



Clinical activity and safety of atezolizumab in patients with recurrent glioblastoma

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Abstract

Purpose Glioblastoma is the most common primary malignant brain tumor. No standard treatment exists for recurrent disease. Glioblastoma is associated with an immunosuppressive tumor microenvironment. Immune checkpoint inhibitors, including atezolizumab (anti-programmed death-ligand 1), have demonstrated clinical activity in various cancers. Here, we present the safety and efficacy of atezolizumab in patients with glioblastoma from the phase 1a PCD4989g clinical trial (NCT01375842).

Methods Eligible patients had confirmed recurrent glioblastoma and measurable disease per RANO criteria. Atezolizumab (1200 mg) was administered intravenously every 3 weeks until progression or unacceptable toxicity. Patients were monitored for safety; response was evaluated at least every 6 weeks. Baseline biomarkers were evaluated.

Results All 16 patients enrolled had received prior chemotherapy, and 50% prior bevacizumab. Ten patients (63%) experienced a treatment-related event. No treatment-related grade 4–5 events were reported. All deaths occurred due to progression or during follow-up. One patient experienced a partial response (5.3 months); 3 experienced disease stabilization. The median overall survival was 4.2 months (range 1.2 to 18.8+ months). Association between peripheral CD4+ T cells and efficacy was observed. Two patients with *IDH1*-mutant tumors and 1 with a *POLE*-mutant tumor experienced ≥ 16 months survival.

Conclusions Atezolizumab was safe and well tolerated in this group of patients with recurrent glioblastoma. Our preliminary findings suggest that biomarkers, including peripheral CD4+ T cells and hypermutated tumor status, may help guide selection of patients with recurrent glioblastoma who might receive most benefit from atezolizumab therapy, supporting further atezolizumab combination studies in glioblastoma.

Keywords Atezolizumab · Glioblastoma (GBM) · Programmed death-ligand 1 (PD-L1) · Tumor mutational burden (TMB)

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Introduction

Glioblastoma (GBM) is the most common malignant primary brain tumor [1]. Despite aggressive treatment consisting of surgery and radiochemotherapy followed by temozolomide [2, 3], the median overall survival (mOS) had been 12–20 months, with a 5-year survival rate of

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approximately 10% [1, 3]. A recent phase 3 trial demonstrated further mOS improvement to approximately 21 months with a 5-year survival rate of 13% [4]. No standard treatment exists for recurrent disease [5]. To date, targeted agents have not demonstrated survival benefit [6], and therapeutic options for newly diagnosed and recurrent GBM are similar [3, 7, 8].

The interaction between programmed death-1 (PD-1), expressed on tumor-infiltrating immune cells (IC), and programmed death-ligand 1 (PD-L1), expressed on both tumor cells (TC) and IC, inhibits T-cell activation in the tumor microenvironment. Atezolizumab is an engineered, humanized anti-PD-L1 monoclonal antibody that targets PD-L1 and blocks its interaction with PD-1 and B7.1, reinvigorating anti-cancer immunity [9–11]. Additionally, direct targeting of PD-L1 with atezolizumab may preserve immune homeostasis in normal tissue by leaving the interaction between PD-1 and programmed death-ligand 2 (PD-L2) intact [12, 13]. The clinical efficacy and safety of atezolizumab have been demonstrated in many cancers [9, 14–16], and led to US Food and Drug Administration approval of atezolizumab as second-line therapy for patients with advanced or metastatic non-small cell lung cancer and urothelial cancer [17].

Some GBM tumor cells (TC) and tumor-infiltrating immune cells (IC) express PD-L1 [18], and higher expression is associated with higher grade and worse outcome [19]. Thus, PD-L1 could serve as a rational therapeutic target in GBM [20]. Targeting the PD-L1/PD-1 pathway has demonstrated significant activity in several cancers [21]. Data have shown clinical benefit of immune checkpoint inhibition in some patients with high tumor mutational burden (TMB) [22–24], including a small subset of patients with GBM [25, 26]. Several clinical trials have examined or are evaluating anti-PD-L1/PD-1 therapy in GBM [27, 28]. Preliminary results from a phase 3 study of nivolumab in recurrent GBM showed a lack of clinical benefit compared with bevacizumab in biomarker-unselected patients [28].

A phase 1a clinical trial (PCD4989g; NCT01375842) evaluated the safety and tolerability of atezolizumab monotherapy in many tumor types [9, 11]. Here we present the clinical safety and efficacy of atezolizumab in patients from the GBM cohort of this study.

