential for therapeutic benefit. Our study incorporated a comprehensive analysis of tumor immune biomarkers and detailed analysis of circulating cytokines as well as prospective integration of the neurologic assessment in neuro-oncology (NANO) scale (22) to monitor neurologic function.

Patients and Methods

Study design and patients

This phase II, multicenter, open-label, two-cohort study enrolled adults with histologically confirmed glioblastoma at first or second relapse and a Karnofsky performance status of ≥70 who were ≥28 days from prior surgery and ≥12 weeks from prior radiation. Patients requiring >4 mg/day of dexamethasone or who received PD-1/PD-L1 or VEGF/VEGFR inhibitors were excluded. Additional eligibility criteria are listed in the Supplementary Data.

The study (NCT02337491) was compliant with the Declaration of Helsinki and guidelines on Good Clinical Practice. Ethics approval was obtained at all participating centers and all patients provided informed consent. The study sponsor was Dana-Farber Cancer Institute (Boston, MA).

Study procedures

Initially a safety lead-in using a 3+3 design was performed to establish the MTD or recommended phase II dose (RP2D) of pembrolizumab combined with bevacizumab because these two agents had not been combined previously for patients with glioblastoma. The initial dose level for the safety lead-in evaluated the established dose of each agent when administered as monotherapy including 200 mg of pembrolizumab i.v. every 3 weeks and 10 mg/kg of bevacizumab i.v. biweekly. Subsequent planned dose levels included deescalation of pembrolizumab dosing if the MTD was exceeded. Thereafter, eligible patients were randomized (5:3) to include a total of 50 patients to receive pembrolizumab plus bevacizumab (cohort A) and 30 patients to receive pembrolizumab monotherapy (cohort B). Patients who were treated at the RP2D during the safety lead-in were included in the

intent-to-treat (ITT) population for cohort A. Each cohort accrued using a single-stage design. Treatment continued until tumor progression, unacceptable toxicity, noncompliance, or withdrawal of consent. Additional information on study procedures is provided in the Supplementary Data.

Toxicity was graded using Common Terminology Criteria for Adverse Events version 4.0. Investigator assessed response occurred every 6 weeks using clinical examination and contrast-enhanced MRI according to the radiologic assessment in neuro-oncology (RANO) criteria (23). Clinically stable patients with radiologic progression were allowed to continue study therapy for up to 3 months pending progression confirmation as per the immunotherapy response assessment criteria in neuro-oncology criteria including backdating to date of initial progression for those patients who had confirmation of progression noted on follow-up imaging (24). Patient neurologic function was assessed at baseline and at MRI assessments using the NANO scale (22).

Biomarker analyses

Immunocorrelative studies including PD-L1 expression, TIL density, immune activation GEP by nanostring, as well as plasma biomarkers were performed as described previously (25–29) and are detailed in the Supplementary Data.

Outcomes

The primary endpoint for each cohort, PFS-6, and secondary endpoints of objective response rate (ORR), PFS, OS, and overall safety, were assessed on the ITT population which included patients who were randomized to receive study therapy. Time-to-event analyses used the Kaplan–Meier method. Exploratory endpoints included association of outcome with tumor PD-L1 expression, TIL density and immune activation GEP, levels of circulating cytokines, as well as changes in NANO scale (22).

Statistical analysis

Cohort A included a safety lead-in with a planned deescalation of pembrolizumab dosing if more than one dose-limiting toxicity (DLT) was observed among the initial 6 patients. Upon determination of the MTD/RP2D in the safety lead-in, patients were randomized 5:3 to the combination of pembrolizumab plus bevacizumab (cohort A) or single-agent pembrolizumab (cohort B). Although a contemporaneous control arm of standard therapy was not included, success for each study arm was defined by a noteworthy improvement in primary endpoint relative to established historical benchmarks. For cohort A, accrual of 50 patients provided 87% power with an overall type I error of 0.05 to detect a PFS-6 ≤40% versus >60%. A PFS-6 rate of 40% was chosen based on phase II data for bevacizumab in the same target population (30, 31). For cohort B, accrual of 30 patients provided 84% power with an overall type I error of 0.05 to detect a PFS-6 ≤10% versus >30%. A 10% PFS-6 rate was chosen based on meta-analysis data for the same target population treated with salvage therapy, excluding antiangiogenic therapy (32).

Additional statistical methods are described in the Supplementary Data.

Results

Patients and treatment

No DLTs were noted during the safety lead-in, which established the RP2D at 200 mg of pembrolizumab i.v. every 3 weeks plus 10 mg/kg of bevacizumab biweekly. Eighty-eight patients enrolled and 80 were deemed eligible between February 2015 and June 2016. Fifty patients were treated on cohort A (including the 6 patients enrolled to the safety lead-in) and 30 on cohort B (Fig. 1). Summary demographics and baseline patient characteristics were comparable for both cohorts (Table 1).

All patients have discontinued study therapy, including 91% due to progressive disease. Six percent electively discontinued study therapy after a median of 102.6 weeks (range, 54.0–114.0). The median number of completed cycles for cohorts A and B were 3.0 and 1.5, respectively. Seventy-three patients have died while 7 remain in active follow-up.

