Original Article

Treatment With Pembrolizumab in Programmed Death Ligand 1–Positive Recurrent Glioblastoma: Results From the Multicohort Phase 1 KEYNOTE-028 Trial

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BACKGROUND: Current treatments for recurrent glioblastoma offer limited benefit. The authors report the antitumor activity and safety of the anti-programmed death 1 (anti–PD-1) immunotherapy, pembrolizumab, in programmed death ligand 1 (PD-L1)–positive, recurrent glioblastoma. **METHODS:** Adult patients with PD-L1–positive tumors were enrolled in the recurrent glioblastoma cohort of the multicohort, phase 1b KEYNOTE-028 study (ClinicalTrials.gov identifier, NCT02054806) and received pembrolizumab 10 mg/kg every 2 weeks for up to 2 years. The primary endpoint was investigator-assessed overall response rate according to the Response Evaluation Criteria in Solid Tumors, version 1.1. Archival tumor samples were assessed for PD-L1 expression levels (prospectively) and T-cell–inflamed gene expression profile score (retrospectively). **RESULTS:** After a median follow-up of 14 months (range, 2-55 months) among the 26 enrolled patients, the overall response rate was 8% (95% CI, 1%-26%). Two partial responses, lasting 8.3 and 22.8 months, occurred. Progression-free survival (median, 2.8 months; 95% CI, 1.9-8.1 months) rate at 6 months was 37.7%, and the overall survival (median, 13.1 months; 95% CI, 8.0-26.6 months) rate at 12 months was 58%. Correlation of therapeutic benefit to level of PD-L1 expression, gene expression profile score, or baseline steroid use could not be established. Treatment-related adverse events occurred in 19 patients (73%), and 5 patients experienced grade 3 or 4 events (there were no grade 5 events). Immune-mediated adverse events and infusion reactions occurred in 7 patients (27%). **CONCLUSIONS:** Pembrolizumab monotherapy demonstrated durable antitumor activity in a subset of patients with manageable toxicity in this small, signal-finding, recurrent glioblastoma cohort. Future studies evaluating rationally designed pembrolizumab combination regimens may improve outcomes in patients with recurrent glioblastoma. ***Cancer*** **2021;127:1620-1629**.

KEYWORDS: glioblastoma, immunotherapy, pembrolizumab, programmed death ligand 1, treatment outcomes.

INTRODUCTION

Glioblastoma, the most common and lethal primary malignant brain tumor in adults,[1] frequently recurs because of tumor heterogeneity, rapid proliferation, and infiltrative lesions.[2,3] Initial treatment in patients with glioblastoma—maximal resection followed by radiotherapy and temozolomide—is associated with 14.6-month median overall survival (OS).[4] Randomized phase 3 studies have shown no improvement in OS after dose-dense temozolomide,[5] or with the vascular endothelial growth factor inhibitor bevacizumab plus temozolomide and radiotherapy.[6,7] Adding tumor-treating fields to temozolomide resulted in a 4.9-month survival benefit.[8] Nitrosoureas or bevacizumab provide median survival of 6 to 9 months for recurrent disease[9-12]; thus clinical trials are the preferred option for eligible patients with recurrent glioblastoma.[13]

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Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA's data sharing policy, including restrictions, is available at http://engagezone.msd.com/ds_documentation.php. Requests for access to the clinical study data can be submitted through the EngageZone site or by email to dataaccess@merck.com.

Additional supporting information may be found in the online version of this article.

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Central nervous system immune-privilege limits the movement of immune cells and drug compounds across the blood-brain barrier[2,3]; however, studies in animal models have shown that brain-derived tumor antigens can prompt a T-cell immune response in central nervous system–draining cervical lymph nodes,[14] with migration of effector T cells and tumor destruction.[15] Glioblastomas express programmed death ligand 1 (PD-L1),[16-20] allowing them to evade immune response via the programmed death 1 (PD-1) pathway.[18,21] Immunotherapies that block the interaction between PD-1 and its ligand PD-L1 help to restore native antitumor immune responses, with encouraging data observed in multiple cancers.[18,21] Orthotopic murine models of glioblastoma have demonstrated significantly prolonged OS with anti−PD-1 antibody plus radiotherapy[22] and with anti−PD-1 monotherapy compared with isotype antibody controls.[23] Moreover, high tumor PD-L1 expression in patients with glioblastoma has been correlated with a poor prognosis,[17,24] whereas PD-L1 expression in tumor-adjacent tissue correlated with better outcomes.[17]

Pembrolizumab, a humanized anti−PD-1 antibody, has shown robust antitumor activity with a favorable safety profile and received US Food and Drug Administration approval as monotherapy across several tumor types, including treatment-refractory, metastatic, microsatellite instability (MSI)-high cancer.[25] Efficacy and safety results with pembrolizumab monotherapy in the glioblastoma cohort of the multicohort KEYNOTE-028 study (ClinicalTrials.gov identifier NCT02054806) are reported here.