Methods

PCD4989g overview

The phase 1a study followed a standard dose-escalation design and then allowed for tumor-specific expansion cohorts, including one for recurrent GBM (Figure S1).

Study objectives

The primary objective was to evaluate the safety and tolerability of single-agent atezolizumab. Key additional objectives were to assess atezolizumab activity as measured by investigator-assessed best radiographic overall response, progression-free survival (PFS), and OS, and to evaluate exploratory biomarkers.

Patients with GBM

Key eligibility criteria included initial histological confirmation of GBM, radiographic evidence of recurrent disease (first or second recurrence as GBM; if disease had progressed from grade 2 or 3 glioma, additional lines of prior therapy were allowed), measurable disease per Response Assessment in Neuro-Oncology (RANO) criteria [29], and Karnofsky performance status ≥ 70 . Patients had to have prior radiation and/or temozolomide treatment. Key exclusion criteria included evidence of recent brain hemorrhage and history or risk of autoimmune disease. At enrollment, patients could be on dexamethasone (or equivalent) ≤ 4 mg daily. After enrollment, steroid dosing was at the investigator's discretion. All Institutional Review Boards approved the study. All patients gave written informed consent.

Dosing and administration

Atezolizumab was administered intravenously every 3 weeks at 1200 mg until disease progression or unacceptable toxicity. Treatment was discontinued in patients who experienced progressive disease (PD) by RANO criteria and did not meet pre-specified criteria for continued dosing past progression. Patients could continue to receive treatment after progression until loss of clinical benefit (investigator-assessed).

Study assessments

Safety

All patients who received a dose of atezolizumab were assessed for safety. Adverse events (AEs) were recorded until 90 days after the last dose of atezolizumab or until initiation of another cancer therapy. AEs were graded according to National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0. AEs of special interest (AESI) were defined in the protocol as conditions suggestive of autoimmune disorder (see Supplementary Appendix Methods).

Efficacy

The efficacy-evaluable population was defined as patients with measurable disease at baseline per RANO criteria. Patients were evaluated by imaging every 6 weeks. Best overall response and PFS were investigator-assessed. PFS was defined as the time from the start of study drug until PD or death. Best overall response was defined as the best confirmed response recorded from the start of treatment.

Exploratory biomarkers

Immunohistochemistry (IHC) for PD-L1 expression and tumor-infiltrating CD8+ Cells

PD-L1 expression on TC (percentage of PD-L1-positive TC) and IC (percentage of PD-L1-positive IC per tumor area) was centrally evaluated using the VENTANA SP142 PD-L1 IHC assay in archival and fresh tumor specimens (Ventana Medical Systems, Inc, Oro Valley, AZ, USA) [30]. Please see the Supplementary Appendix for more details. All pretreatment samples were archival, with 14 out of 16 collected at diagnosis. The median time between tissue collection and study initiation was 407 days (range 49–1291). Postprogression studies were performed on fresh tumor samples. PD-L1 expression was scored based on the percentage of TC or IC expressing PD-L1 (see Supplementary Appendix Methods). IHC was performed to detect CD8+ T cells in the tumor bed (clone C8/144B [Dako, Santa Clara, CA, USA]) (performed by HistoGeneX, Naperville, IL, USA).

Immune cell quantification in peripheral blood

Lymphocyte subsets were measured using the BD Multitest TBNK Reagent with BD Trucount tubes (BD Biosciences, San Jose, CA, USA). Total B-cell and CD4+ and CD8+ T-cell counts were quantified by flow cytometry (performed by QLAB, [Koninklijke Philips N.V.]).

Tumor mutation analyses

Somatic mutations, microsatellite instability (MSI) status, and TMB were centrally assessed using the FoundationOne DNA-based assay (Foundation Medicine, Inc, Cambridge, MA, USA) [14, 15, 31]. Briefly, after extraction from formalin-fixed paraffin-embedded biopsy or surgical specimens, 50–200 ng of DNA underwent whole-genome shotgun library construction and hybridization-based capture of 4557 exons from 287 cancer-related genes and 47 introns from 19 genes. TMB was defined as the number of

somatic, coding, base substitution, and insertion/deletion mutations per megabase of DNA sequenced.