Efficacy

With a median follow-up for cohort A of 48.6 months [95% confidence interval (CI), 48.6–not reached], PFS-6 rate was 26% (95% CI, 16.3–41.5), while median PFS and OS were 4.1 months (95% CI, 2.8–5.5) and 8.8 months (95% CI, 7.7–14.2), respectively (Fig. 2). With a median follow-up for cohort B of 49.4 months (95% CI, 48.6–not reached), the PFS-6 rate was 6.7% (95% CI, 1.7–25.4), while the median PFS and OS were 1.43 months (95% CI, 1.4–2.7) and 10.3 months (95% CI, 8.5–12.5), respectively.

Maturity of follow-up allowed for detection of a possible “tail of the curve” for OS which was not observed for either study cohort. Ten patients, all on cohort A (20.0%), achieved a radiographic response by RANO criteria including nine partial responses and one complete response. Most responses occurred in patients not receiving dexamethasone (90%), were treated at first progression (80%), or had an *IDH1* wild-type tumor (90%). Age, *MGMT* methylation, and tumor location were not associated with response. The median duration of radiographic response was 48 weeks (range, 10.9–174.4+). A summary of univariate factors and association with OS are summarized in Fig. 3. Poor survival was associated with baseline dexamethasone use (HR = 3.27; 95% CI, 1.6–6.7) for cohort A and *IDH1* mutation (HR = 6.4; 95% CI, 1.7–23.3) or lack of *MGMT* promoter methylation (HR = 3.4; 95% CI, 1.1–10.5) for cohort B. Median OS for patients on baseline dexamethasone versus not for cohort A were 6.23 months (95% CI, 4.44–NA) and 12.8 (95% CI, 8.25–17.4; $P = 0.0011$), respectively. No difference in OS ($P = 0.777$) was observed among cohort B patients when stratified by baseline dexamethasone use. A trend for worse OS was also associated with enrollment at second relapse for cohort A but did not reach statistical significance (HR = 1.86; 95% CI, 0.97–3.59).

Safety

Most treatment-related adverse events (TRAE) were low grade (Table 2A), including immune-related adverse events (AE; Table 2B). No grade 5 TRAEs occurred. For cohort A, no grade 4 TRAEs occurred and hypertension was the most common grade 3 event (20%). For cohort B, a single grade 4 TRAE occurred and was cerebral edema that developed at

tumor progression during a dexamethasone taper. Headache was the most common grade 3 event (10%). One patient who was treated on cohort B discontinued study therapy due to a TRAE (grade 2 arthralgia).

Tumor immune biomarker analyses

Tumor was available from either original diagnosis (archival; $n = 46$; 58%) or relapse prior to study enrollment ($n = 26$; 33%) for 72 patients (90%; Table 1). There was no difference in OS for patients with archival versus relapsed tumor samples ($P = 0.34$). PD-L1 expression, detected in 31 tumors (39%), correlated with PFS for cohort B but not cohort A, and was not associated with OS for either cohort (Fig. 3). TIL density, which was low (IHC score 0–1) in 35 tumors (44%) and increased (IHC score 2–3) in 35 tumors (44%), was not associated with outcome for either cohort. Immune activation GEP was evaluable for 63 tumors (79%), including 40 (80%) from cohort A and 23 (77%) from cohort B. GEP scores below −0.3 predict rapid progression with pembrolizumab whereas scores above −0.3 associate with variable progression times including a higher likelihood of longer PFS (26, 28). Of note, 66% of our study patients had a GEP score below −0.3. The median GEP score was higher among relapsed compared with archival tumors but distributions were overlapping (Supplementary Fig. S1; $P = 0.62$). Similarly, median GEP score trended higher with increased PD-L1 expression ($P = 0.11$) and TIL score ($P = 0.52$) but wide, overlapping distributions without statistical significance were observed (Supplementary Fig. S2A and S2B). For both cohorts, GEP score was not associated with ORR, PFS, or OS.

Plasma biomarker analyses

Plasma samples were available from 32 cohort A (64%) and 19 cohort B patients (63%). Posttreatment (day 84), cohort A had decreased ANG-2 and VEGF levels and increased PlGF levels, whereas no significant changes were noted for cohort B (Supplementary Table S1). Baseline VEGF was higher for cohort A versus cohort B ($P = 0.0052$). When evaluated for correlation with outcome, elevated baseline PlGF and sVEGFR1 for cohort B and posttreatment VEGF for cohort A correlated with poor OS (Supplementary Table S2).

Integration of NANO

The overall NANO compliance rate for all visits was 94%. Seven patients (9%) lacked a baseline NANO evaluation and were excluded from NANO analyses. Fifteen patients (19%) lacked an end of treatment NANO evaluation. At baseline, 35 patients (43%) had a normal NANO examination. Two NANO domains (strength and language) accounted for most changes in neurologic function during study treatment. Eighteen patients (25%, 9 per cohort) met NANO criteria for progression, including 2 without radiographic progression. Three patients (4%, all in cohort A) had NANO response and SD by MRI; these patients survived between 13.0 and 49.6 months. NANO assessment before cycle 3 correlated with RANO response ($P = 0.011$) and change in either Karnofsky performance score (KPS; $P = 0.002$) or dexamethasone requirement ($P = 0.007$). Patients with NANO progression at this time point had poorer median survival (9.57 months) compared with those without NANO progression (10.65 months), but this trend did not achieve statistical significance ($P = 0.2$).