MATERIALS AND METHODS

Study Design and Patients

The international, single-arm KEYNOTE-028 trial evaluated the efficacy and safety of pembrolizumab across 20 PD-L1−positive solid tumor cohorts, as previously described.[26] The glioblastoma cohort enrolled patients aged ≥18 years who had histologically confirmed disease that was recurrent and failed prior standard therapy or for which no standard therapy existed. Patients may have received ≥1 prior treatment (except bevacizumab), and must have had a PD-L1−positive tumor, measurable disease according to Response Evaluation Criteria in Solid Tumors, version 1.1 (RECIST v1.1), an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, and adequate organ function. Exclusion criteria included the following: immunodeficiency; immunosuppressive therapy within 7 days of starting treatment (≤4 mg concomitant dexamethasone or equivalently dosed steroids were permitted); active autoimmune disease requiring systemic treatment in the previous 2 years; prior anticancer monoclonal antibody within 4 weeks, chemotherapy or targeted small molecule therapy within 2 weeks, or radiation therapy within 3 months; prior treatment with any immune checkpoint inhibitor; known additional malignancy or an active infection requiring treatment, and carcinomatous meningitis. All patients provided written informed consent before participation. An independent institutional review board/ethics committee approved the protocol (MK-3475-028) (see Supporting Materials) at each study site, and the trial was conducted in compliance with Good Clinical Practice guidelines and the Declaration of Helsinki.

Patients received intravenous pembrolizumab 10 mg/kg every 2 weeks (this was the highest dose tested in KEYNOTE-001 [ClinicalTrials.gov identifier NCT01295827] without dose-limiting toxicities).[27] Treatment continued for up to 24 months, until documented disease progression (PD), unacceptable adverse events (AEs), intercurrent illness precluding treatment, consent withdrawal, pregnancy, noncompliance, or administrative reasons.

Endpoints

The primary endpoint was the overall response rate (ORR) (the proportion of patients with a complete response [CR] or a partial response [PR] according to RECIST v1.1 by investigator review). Secondary endpoints included safety, duration of response (DOR) (the time from the first documented PR or CR to PD), progression-free survival (PFS) (the time from baseline to the earlier of either first documented PD or death from any cause), and OS. Exploratory endpoints included response evaluation by central review according to Response Assessment in Neuro-Oncology (RANO) criteria.[28] When possible, the best overall response (BOR) was determined retrospectively by central review according to RECIST v1.1.

Assessments

Tumor response was assessed by computed tomography or magnetic resonance imaging every 8 weeks for the first 6 months of treatment and every 12 weeks thereafter, with assessments confirmed ≥4 weeks after the first documented response or PD. Clinically stable patients with evidence of progression (eg, symptoms absent, slow

disease progression, ECOG performance status maintained) could continue treatment until radiographically confirmed PD; if confirmed, the date of PD was backdated to the first documented PD. Safety was evaluated from baseline through 30 days after treatment discontinuation (90 days for serious AEs and events of clinical interest). AEs were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0.

QualTek Molecular Laboratories evaluated archived or newly obtained formalin-fixed, paraffin-embedded tumor samples using the 22C3 PD-L1 antibody clone (Merck & Co., Inc., Kenilworth, NJ, USA) and a laboratory-developed prototype immunohistochemical assay. PD-L1 positivity was defined as membranous PD-L1 expression in ≥1% of tumor and associated inflammatory cells or positive staining in stroma (band-like/lichenoid staining pattern at the interface of tumor and stroma), as previously described (see Supporting Fig. 1).[29,30]

RNA samples were extracted from pretreatment formalin-fixed, paraffin-embedded slides and analyzed retrospectively on the NanoString nCounter system to assess tumor T-cell–inflamed gene expression profiles (GEPs). GEP scores were calculated as a weighted sum using normalized expression values for 18 inflammatory genes (*CCL5*, *CD27*, *CD274* [*PD-L1*], *CD276* [*B7-H3*], *CD8A*, *CMKLR1*, *CXCL9*, *CXCR6*, *HLA.DQA1*, *HLA.DRB1*, *HLA.E*, *IDO1*, *LAG3*, *NKG7*, *PDCD1LG2* [*PD-L2*], *PSMB10*, *STAT1*, and *TIGIT*) related to antigen presentation, chemokine expression, cytolytic activity, and adaptive immune resistance, as previously described.[31]

Statistical Analysis

The primary endpoint of ORR was evaluated separately in each KEYNOTE-028 cohort by sequential monitoring after ≥6 patients had undergone ≥1 postbaseline imaging assessment. A sample size of 22 evaluable patients per cohort was planned, as determined using the binomial exact method, with power set at 80% to detect an ORR >10% and 1-sided α set at .08. As prespecified in the protocol, all patients with measurable disease per RECIST v1.1 at baseline who received ≥1 pembrolizumab dose were included in efficacy and safety analyses; those with missing response data were counted as nonresponders. Response rates were provided as point estimates, with 95% CIs based on the binomial exact method and *P* values for the ORR based on the exact binomial distribution. DOR, PFS, and OS were estimated using the Kaplan-Meier method.