Statistical analysis

The clinical cutoff date was December 31, 2016. Sample size considerations were not made with explicit power and type I error considerations but were made to obtain preliminary safety, pharmacokinetic, and pharmacodynamic information. The Kaplan–Meier method was used to estimate the OS and PFS survival curves, including the median. The Brookmeyer–Crowley method was used to construct 95% confidence intervals for the median OS and PFS.

Study oversight

F. Hoffmann-La Roche, Ltd./Genentech, Inc. sponsored the study, provided study drugs, and collaborated with academic authors regarding study design and the collection, analysis and interpretation of data. All drafts of the manuscript were prepared by the authors, with editorial assistance from a professional medical writer funded by the sponsor. All authors approved submission. All authors verified that the study was conducted per protocol (available with the statistical analysis plan online) and vouched for data accuracy and completeness.

Results

Patients

Sixteen patients were enrolled in the GBM cohort (Table 1); all 16 were evaluated for atezolizumab safety and efficacy. The median age was 52 years (range 31–75), and 13 patients (81%) were men. An equal number of patients had KPS 70–80 and 90–100. Seven patients (44%) were previously treated with systemic steroids; 12 patients (75%) received systemic steroids while on study treatment. Eight patients (50%) had received 1 prior line of therapy, and 8 (50%) had received two. All patients had received prior radiotherapy and temozolomide; 8 (50%) had also received bevacizumab. The median atezolizumab treatment duration was 2 months (Fig. 1a).

Safety

All 16 patients experienced ≥ 1 AE; 10 patients (63%) had a treatment-related AE (TRAE; Table 2A). Three patients (19%) had grade 3 TRAEs [asthenia, increased aspartate aminotransferase (AST) and brain edema]. One patient (6%) with PD had a serious TRAE (grade 3 brain edema), which was treated with an increased dose of steroids but

Table 1 Patient demographics and baseline characteristics

Characteristics	N=16
Median age, years	52
Range	31–75
Sex, n (%)	
Male	13 (81%)
Female	3 (19%)
Karnofsky performance status, n (%)	
70–80	8 (50%)
90–100	8 (50%)
Race, n (%)	
White	13 (81%)
Other	3 (19%)
Systemic steroid use at baseline, n (%)	
Yes	7 (44%)
No	9 (56%)
Systemic steroid use during study, n (%)	
Yes	12 (75%)
No	4 (25%)
Prior lines of therapy for GBM, n (%)	
1	8 (50%)
2	8 (50%)
Prior systemic therapies received, n (%)	
Bevacizumab	8 (50%)
Chemotherapy (includes temozolomide)	16 (100%)
Tyrosine kinase inhibitor	2 (13%)
Prior radiotherapy received, n (%)	
Radiotherapy	16 (100%)
PD-L1 IC score, n (%)	
IC0	11 (69%)
IC1	3 (19%)
IC2	2 (13%)
IC3	0
PD-L1 TC score, n (%)	
TC0	14 (87%)
TC1	2 (13%)
TC2 or TC3	0

IC tumor-infiltrating immune cells, PD-L1 programmed death-ligand 1, TC tumor cells

did not lead to atezolizumab discontinuation. One patient (6%) interrupted atezolizumab due to treatment-related grade 3 asthenia (Table 2A). No grade 4–5 TRAEs were reported. All patients discontinued atezolizumab treatment due to PD. All deaths occurred due to PD or during follow-up.

Consistent with what is typically observed in patients with primary brain tumors [32], the most common all-grade TRAE was fatigue (25%), followed by malaise (19%) (Table 2B). Six patients (38%) experienced an AESI; 3 of these patients (19%) had grade 3 AESIs: 2 (13%)

had increased alanine aminotransferase, and 1 (6%) had increased AST (Table S1).

Efficacy

The overall response rate (ORR) was 6% (Fig. 1b). After 165 days on study, 1 patient (6%) developed a partial response (PR), with a duration of 5.3 months and a maximum 69% reduction in the sum of the largest diameters (SLD) from baseline (Fig. 1b, Figure S2). Three patients (19%) had stable disease (SD). An additional patient had a 63% reduction in SLD from baseline, but was classified as having PD due to the appearance of new lesions.

The median PFS (mPFS) was 1.2 months (range 0.7–10.7 months) (Figure S3). The mOS was 4.2 months (range 1.2 to 18.8+ months) (Fig. 1c). The landmark 12-month survival rate was 21% (95% CI 0.3–42.6). Longer-term survival (range 16.0 to 18.8+ months) was observed in three patients. At data cutoff, all patients had discontinued atezolizumab treatment, but two patients were alive; 1 had SD as best response, the other had PD.