Discussion

Glioblastoma has emerged as a profoundly immunotherapy refractory tumor that likely reflects multiple, complementary mechanisms of tumor-induced immunosuppression (3, 33–36). VEGF, which is highly expressed in glioblastoma and drives angiogenesis, also contributes to tumor immunosuppression via multiple mechanisms (7, 37–43). Clinical benefit of bevacizumab to block VEGF for glioblastoma is limited to an improvement in PFS but not OS (4–6). Our study evaluated the hypothesis that concurrent VEGF blockade may enhance the antitumor activity of pembrolizumab for patients with recurrent glioblastoma. The rationale underlying our study hypothesis is based on extensive preclinical (8–11) and clinical (12–14) data in multiple cancer types that has led to the approval of five such combinations for extracranial malignancies in the past 18 months (15–18, 44).

Our study data demonstrate that standard dosing of bevacizumab and pembrolizumab in combination failed to improve both PFS or OS relative to bevacizumab monotherapy indicating that a definitive study randomizing patients to bevacizumab versus bevacizumab plus anti-PD-1 therapy is not indicated (2). Among patients who achieved a response, however, its duration was significantly longer than that associated with bevacizumab monotherapy. This observation aligns with recently reported data indicating that response to anti-PD-1 therapy with nivolumab, although noted in a small percentage of patients with recurrent glioblastoma, was of noteworthy duration (2). In addition, our study confirmed that single-agent anti-PD-1 therapy was ineffective for recurrent glioblastoma with most patients progressing rapidly, within two cycles of therapy (2).

The observed failure of bevacizumab to improve outcome when combined with pembrolizumab in our study likely involves a number of possible explanations. First, our study results argue that the potential complementary benefit of dual blockade of VEGF and PD(L)-1 is dependent on tumor context and not applicable to glioblastoma. Second, our trial used an established, and relatively high bevacizumab dose, which is feasible due to its safety profile. However, preclinical studies suggest that lower dosing of antiangiogenics may induce normalization of the tumor vasculature with improved antitumor immune responses and survival whereas higher doses do not (9). Relatively high doses of anti-VEGF drugs can augment preexisting hypoxia within the tumor microenvironment that could worsen immunosuppression (7, 45, 46) and promote an intratumoral influx of immunosuppressive cells (47, 48). Third, antiangiogenic agents may also decrease intratumoral penetration of therapeutic mAbs such as cetuximab or trastuzumab (49), although it is unknown whether benefit of anti-PD-1 blocking antibodies requires intratumoral distribution in glioblastoma tumors. It is also possible that timing of anti-PD-1 therapy may be relevant based on two recent reports demonstrating that neoadjuvant anti-PD-1 therapy can trigger infiltration and activation of TILs in the tumor microenvironment which may enhance efficacy (50, 51).

Immune biomarkers including PD-L1 expression, TIL analysis, and immune activation GEP have been shown to predict a likelihood of therapeutic benefit with immune checkpoint blockade for some malignancies (26, 28, 52), but have not been previously evaluated prospectively in a comprehensive manner for glioblastoma. We noted that PD-L1 expression

and increased TIL infiltrate were present in nearly 50% of tumor samples in our study but did not correlate with OS. Immune activation GEP was markedly low in most glioblastoma tumors in our study and also did not correlate with outcome. Insufficient tumor material precluded evaluation of tumor mutational burden or microsatellite instability status as potential biomarkers; however, a recent retrospective study indicates that tumor hypermutation following temozolomide chemotherapy in patients with recurrent glioblastoma was not associated with improved benefit following immune checkpoint blockade (53). Our findings indicate that tumor PD-L1 expression, TIL infiltrate, and immune activation GEP may not be informative to predict therapeutic benefit of anti-PD-1 therapy for patients with recurrent glioblastoma. A limitation of these analyses is that some tumor samples were from original diagnosis; however, archival tumor samples or a mixture of archival and at-treatment samples, as was incorporated for our study, have been utilized effectively for immunocorrelative analyses in solid tumor studies (54, 55) including glioblastoma (56).

Our study also incorporated a detailed analysis of clinical and tumor-associated biomarkers. Baseline dexamethasone use (cohort A) as well as lack of tumor *MGMT* methylation and *IDH1* mutation (cohort B) correlated with poorer OS. Baseline dexamethasone use and tumor *MGMT* methylation status were also associated with outcome among patients with recurrent glioblastoma treated with nivolumab on a randomized phase III study (2). The detrimental effect of dexamethasone observed in our study may have been due to its ability to quantitatively and qualitatively decrease effector immune cells in patients with glioblastoma (57) or it may have reflected other relevant confounding factors such as a larger tumor burden. The effect of *IDH1* mutation status should be interpreted cautiously due the small number of patients with *IDH1*-mutant tumors enrolled on cohort B ($n = 4$) and that the median time from original glioblastoma diagnosis to study enrollment was much longer in the *IDH1*-mutant patients compared with wild-type (1,238 days vs. 783 days). Our analysis of plasma angiokines revealed that elevated baseline PlGF and sVEGFR1 (cohort B) and posttherapy VEGF (cohort A) levels correlated with poorer survival. These inducible factors may reflect increased tumor hypoxia at baseline (in the anti-PD-1 therapy alone group) and posttreatment (in the combination group), which can contribute to immunosuppression (45, 46). Overall our findings are limited by sample size, thus are hypothesis generating and may warrant further study.