RESULTS

Patients

Between March 2014 and February 2015, 111 patients with recurrent glioblastoma were screened. Among them, 63 of 102 patients (62%) who were screened for tumor PD-L1 expression had PD-L1–positive tumors, of whom 26 met remaining eligibility criteria and were enrolled and treated (see Supporting Fig. 2). At baseline, the median patient age was 55.5 years (range, 33-76 years), and 54% of patients were men (Table 1). All patients had received prior chemotherapy, and most (25 of 26 patients) had received prior radiation therapy. Most patients had received ≥1 prior treatment (excluding adjuvant or neoadjuvant therapy): 20 (77%) had received prior first-line treatment (temozolomide [n = 18], carmustine [n = 1], procarbazine [n = 1], vincristine [n = 1], and/or unspecified investigational treatment [n = 2]); and 11 (42%) had received prior second-line or later treatment (temozolomide [n = 8], lomustine [n = 2], buparlisib [n = 1], and/or tyrosine kinase inhibitor [n = 1]). At baseline, 7 patients (27%) were receiving concomitant steroid for the management of cerebral edema-associated symptoms. All patients provided archived tumor samples. Retrospectively collected data on tumor promoter O^6-methylguanine-DNA methyltransferase (MGMT) status, as available per local testing, indicated that 7 patients had tumors with methylated or partially methylated MGMT, and 4 patients had tumors with unmethylated MGMT. Comparable information on *IDH* mutational status was not available.

As of January 23, 2019 (data cutoff), the median follow-up was 14.0 months (range, 2.3-55.1 months). Twenty-four patients (92%) discontinued treatment primarily because of PD (n = 16; 62%) (see Supporting Fig. 2), whereas 2 patients (8%) had completed pembrolizumab treatment. After pembrolizumab treatment, 7 patients (27%) underwent tumor resections (1 underwent surgery while enrolled in the trial, and 2 underwent complete tumor resections), 12 (46%) received subsequent anticancer drug therapies, and 1 (4%) received radiotherapy for refractory disease during pembrolizumab treatment.

Efficacy

The ORR by investigator assessment according to RECIST v1.1 (primary endpoint) was 8.0% (95% CI, 1.0%-26.0%) (see Supporting Table 1), including 2 PRs (see imaging example in Figure 1) and no CRs. The 2 PRs were also observed when evaluated by RANO criteria

TABLE 1. Baseline Demographics and Disease Characteristics, N = 26

Characteristic	No. of Patients (%)
Age: Median [range], y	55.5 [33-76]
Sex	
Men	14 (53.8)
Women	12 (46.2)
Race	
White	19 (73.1)
Asian	3 (11.5)
Missing	4 (15.4)
ECOG performance status	
0	12 (46.2)
1	14 (53.8)
Time from primary diagnosis[a]: Median [range], mo	21.8 [7.6-305.3]
No. of recurrences[b]	
1	18 (69.2)
2	6 (23.1)
≥3	1 (3.8)
Prior lines of therapy[c]	
1	10 (38.5)
2	13 (50.0)
3	3 (11.5)
Prior therapies[d]	
Chemotherapy	26 (100.0)
Temozolomide	25 (96.2)
Investigational therapy[e]	3 (11.5)
Supportive therapy	1 (3.8)
Radiation	25 (96.2)
Concomitant steroid therapy[f]	
None	19 (73.1)
≤2 mg	2 (7.7)
>2 mg	5 (19.2)
MGMT status[g]	
Methylated	6 (23.1)
Partially methylated	1 (3.8)
Unmethylated	4 (15.4)
Unknown	2 (7.7)
Not done	13 (50.0)
MGMT assessment method[g]	
qPCR	6 (23.1)
Immunohistochemistry	1 (3.8)
Methylation-specific PCR	2 (7.7)
Unknown	4 (15.4)
Not done	13 (50.0)

Values are n (%) unless otherwise noted.
Abbreviations: ECOG, Eastern Cooperative Oncology Group; MGMT, O^6-methylguanine-DNA methyltransferase; PCR, polymerase chain reaction; qPCR, quantitative polymerase chain reaction.
[a]The exact date of primary diagnosis was unknown in 3 patients.
[b]One patient had an unknown number of recurrences.
[c]Adjuvant/neoadjuvant therapy was regarded as 1 line of prior therapy.
[d]Patients could have received ≥1 type of prior therapy.
[e]This includes 1 patient who received tyrosine kinase inhibitors.
[f]Steroid therapies include dexamethasone or an equivalently dosed steroid.
[g]MGMT data were collected retrospectively from the study sites based on local testing.