Exploratory biomarkers

We evaluated the relationship between baseline peripheral lymphocyte levels, systemic steroid use, and outcome. Patients who were taking systemic steroids at the time of study drug initiation had fewer baseline B cells and CD4+ T cells than those who did not, while no association was observed for CD8+ T cells (Fig. 2a). Patients with lymphopenic baseline CD4+ T cells and B cells ($<0.40 \times 10^6$ cells/mL and $<0.19 \times 10^6$ cells/mL, respectively) showed a trend toward reduced PFS and OS (Fig. 2b).

A non-significant trend toward improved OS was observed in patients who were not taking steroids at the time of treatment initiation (mOS, 5.0 months), versus those who were on steroids (mOS, 3.9 months; Figure S4). No difference was seen in PFS between patients with and without steroid treatment at baseline (Figure S4). A non-significant trend toward shorter PFS and OS was observed in patients who had received prior bevacizumab (mOS, 3.6 months; mPFS, 1.0 months) versus those who did not receive prior bevacizumab (mOS, 5.0 months; mPFS, 2.4 months; Figure S5).

Most patients had low or no PD-L1 expression on TC and IC (Table 1). Of note, levels of tumor-infiltrating CD8+ T cells were low (mean 0.19% cells/center of the tumor) compared with tumor types known to have high levels of immune infiltration [9, 14]. Postprogression tumor tissue was collected from the patient who experienced a PR and one patient who had PD as best response. In both, tumor-infiltrating CD8+ T cells were similarly low in pretreatment and postprogression samples (PR patient, 0.06% and 0.02%; PD