The NANO scale was developed by a multidisciplinary panel of neuro-oncology experts as an objective, user-friendly measure of neurologic function that provides a broader neurologic assessment than measures of function traditionally utilized for patients with glioblastoma such as KPS or the mini–mental status exam (22). We demonstrated that NANO, which has not been previously evaluated in a neuro-oncology clinical trial, can be performed efficiently in a multicenter trial as indicated by an overall acceptable compliance rate (94%); however, the lack of baseline and end-of-study assessments in some patients precluded accurate assessment of whether neurologic changes correlated with outcome. Nonetheless, in general, we observed preservation of baseline neurologic status by NANO during treatment in nonradiographic progressors and neurologic decline among radiographic progressors although this was impacted by tumor anatomic location relative to functional cortex as expected.

Several limitations of this study exist. Although our study did not incorporate a standard-of-care control arm, failure to generate a signal of improved outcome relative to established, historical benchmarks, argues that further investigation of our study regimen relative to a contemporaneous control arm is not justified. We incorporated a comprehensive analysis of relevant immunocorrelative biomarkers and demonstrated that these were not informative among patients with recurrent glioblastoma; however, the sample size for these analyses was relatively small and included some archival tumor samples which may not have reflected the tumor microenvironment at the time of study therapy. Finally, missed key assessment time points including those at baseline and end of study likely diminished our ability to fully assess the utility of NANO and future studies incorporating NANO should strive to improve compliance especially at these critical points of investigational therapy.

Overall, our results do not support future studies of anti-PD-1 monotherapy or in combination with bevacizumab administered using the FDA-approved, established dosing schedule. PD-L1, TIL density, and GEP score do not appear to be useful biomarkers among patients with recurrent glioblastoma undergoing anti-PD-1 therapy. Ongoing clinical trials (NCT02336165 and NCT03452579) are assessing whether a reduced bevacizumab dosing schedule may improve outcome with anti–PD-1/PD-L1 therapy for patients with glioblastoma. Future efforts should consider targeting alternative potential modulators of immunosuppression in glioblastoma tumors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the Ben and Catherine Ivy Foundation and the Jennifer Oppenheimer Cancer Research Initiative for their support of this clinical trial, Anna Khachatryan for outstanding technical support with biomarker analyses, Kathleen Shedlock, ANP for substantial collaborative input, and Debbie Ferreira for administrative support. We gratefully acknowledge Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc. for provision of pembrolizumab and financial support of the conduct of this study. We also thank the study patients, their families, and all the participating investigators and research staff. This research was funded in part through the NIH/NCI Cancer Center Support Grant P30 CA008748. L. Nayak acknowledges support from the Leukemia and Lymphoma Society Scholar in Clinical Research Award (LLS Grant ID: 2322–19). R.K. Jain acknowledges support from the NIH (R35CA197742, R01CA208205, U01CA224173), the Ludwig Center at Harvard, the Jane’s Trust Foundation, National Foundation for Cancer Research, and the Advanced Medical Research Foundation.

We gratefully acknowledge the following organizations for funding support: The Ben and Catherine Ivy Foundation; The Jennifer Oppenheimer Cancer Research Initiative; Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc.; and NIH/NCI Cancer Center Support Grant P30 CA008748.

Authors’ Disclosures

L. Nayak reports grants from The Leukemia and Lymphoma Society and NIH/NCI outside the submitted work. K. Peters reports grants from Merck during the conduct of the study. J.L. Clarke reports other from Agios, Merck, and Novartis outside the submitted work. J.T. Jordan reports personal fees from Navio Theragnostics Inc., CereXis Inc, Health2047 Inc., and Neurofibromatosis Network, and grants from The Burke Foundation outside the submitted work. J. de Groot reports other from Carthera, HiaHe Pharma, Taiho Pharma, Janssen Global Services, Prelude Therapeutics, Merck Sharp & Dohme Co., Kiyatec, Novartis, and Debiopharm Therapeutics Inc., as well as personal fees from ResTORbio Inc., Roche, Magnolia Innovation LLC, Insightec, Syneos Health, Bioasis Technologies Inc., GenomiCare, Merk & Co., Del Mar Pharmaceuticals, and Tocagen outside the submitted work. L. Nghiemphu reports grants from NIH and Novartis outside the submitted work. T. Kaley reports nonfinancial support from NIH during the conduct of the study. H. Colman reports grants from Merck during the conduct of the study, as well as personal fees from Best Doctors/Teladoc, Karyopharm Therapeutics, Private Health, Orbus, Bayer,

Forma Therapeutics, and Adastra Pharmaceuticals outside the submitted work. S. Gaffey is employed by Foundation Medicine, Inc. J.H. Yearley reports a patent 10647771 issued to Merck Sharp & Dohme and a patent 0241115 issued to Merck Sharp & Dohme. D.G. Duda reports grants and personal fees from Bayer and BMS; grants from Exelixis; and personal fees from Surface Oncology and Simcere during the conduct of the study. R.K. Jain reports grants from NIH, National Foundation for Cancer Research, The Ludwig Center at Harvard, The Jane's Trust Foundation, and The Advanced Medical Research Foundation during the conduct of the study, as well as personal fees and other from Amgen, Chugai, Ophthotech, Merck, SPARC, SynDevRx, XTuit, Enlight, Accurius Therapeutics, Tekla Healthcare Investors, Tekla Life Sciences Investors, Tekla Healthcare Opportunities Fund, and Tekla World Healthcare Fund outside the submitted work. P.Y. Wen reports personal fees and other from Agios, AstraZeneca/Medimmune, Vascular Biogenics, and VBI Vaccines; other from Beigene, Eli Lily, Genentech/Roche, Kazia, MedicaNova, Merck, Novartis, and Oncoceutics; personal fees from Bayer, Blue Earth Diagnostics, Immunomic Therapeutics, Karyopharm, Kiyatec, Puma, Taiho, Deciphera, Tocagen, Voyager, QED, Imvax, and Elevate io; and other from Prime Oncology, UpToDate, and Elsevier during the conduct of the study. D.A. Reardon reports other from Merck during the conduct of the study; Dr. Reardon also reports other from Acerta Pharmaceuticals, Incyte, Omnio, and Tragara; personal fees and other from Agenus, Celldex, EMD Serono, and Inovio; and personal fees from Abbvie, Advantagene, Amgen, Bayer, Bristol-Myers Squibb, DelMar, Genentech/Roche, Imvax, Medicenna, Merck, Merck KGaA, Monteris, Novocure, Oncours, Oxigene, Regeneron, Stemline, Sumitono Dainippon Pharma, Taiho Oncology Inc, and Boston Biomedical outside the submitted work. No disclosures were reported by the other authors.