(exploratory endpoint).[28] When assessed by central review (exploratory endpoint), there were 3 PRs and no CRs (ie, 1 additional patient had a PR by central review).

The disease control rate (ie, the percentage of patients with a PR/CR or with stable disease [SD] ≥6 months), determined by investigator review, was 36.0% (95% CI, 18.0%-57.5%). PD was the BOR in 11 patients (44.0%; 95% CI, 24.4%-65.1%) according to investigator review. The time to response for 1 of the 2 patients who had a PR, according to investigator review, was 8.6 months, and the DOR was 8.3 months. For the second patient, the response occurred at 20.3 months, and the DOR was 22.8 months. Four patients remained on treatment beyond 1 year (Fig. 2A). The time to response for the additional patient who had a PR by central review was 3.7 months, and the DOR was 7.4 months.

In 1 patient, tumor size initially increased, then decreased according to investigator review (Fig. 2B). This patient had a BOR of SD with PFS of 8.1 months. According to central review, 1 additional patient had an initial increase in tumor lesions from baseline and a subsequent decrease, with a BOR of SD and PFS of 22.5 months.

Events of PD or death occurred in 24 patients (96%), and the median PFS was 2.8 months (95% CI, 1.9-8.1 months). The PFS rates at 6, 12, and 24 months were 37.7%, 16.8%, and 8.4%, respectively (Fig. 3A). At the time of the current analysis, 23 patients (88%) had died, and the median OS was 13.1 months (95% CI, 8.0-26.6 months). The 6-month, 12-month, and 24-month OS rates were 75.8%, 58.0%, and 31.2%, respectively (Fig. 3B).

Exploratory biomarker assessments in available tumor samples (all archival) indicated that baseline tumor PD-L1 expression in the 2 responders was 1% and 100%. One response occurred among 10 patients who were assessed for MSI; none had MSI-high status. Among 22 patients who were evaluated using the 18-gene GEP panel, GEP scores were very low; nonetheless, 5 patients (23%) achieved clinical benefit, including 4 who had SD for ≥200 days and 1 who had a PR for ≥500 days (see Supporting Fig. 3). Retrospective assessments indicated methylated MGMT tumor status in 1 patient who had a confirmed response (according to investigator and central reviews). Tumor samples for the remaining responders were not tested for MGMT methylation status.

A post hoc data review indicated no association between treatment outcomes and baseline dexamethasone use (see Supporting Table 2). Although neither of the 2 patients who had a PR was receiving dexamethasone at baseline, 1 of these patients received 21 days of concomitant prednisone treatment (equivalent to 3-9 mg dexamethasone) between 5.8 and 6.5 months from baseline and had subsequent treatment response at 20.3 months. Although this data review was limited by small sample size, baseline dexamethasone use did not appear to correlate with BOR of either SD or PD (see

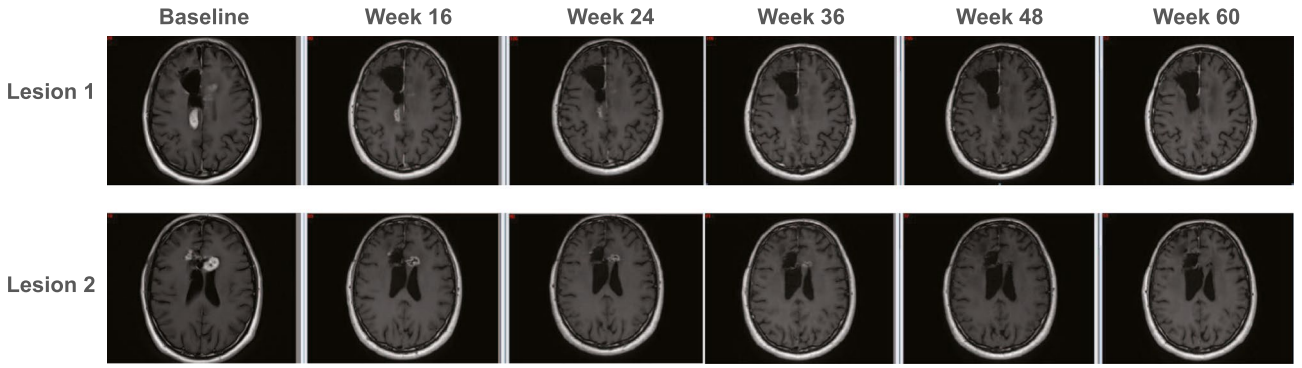

Figure 1. Target lesion changes from baseline through 60 weeks are illustrated in a patient who had a confirmed partial response based on Response Evaluation Criteria in Solid Tumors, version 1.1, by investigator review.