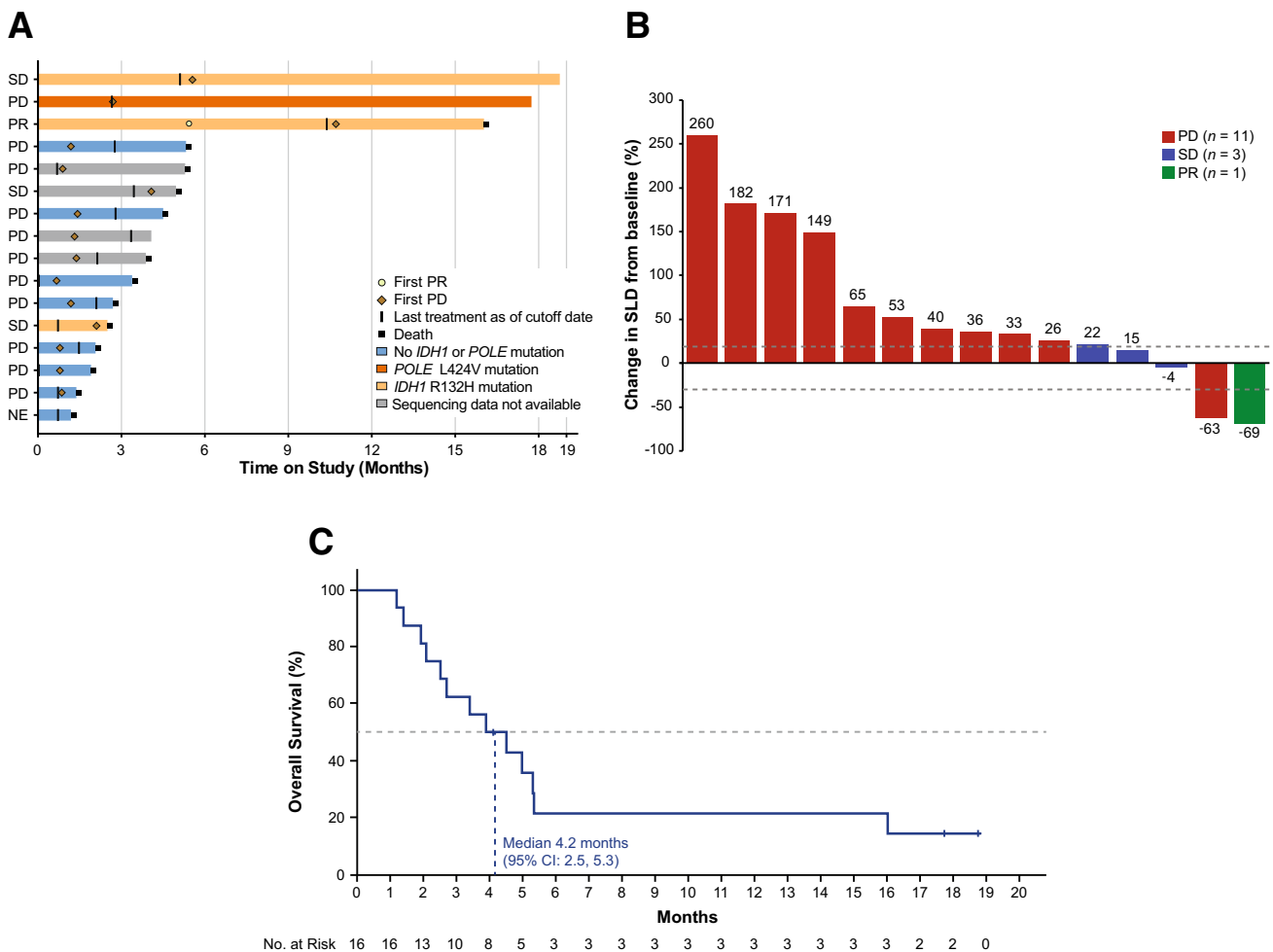


Fig. 1 Clinical activity of atezolizumab in patients with glioblastoma. **a** Duration of treatment and time to response. **b** Maximum change from baseline in the SLD for target lesions; +20% and -30% are

marked by dashed lines. **c** Kaplan–Meier estimate of overall survival. *NE* not evaluable, *PD* progressive disease, *PR* partial response, *SD* stable disease, *SLD* sum of the largest diameters

patient, 0.04% and 0.05%; respectively). No notable changes in PD-L1 IHC expression on TC or IC were observed in archival versus post-progression samples.

In GBM, *IDH1/2* mutations and MGMT promoter methylation are associated with increased survival and benefit from standard treatments [33]. Twelve patients had tumor tissue available for genomic analyses. Three patients had R132H mutations in *IDH1* (1 PR, 2 SD; Table 3); none had *IDH2* mutations. Patients with *IDH1*-mutant tumors had better PFS with a trend toward longer OS than patients with *IDH1*-wild-type tumors (mPFS, 5.5 versus 1.2 months; mOS, 16.0 versus 2.7 months; Fig. 2c, d). Of the patients with *IDH1*-wild-type tumors, 8 experienced PD and 1 was not evaluable due to death prior to the first tumor assessment (Fig. 1a).

All tumors evaluated were microsatellite stable (MSS). In the 12 patients assessed, the median TMB was 2.7 mutations per megabase (Mut/Mb) (95% CI 1.80–4.51), with 1

outlier (183 Mut/Mb). This patient’s tumor was *IDH1*-wild-type but had a deleterious somatic mutation (L424V) in the DNA polymerase gene *POLE* (Table 3), which was previously identified in hypermutated tumors [34, 35]. Despite a reduction of target lesions (63% decrease in SLD; Fig. 1b) and prolonged survival (17.7+ months), this patient had confirmed PD due to new lesions.

Discussion

In this phase 1a study, atezolizumab monotherapy was well tolerated in patients with recurrent GBM. No unexpected AEs were reported. Durable radiographic response and SD were observed in a subset of patients with GBM, with 1 patient experiencing a sustained PR and 1 exhibiting prolonged SD. The landmark 12-month OS rate (21%)

Table 2 Safety summary and treatment-related AEs

A. Safety summary, n (%)		All grades N= 16		Grade 3 N= 16
Treatment-related AEs, all grades				
Grade 3 treatment-related AEs	10 (63%)			0
Grade 4–5 treatment-related AEs	3 (19%)			0
Treatment-related serious AEs	0			0
Treatment-related AEs leading to interruption of atezolizumab	1 (6%)			1 (6%)
Treatment-related AEs leading to discontinuation of atezolizumab	1 (6%)			1 (6%)
	0			0
B. Treatment-related AE, n (%)				
Fatigue	4 (25%)			0
Malaise	3 (19%)			0
Asthenia	2 (13%)			2 (13%)
Diarrhea	2 (13%)			0
Headache	2 (13%)			0
Nausea	2 (13%)			0
AST increase	1 (6%)			1 (6%)
Brain edema	1 (6%)			1 (6%)
ALT increase	1 (6%)			0
Apraxia	1 (6%)			0
Arthralgia	1 (6%)			0
Back pain	1 (6%)			0
Constipation prophylaxis	1 (6%)			0
Decreased appetite	1 (6%)			0
Dehydration	1 (6%)			0
Dermatitis	1 (6%)			0
Dry mouth	1 (6%)			0
Dysgeusia	1 (6%)			0
Flushing	1 (6%)			0
Glossodynia	1 (6%)			0
Hyperhidrosis	1 (6%)			0
Myalgia	1 (6%)			0
Muscular weakness	1 (6%)			0
Night sweats	1 (6%)			0
Rash macular	1 (6%)			0
Rash maculo-papular	1 (6%)			0