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Translational Relevance

VEGF promotes angiogenesis in glioblastoma but there is accumulating evidence that also implicates VEGF as a mediator of tumor-induced immunosuppression. Preclinical and clinical data in some cancers support a hypothesis that dual VEGF and immune checkpoint blockade may enhance antitumor immune responses and has led to approval of five such combinations for extracranial malignancies. Our randomized phase II trial in patients with recurrent glioblastoma demonstrates that bevacizumab administered using an established dosing schedule fails to improve outcome when added to programmed-death 1 (PD-1) blockade, which is ineffective as monotherapy. In addition, we show that tumor programmed death-ligand 1 expression, tumor-infiltrating lymphocyte density, and immune gene activation score do not identify patients more likely to benefit from anti-PD-1 therapy. Finally, better assessment of quality of life including preservation of neurologic function is needed for patients with glioblastoma. We demonstrate the feasibility of integrating the neurologic assessment in neuro-oncology scale to prospectively assess neurologic function in a clinical trial.

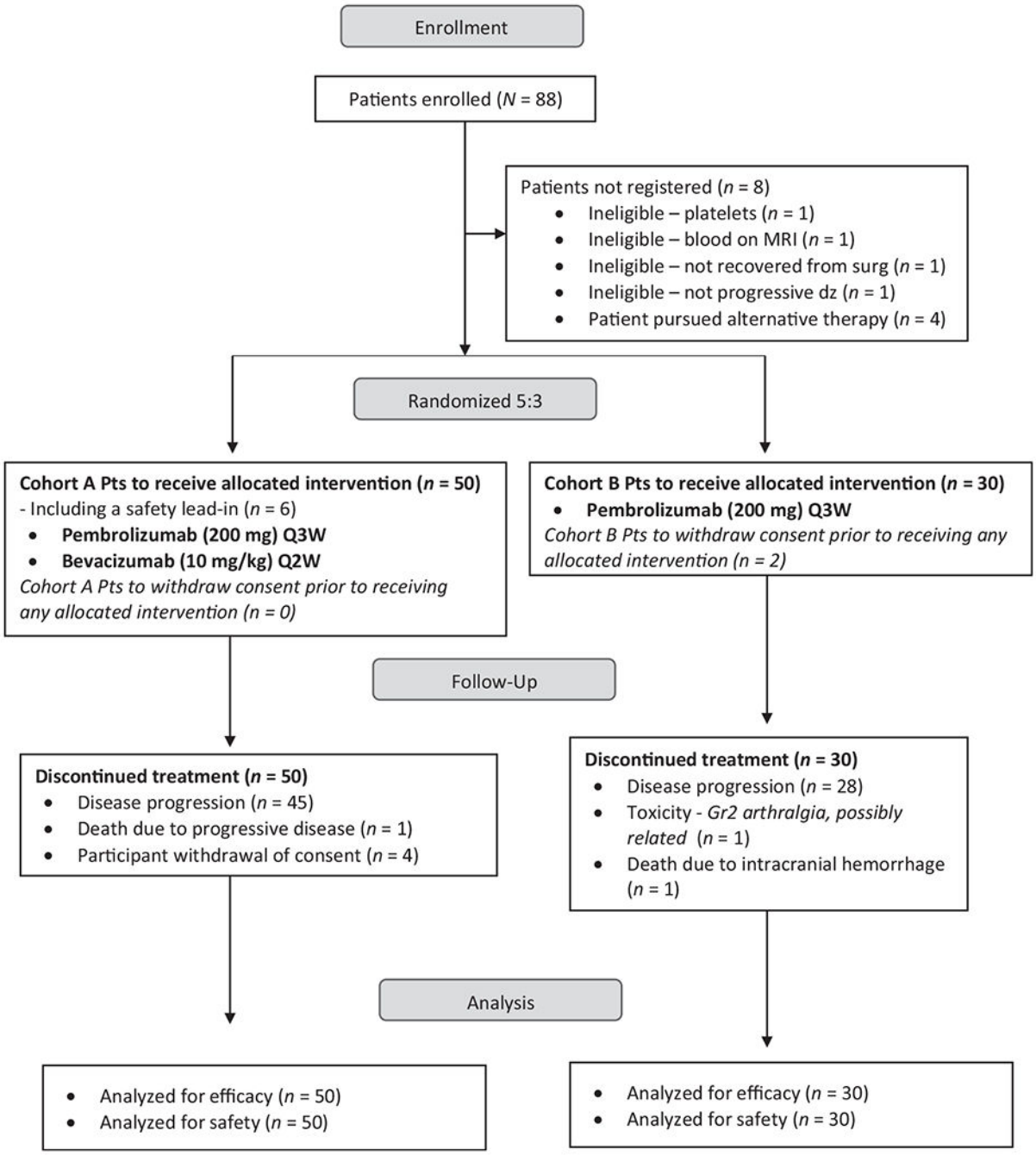

Figure 1.