Supporting Table 2). Similarly, no effect of baseline absolute lymphocyte count (ALC) was observed, as baseline ALC values (0.8×10^9/L and 1.15×10^9/L) in the 2 responders were similar to the median baseline ALC in the glioblastoma cohort overall (0.94×10^9/L; range, 0.3-2.41×10^9/L).

Safety

Of 26 patients who received pembrolizumab, 19 (73%) experienced ≥1 treatment-related AE, and no AEs led to treatment discontinuation or death (Table 2). The most common treatment-related AEs were fatigue and rash (6 patients each [23%]). Serious treatment-related AEs occurred in 4 patients (15%), and 5 patients (19%) had grade 3 or 4 treatment-related AEs. No drug-related worsening of neurologic deficits or cerebral edema were recorded in this cohort. Seven patients (27%) had immune-mediated AEs and infusion reactions, irrespective of association with treatment (Table 2). Of these, 1 patient had a grade ≥3 event (grade 3 colitis that resolved without treatment interruption).

DISCUSSION

The 8% ORR according to RECIST v1.1 (or RANO) and the durable therapeutic benefit observed in this signal-finding glioblastoma cohort of KEYNOTE-028 suggest that pembrolizumab monotherapy had antitumor activity in a small proportion of patients with recurrent glioblastoma, for whom treatment options are limited. Meta-analyses evaluating data from several hundred patients with recurrent glioblastoma have demonstrated that salvage therapies typically offer limited therapeutic benefit and do not improve OS beyond a median of 9 months, setting a very low standard for anticipated outcomes.[9,32] Results from this small, single-arm study demonstrating a 37.7% PFS rate at 6 months, median OS of 13.1 months, and DOR >8 months indicate preliminary evidence of antitumor activity with pembrolizumab monotherapy in some patients with recurrent glioblastoma. The ORR was similar to that observed in a phase 1a study of the anti–PD-L1 agent atezolizumab in recurrent glioblastoma (6%; regardless of PD-L1 expression).[33] In addition, in a pooled analysis of 559 patients from 11 studies evaluating angiogenesis inhibitors in recurrent glioblastoma, the 1-year OS rate was 29% in comparison to the 1-year OS rate in the current study of 58% (approximately 2-fold higher); however, it is unclear how baseline characteristics between study populations may have differed, thus cross-study comparisons should be made cautiously.[32] Moreover, the safety profile in this cohort was consistent with that previously observed for pembrolizumab in other tumor types.[26,34-37] No deaths due to AEs occurred. There were also few treatment-related AEs of grade 3 or 4 severity, and none resulted in treatment discontinuation, demonstrating the tolerability of pembrolizumab.

On the basis of preclinical data demonstrating therapeutic benefit with anti–PD-1 antibodies in syngeneic, orthotopic, murine glioblastoma models[22,23] and the association between PD-L1 expression and clinical outcomes observed with pembrolizumab in other tumor types,[38] for the current study, we enrolled only patients who had PD-L1–positive tumors. The proportion of PD-L1–positive glioblastoma tumors reported varies in the literature from 35% to 100%.[16,20,39] Approximately 60% of patients who were screened for the KEYNOTE-028 glioblastoma cohort had PD-L1–positive tumors, suggesting that the PD-1 pathway may offer a potential therapeutic target in patients with recurrent glioblastoma. However,