Table 2 (continued)

B. Treatment-related AE, n (%)	All grades N = 16	Grade 3 N = 16
Rhinorrhea	1 (6%)	0
Rosacea	1 (6%)	0
Sinus congestion	1 (6%)	0
Urticaria	1 (6%)	0
Vascular purpura	1 (6%)	0
Vomiting	1 (6%)	0

AE adverse event, ALT alanine aminotransferase, AST aspartate aminotransferase

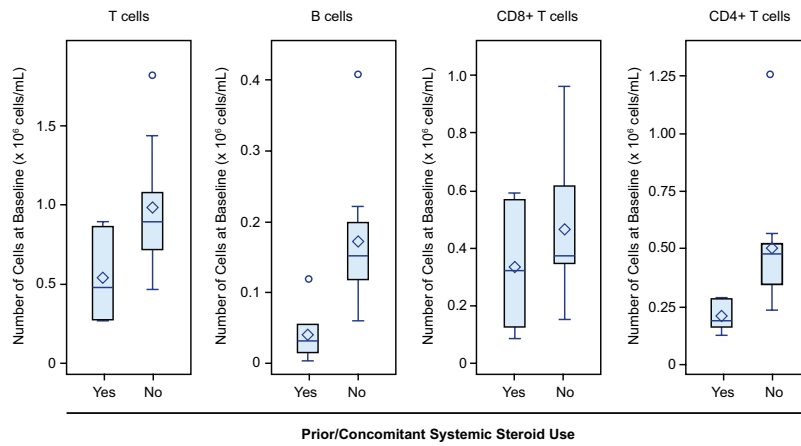
was comparable to that seen with bevacizumab or cytotoxic chemotherapies [7, 36, 37].

GBM is a historically difficult disease to treat, with few advances in treatment options in the last 30 years [3, 6]. Bevacizumab-refractory patients are particularly resistant to subsequent therapies and were included in this clinical trial as additional therapeutic options are needed. Furthermore, inhibition of VEGF signaling has led to enhanced antitumor immune responses in preclinical models [38], suggesting that patients previously treated with bevacizumab may derive benefit from immune checkpoint inhibitors. Recently, immunotherapy has improved survival in patients with a variety of cancer types [21], however a number of potential issues may limit its efficacy in GBM. The first is the highly immune-suppressed tumor microenvironment, influenced by both tumor intrinsic factors such as PD-L1 expression, as well as iatrogenic factors such as steroid administration. Second, GBM has relatively low TMB, differentiating it from malignancies with higher TMB that have shown sustained responses to immunotherapy. Third, the challenge in achieving adequate doses of the therapeutic agent in the target organ. Fourth is difficulty interpreting imaging studies. Finally, there is concern for CNS toxicity from either increased intracranial pressure due to a robust immune response or a misdirected immune-mediated injury.