Study profile. CONSORT diagram showing the number of patients who were enrolled, treated with pembrolizumab plus bevacizumab (cohort A) or pembrolizumab (cohort B), discontinued treatment, and were analyzed for efficacy and safety.

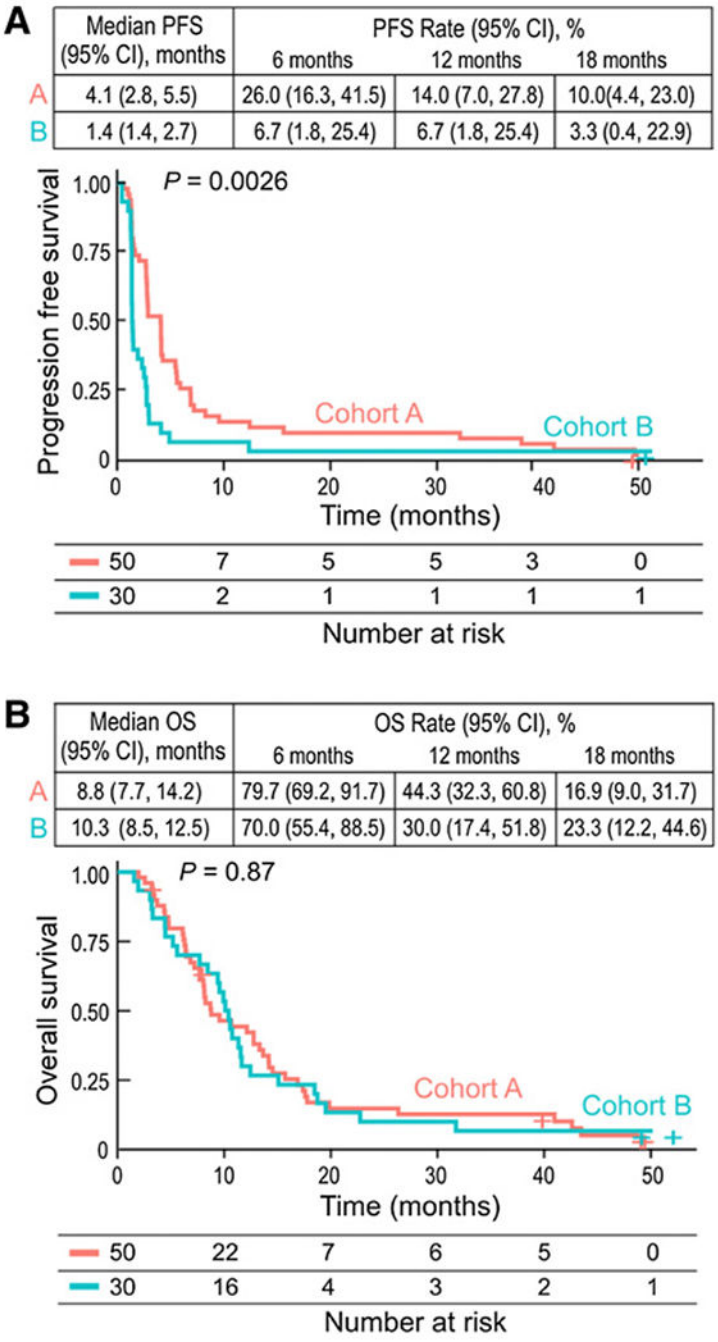

Figure 2.

PFS and OS in all patients. **A,** The number of events; median PFS; PFS rates at 6, 12, and 18 months; and the Kaplan–Meier curve for PFS in all patients treated with nivolumab or nivolumab plus bevacizumab. **B,** The number of events; median OS; OS rates at 6, 12, and 18 months; and the Kaplan–Meier curve for OS per investigator assessment in patients treated with nivolumab or nivolumab plus bevacizumab. Symbols indicate censored observations. HRs and CIs were estimated using a Cox proportional hazards model. HR, hazard ratio; OS, overall survival; PFS, progression-free survival.