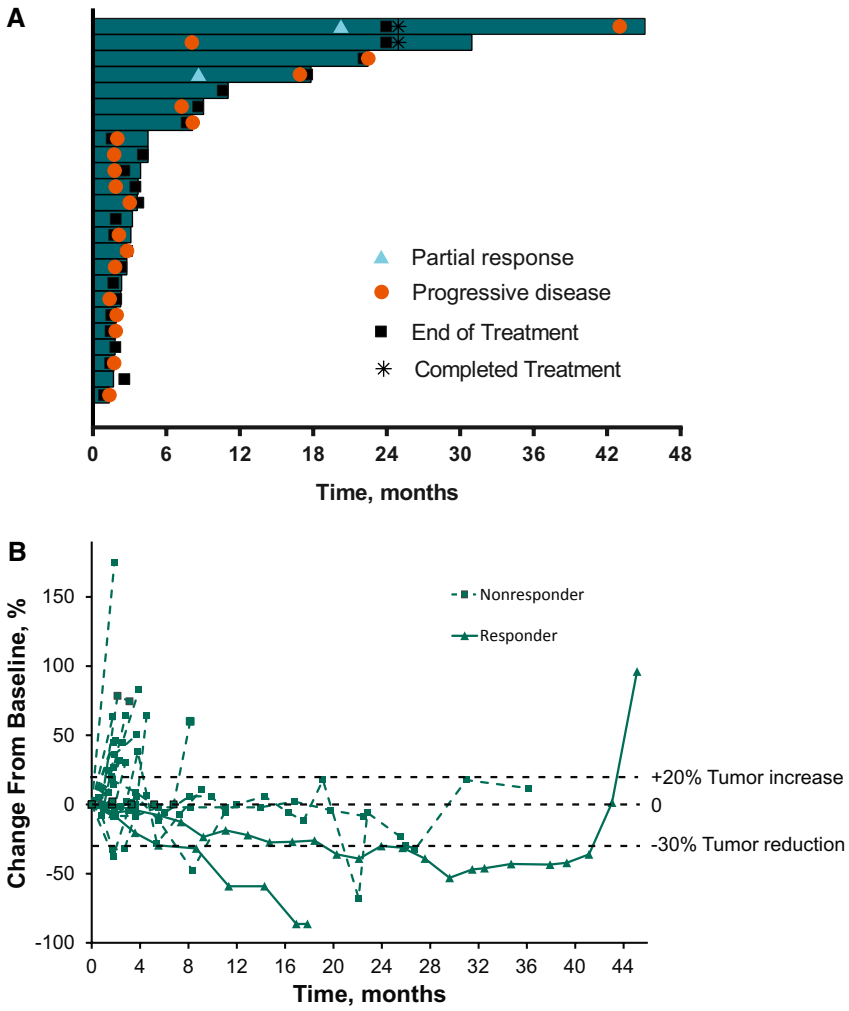

Figure 2. Treatment exposure and responses are illustrated in individual patients based on Response Evaluation Criteria in Solid Tumors, version 1.1, by investigator review. Only patients who had ≥1 evaluable postbaseline tumor assessment are included (n = 24). (A) Treatment and response duration, including confirmed and unconfirmed responses, are illustrated. Horizontal bars indicate the duration of follow-up. (B) Longitudinal percentage change from baseline in tumor size are illustrated.

because tumor PD-L1 expression was required for enrollment, it is not possible to draw conclusions regarding a potential correlation of PD-L1 expression with response in this study.

The response rate in this small, signal-finding cohort was similar to the 8% response rate observed with single-agent nivolumab in the recurrent glioblastoma cohort of the CheckMate-143 study (ClinicalTrials.gov identifier, NCT02017717), which enrolled patients irrespective of tumor PD-L1 expression and found no survival benefit with nivolumab compared with bevacizumab.[40] In addition, the median DOR (evaluated by RANO criteria) with anti–PD-1 therapy in CheckMate-143 and in the current study (11 and 16 months, respectively) was at least twice that of bevacizumab in CheckMate-143 (5 months).[40] Although only 2 responses were observed in the current study, our findings combined with those from CheckMate-143 indicate that durable responses may be achieved with anti–PD-1 monotherapy in a subset of patients with recurrent glioblastoma. In addition, increased understanding of potential biomarkers that can identify patients with glioblastoma who are most likely to respond to anti–PD-1 therapy is also needed. Importantly, despite prior evidence suggesting an association between PD-L1 tumor expression and worse prognosis in glioblastoma,[17] we observed durable responses in 2 patients who had PD-L1–positive glioblastoma, although, as noted above, the exclusion of patients with PD-L1–negative glioblastoma in this study precludes interpretation of the exact role of tumor PD-L1 expression in predicting a response

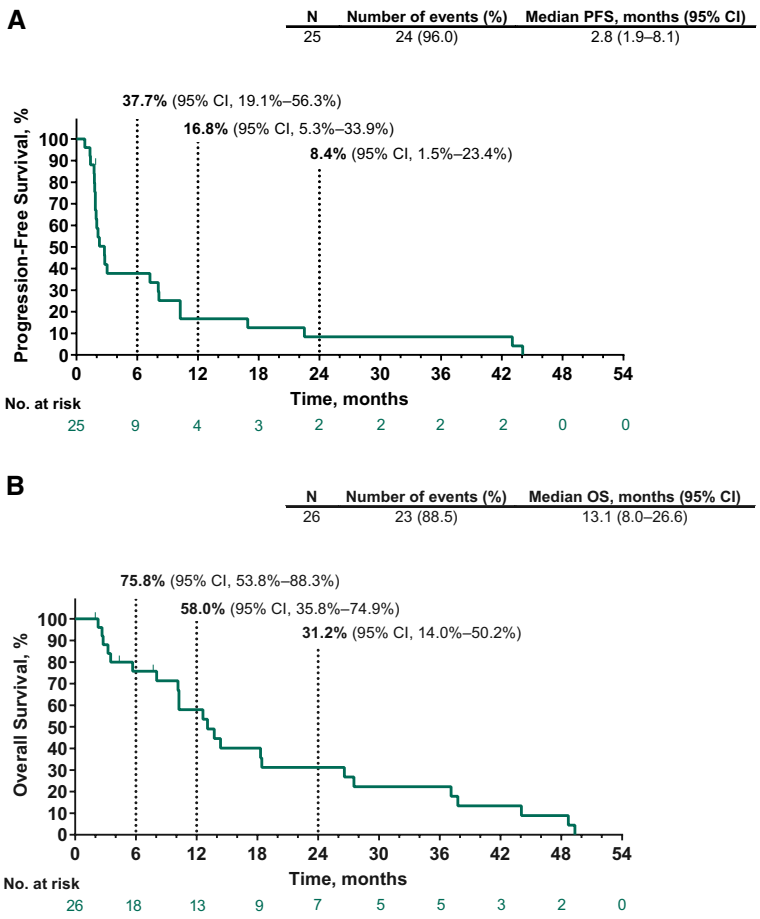

Figure 3. Survival estimates are illustrated in patients who received ≥1 dose of pembrolizumab, including (A) progression-free survival (PFS) based on Response Evaluation Criteria in Solid Tumors, version 1.1, by investigator review, and (B) overall survival (OS).

to pembrolizumab. Moreover, given the limited number of responses in this small cohort of patients with recurrent glioblastoma, additional biomarkers may need to be considered. Notably, GEP scores in the glioblastoma cohort were exceptionally low compared with those in other tumor cohorts from KEYNOTE-028[30] (including anal canal, biliary, colorectal, esophageal, and ovarian cancer) and KEYNOTE-012[31] (bladder, gastric, head and neck squamous cell carcinoma, and triple-negative breast cancer; ClinicalTrials.gov identifier, NCT01848834) and did not appear to correlate with therapeutic benefit, although these analyses were based on archival samples, which may not accurately reflect the recurrent glioblastoma tumor microenvironment.

One patient in our current study experienced an initial increase in tumor size, based on investigator review, that later reversed, suggesting that pseudoprogression may have occurred at a rate (4%) similar to that reported across various cancers treated with immune checkpoint inhibitors (range, 2%-14%).[41] However, interpreting the etiology of progressive imaging changes is challenging. The RANO criteria[28] address this limitation because evaluation by imaging complicates the differentiation of tumor progression from pseudoprogression,[42] and contrast enhancement caused by inflammation should be considered when using immunotherapies.[42,43]

Several limitations potentially affected this signal-finding study. The small size of the glioblastoma cohort and the lack of an active comparator in this study preclude definitive conclusions regarding efficacy. Among the 26 patients enrolled in this study, more than half were not receiving concomitant steroids at baseline, and an additional 2 patients received low doses of steroid, which may suggest that the patients enrolled in this cohort had comparatively lower disease burden than their excluded counterparts. PD-L1 expression levels and GEP scores were assessed using archival samples (obtained primarily at original diagnosis) and thus may not accurately reflect

TABLE 2. Patients With Treatment-Related Adverse Events, N = 26

Adverse Event	No. of Patients (%)
AEs of any grade	19 (73.1)
Serious AEs	4 (15.4)
AEs leading to discontinuation	0 (0.0)
Grade 3-4 AEs[a]	5 (19.2)
Arthritis	1 (3.8)
Colitis	1 (3.8)
Lymphopenia	1 (3.8)
Syncope	1 (3.8)
Type 2 diabetes mellitus[b]	1 (3.8)
AEs occurring in ≥5% of patients, any grade	
Fatigue	6 (23.1)
Rash	6 (23.1)
Asthenia	3 (11.5)
Headache	3 (11.5)
Neutrophil count decreased	3 (11.5)
WBC count decreased	3 (11.5)
Hypothyroidism	2 (7.7)
Colitis	2 (7.7)
Diarrhea	2 (7.7)
Erythema	2 (7.7)
Herpes zoster	2 (7.7)
AEs affecting the nervous system, any grade	
Headache	3 (11.5)
Cognitive disorder	1 (3.8)
Dizziness	1 (3.8)
Syncope	1 (3.8)
Immune-mediated AEs and infusion reactions[c]	7 (26.9)
Hypothyroidism	3 (11.5)
Colitis	2 (7.7)
Hyperthyroidism	2 (7.7)
Hypersensitivity	1 (3.8)
Infusion-related reaction	1 (3.8)

Abbreviations: AEs, adverse events; WBC, white blood cell.
[a]No grade 5 events occurred.
[b]This event occurred during the safety follow-up period and resolved within 2 weeks.
[c]Immune-mediated AEs and infusion reactions, irrespective of association with treatment, are listed.