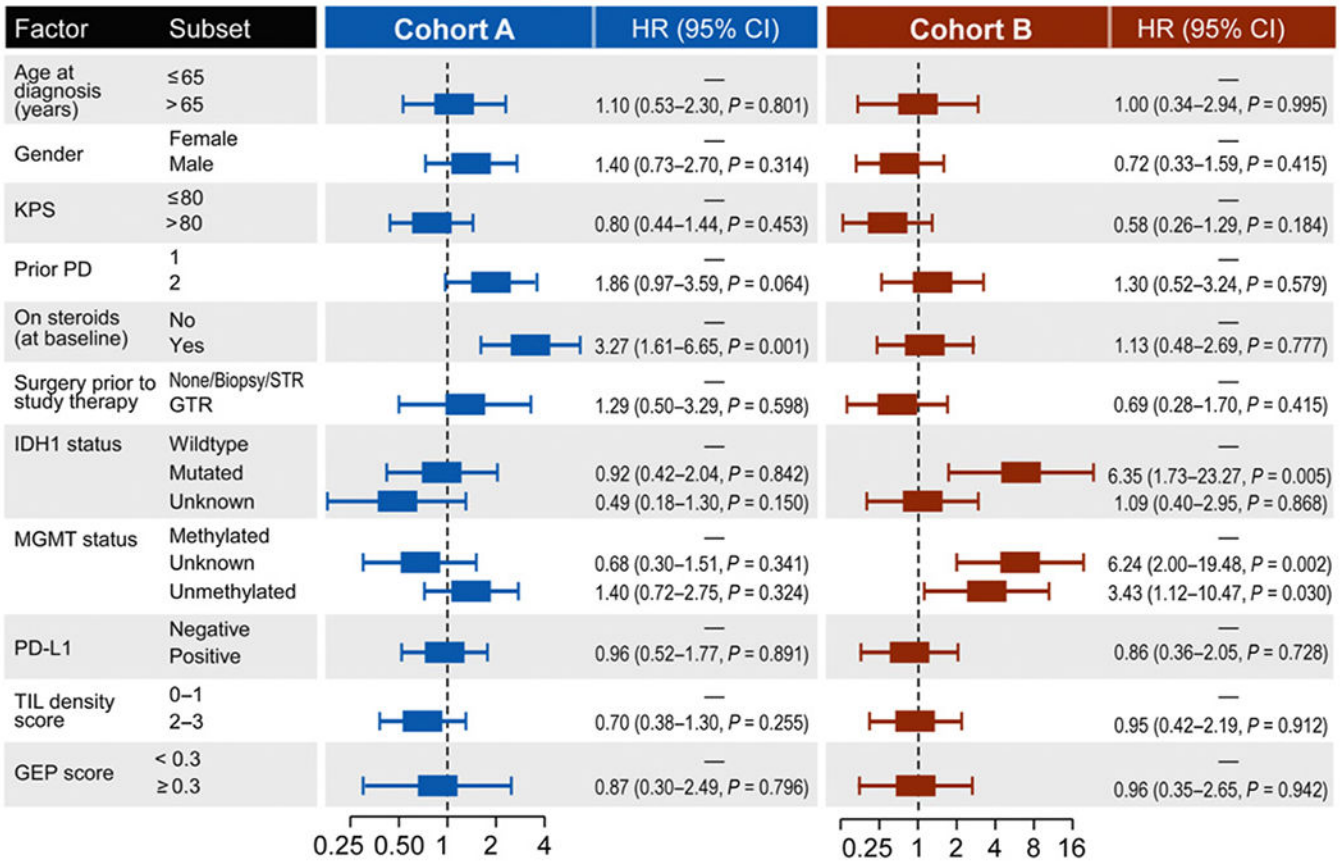

Figure 3.

OS in patient subgroups. Forest plot of univariate HR for death of patient and tumor characteristics in the analysis of treatment effect in patient subgroups by cohort.

Table 1.

Patient characteristics and study disposition[a].

Characteristic	Cohort A: P+B (*n* = 50)	Cohort B: P alone (*n* = 30)	Total (*n* = 80)
Median age (years, IQR)	52 (42–59)	55 (42–62)	53 (42–60)
<65 years (%)	41 (82.0)	26 (86.7)	67 (83.8)
≥65 years (%)	9 (18.0)	4 (13.3)	13 (16.2)
Gender, male (%)	35 (70.0)	19 (63.3)	54 (67.5)
Diagnosis at enrollment			
GBM	50 (100)	30 (100)	80 (100)
GS	0 (0)	0 (0)	0 (0)
KPS (%)			
90–100	26 (52.0)	13 (43.4)	39 (48.8)
80	17 (34.0)	16 (53.3)	33 (41.2)
70	7 (14.0)	1 (3.3)	8 (10.0)
# prior PD			
1	35 (70.0)	24 (80.0)	59 (73.8)
2	15 (30.0)	6 (20.0)	21 (26.2)
Resection prior to study			
Gross total	6 (12.0)	7 (23.3)	13 (16.2)
Subtotal	2 (4.0)	2 (6.7)	4 (5.0)
Biopsy	1 (2.0)	5 (16.7)	6 (7.5)
Not done	41 (82.0)	16 (53.3)	57 (71.2)
Initial glioma diagnosis			
Grade II glioma	1 (2.0)	1 (3.3)	2 (2.5)
Grade III glioma	2 (4.0)	5 (16.7)	7 (8.8)
Grade IV glioma	47 (94.0)	24 (80.0)	71 (88.8)
Dexamethasone use			
At study entry	12 (24.0)	7 (23.3)	19 (23.8)
Required initiation after study start	5/38 (13.2)	5/23 (21.7)	10/61 (16.4)
Required increase after study start	0/12 (0)	1/7 (14.3)	1/19 (5.3)
Mean time from initial GBM diagnosis to enrollment in weeks (SD)	75.9 (70.9)	85.0 (78.2)	79.3 (73.4)
MGMT status			
Methylated	20 (40.0)	9 (30.0)	29 (36.2)
Unmethylated	17 (34.0)	10 (33.3)	27 (33.8)
Unknown	13 (26.0)	11 (36.7)	24 (30.0)
IDH1 status			
Mutant	8 (16.0)	4 (13.3)	12 (15.0)
Wild-type	35 (70.0)	21 (70.0)	56 (70.0)
Unknown	7 (14.0)	5 (16.7)	12 (15.0)
Tumor source for immunocorrelatives			
Archival	31 (62.0)	15 (50.0)	46 (57.5)

Characteristic	Cohort A: P+B (*n* = 50)	Cohort B: P alone (*n* = 30)	Total (*n* = 80)
Relapse	15 (30.0)	11 (36.7)	26 (32.5)
None	4 (8.0)	4 (13.3)	8 (10.0)
PD-L1 expression			
Present	22 (44.0)	9 (30.0)	31 (38.8)
Absent	23 (46.0)	16 (53.3)	39 (48.8)
Not done	5 (10.0)	5 (16.7)	10 (12.5)
TIL density			
0–1	25 (50.0)	10 (33.3)	35 (43.8)
2–3	20 (40.0)	15 (50.0)	35 (43.8)
Not done	5 (10.0)	5 (16.7)	10 (12.5)
Inflammatory gene expression signature			
≥ −0.3	4 (8.0)	6 (20.0)	10 (12.5)
> −0.3	36 (72.0)	17 (56.7)	53 (66.3)
Not done	10 (20.0)	7 (23.3)	17 (21.3)
# study cycles completed			
Median (IQR)	3.0 (2.0–4.0)	1.5 (1.0–3.0)	2.0 (1.0–4.0)
Reason off study			
PD	45 (90.0)	28 (93.3)	73 (91.3)
Toxicity	0 (0)	1 (3.3)	1 (1.2)
Consent withdrawal	4 (8.0)	0 (0)	4 (4.9)
Death	1 (2.0)	1 (3.3)	2 (2.4)
Current status			
Dead	45 (90.0)	28 (93.3)	73 (91.2)
Alive	5 (10.0)	2 (6.7)	7 (8.8)

Abbreviations: B, bevacizumab; GBM, glioblastoma; GS, gliosarcoma; IDH1, isocitrate dehydrogenase 1; IQR, interquartile range; KPS, Karnofsky performance score; MGMT, methylguanine methyltransferase; P, pembrolizumab; PD, progressive disease; PD-L1, programmed death-ligand 1; SD, standard deviation; TIL, tumor-infiltrating lymphocytes.

[a]Percents are correct for denominators in each cell.

Table 2A.

Grade ≥2 adverse events at least possibly related to study therapy in ≥5% of patients by cohort.

	Cohort A: P+B (*n* = 50)				Cohort B: P alone (*n* = 30)			
Grade	**2 (%)**	**3 (%)**	**4 (%)**	**Total (%)**	**2 (%)**	**3 (%)**	**4 (%)**	**Total (%)**
Event								
Hypertension	15 (30)	10 (20)	0	25 (50)	0	0	0	0
Headache	4 (8)	4 (8)	0	8 (16)	6 (20)	3 (10)	0	9 (30)
Fatigue	9 (18)	0	0	9 (18)	5 (17)	0	0	5 (17)
Infection	6 (12)	1 (2)	0	7 (14)	0	0	0	0
Proteinuria	7 (14)	0	0	7 (14)	0	0	0	0
Arthralgia	4 (8)	0	0	4 (8)	1 (3)	0	0	1 (3)
Diarrhea	2 (4)	1 (2)	0	3 (6)	1 (3)	0	0	1 (3)
Seizures	3 (6)	0	0	3 (6)	2 (7)	0	0	2 (7)
Anorexia	1 (2)	0	0	1 (2)	2 (7)	0	0	2 (7)
Hyperglycemia	2 (4)	0	0	2 (4)	1 (3)	1 (3)	0	2 (7)
Lymphopenia	2 (4)	0	0	2 (4)	2 (7)	0	0	2 (7)

Abbreviations: B, bevacizumab; *n*, number; P, pembrolizumab.

Table 2B.

Immune-related adverse events at least possibly related to study therapy.

		A: P+B	B: P alone	A: P+B	B: P alone	A: P+B	B: P alone	A: P+B	B: P alone
Event	**Cohort Grade**	**1**		**2**		**3**		**4**	
Abdominal pain		3 (6%)	0	1 (2%)	0	0	0	0	0
Alkaline phosphatase elevation		2 (4%)	1 (3%)	0	0	0	0	0	0
ALT elevation		7 (14%)	1 (3%)	0	0	1 (2%)	0	0	0
AST elevation		7 (14%)	0	0	0	1 (2%)	0	0	0
Arthralgia		6 (12%)	0	4 (8%)	1 (3%)	0	0	0	0
Bilirubin increase		1 (2%)	0	1 (2%)	0	0	0	0	0
Cerebral edema		0	0	0	0	0	0	0	1 (3%)
Colitis		0	0	1 (2%)	0	0	0	0	0
Conjunctivitis		1 (2%)	1 (3%)	0	0	0	0	0	0
Creatinine increase		2 (4%)	0	0	0	0	0	0	0
Diarrhea		7 (14%)	0	2 (4%)	1 (3%)	0	0	0	0
Dyspnea		1 (2%)	0	1 (2%)	0	0	0	0	0
Hyperglycemia		5 (10%)	0	2 (4%)	1 (3%)	0	1 (3%)	0	0
Hyperthyroidism		4 (8%)	0	1 (2%)	0	0	0	0	0
Hypothyroidism		3 (6%)	1 (3%)	2 (4%)	1 (3%)	0	0	0	0
Infusion reaction		0	0	1 (2%)	0	0	0	0	0
Myalgia		6 (12%)	0	1 (2%)	0	0	0	0	0
Rash		5 (10%)	1 (3%)	1 (2%)	0	0	0	0	0

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; B, bevacizumab; P, pembrolizumab.