the status of these tumor biomarkers at the time of study enrollment. Tumor PD-L1 expression was evaluated in this trial using a prototype assay. Although the US Food and Drug Administration has approved a companion diagnostic assay, it has not been validated for glioblastoma. Characterization of potential immunocorrelative factors, such as tumor PD-L1 expression, extent/composition of immune infiltrate, tumor mutational burden, and neoantigen expression in the recurrent tumor are critical to evaluate, not only as potential biomarkers for identifying the patients most likely to benefit from anti–PD-1 therapy, but also to guide rational combinatorial immunotherapeutic regimens to maximize therapeutic effect. Notably, because KEYNOTE-028 was a large, multicohort study of several solid tumor types, prospective evaluation of tumor-specific characteristics, such as MGMT-methylation status and mutation status for *IDH1* and *IDH2*, was not done. In addition, the limited number of responses made assessment of the impact of biomarkers (including MSI, GEP, and PD-L1 expression) challenging; furthermore, all patients had tumors with PD-L1 expression, limiting assessment of the role of PD-L1 expression. The time to response in this study was long for the 2 responses observed, including 1 response that occurred after 20.3 months, suggesting that antitumor effects can be seen with long-term pembrolizumab treatment in glioblastoma. Given the aggressive growth kinetics of recurrent glioblastoma, single-agent immunotherapy may be insufficient for a timely immune response and full benefit of treatment. Finally, the timing of anti–PD-1 administration relative to other therapeutic interventions for these patients may be a relevant consideration. Recent evaluation of neoadjuvant anti–PD-1 dosing before planned surgical debulking demonstrated evidence of immune activation within the resected tumor,[44,45] and benefits were observed in patients with recurrent glioblastoma,[44] as observed in other cancer types.[46-48]

In conclusion, this signal-finding study demonstrated durable antitumor activity and manageable toxicity with pembrolizumab monotherapy in a small subset of patients with recurrent glioblastoma. The therapeutic benefit of PD-1 blockade may be enhanced in rationally designed combinatorial regimens. Several trials are evaluating anti−PD-1 or anti−PD-L1 therapy combined with other treatments in patients with glioblastoma and may clarify whether targeting PD-1 in glioblastoma improves survival.

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CONFLICT OF INTEREST DISCLOSURES

David A. Reardon reports institutional support from Merck Pharmaceuticals during the conduct of the study and personal fees from AbbVie, Agenus, Bristol Myers Squibb, Celldex, EMD Serono, Genentech/Roche, Inovio, Merck, Merck KGaA, Monteris, Novocure, Oncorus, Oxigene, Regeneron, Stemline, and Taiho Oncology, Inc, outside the submitted work. The Dana-Farber Cancer Institute, a participating site of this trial, has a proprietary and financial interest in pembrolizumab. Tae Min Kim reports institutional support from Merck Pharmaceuticals during the conduct of the study and research funding from the AstraZeneca-Korea Health Industry Development Institute program, outside the submitted work. Jean-Sebastien Frenel reports personal fees from Biocad, Lilly, Novartis, Pfizer, and Roche, outside the submitted work. Matteo Simonelli reports personal fees from AbbVie, outside the submitted work. Juanita Lopez reports personal fees from Eisai, Novartis, and Genmab, and grants and personal fees from Roche, Basilea Pharmaceutica, and Genmab, outside the submitted work. Deepa S. Subramaniam reports institutional support from Genentech, Lilly, Mirati, Halozyme, Bristol Myers-Squibb, EMD Serono, and personal fees from AstraZeneca, Bristol-Myers Squibb, and Takeda Oncology outside the submitted work during the conduct of the study; and is currently an employee of AstraZeneca, and owns stock options. Lillian L. Siu reports grants and research support to institution from Novartis, Bristol-Myers Squibb, Pfizer, Boehringer-Ingelheim, GlaxoSmithKline, Roche/Genentech, Karyopharm, AstraZeneca/Medimmune, Merck, Celgene, Astellas, Bayer, AbbVie, Amgen, Symphogen, Intensity Therapeutics, Mirati Therapeutics,

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AUTHOR CONTRIBUTIONS

David A. Reardon: Conceptualization, investigation, resources, writing–original draft, and writing–review and editing. **Tae Min Kim:** Investigation, resources, writing–original draft, and writing–review and editing. **Jean-Sebastien Frenel:** Investigation, resources, writing–original draft, and writing–review and editing. **Matteo Simonelli:** Investigation, resources, writing–original draft, and writing–review and editing. **Juanita Lopez:** Investigation, resources, writing–original draft, and writing–review and editing. **Deepa S. Subramaniam:** Investigation, resources, writing–original draft, and writing–review and editing. **Lillian L. Siu:** Investigation, resources, writing–original draft, and writing–review and editing. **Hui Wang:** Formal analysis and writing–review and editing. **Suba Krishnan:** Investigation and writing–review and editing. **Karen Stein:** Investigation, writing–original draft, and writing–review and editing. **Christophe Massard:** Conceptualization, investigation, resources, writing–original draft, and writing–review and editing.

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