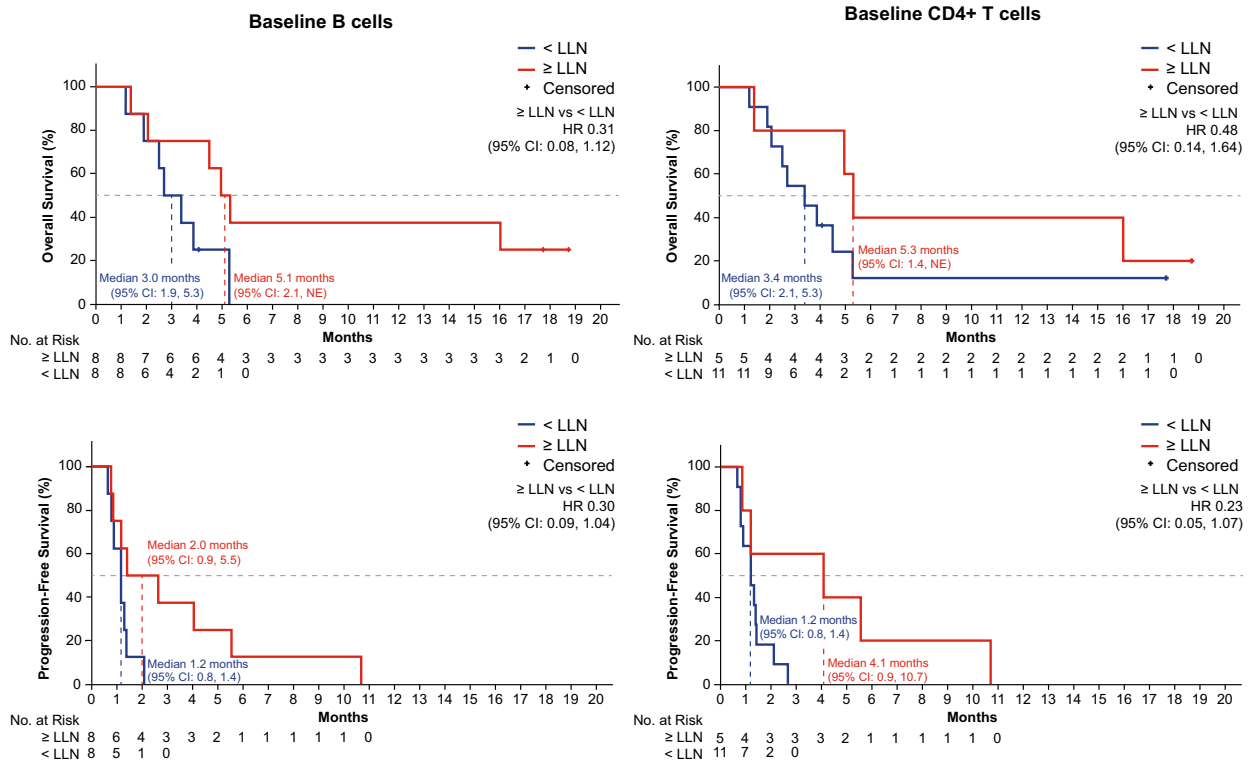
PD-L1 is frequently expressed on tumor infiltrating lymphocytes, and the majority of GBM tumor cells have at least some PD-L1 expression [39]. These findings, cumulatively, suggest that targeting PD-L1 may provide clinical benefit. This study used the VENTANA SP142 PD-L1 IHC assay, designed to maximize detection of PD-L1 on IC. In a recent, small lung cancer study (N = 37), the SP142 assay detected fewer PD-L1-expressing TC compared with other PD-L1 IHC assays [40]. A prior study reported that PD-L1 expression was detected on diffuse/fibrillary cells (84%) and on TC (38%) in glioblastoma [18]. The difference in PD-L1 prevalence between that study and ours may be, in part, due to different IHC assays.

Patients who were taking systemic steroids at the time of study drug initiation had lower circulating lymphocyte levels than those who were not, consistent with the known mechanism of action of steroids [41, 42], and trended toward reduced clinical benefit from atezolizumab. The three patients who had long-term survival, including 1 who experienced a PR, were not receiving steroids at baseline and did not require steroids during study treatment. This may be secondary to the influence of steroids on anti-tumor immune activity within the context of an immune checkpoint inhibitor. Alternatively, either the steroid requirement may merely be an independent prognostic factor *or* the need for steroids may be reduced in patients with more indolent, *IDH*-mutant tumors (two of three long-term survivors). Patients with normal levels of peripheral CD4+ T cells and B cells had a trend

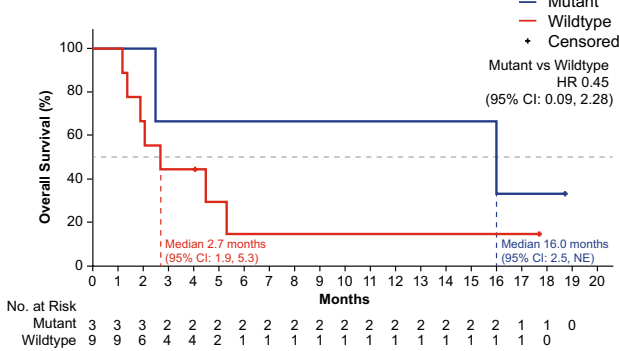
A



B



C



D

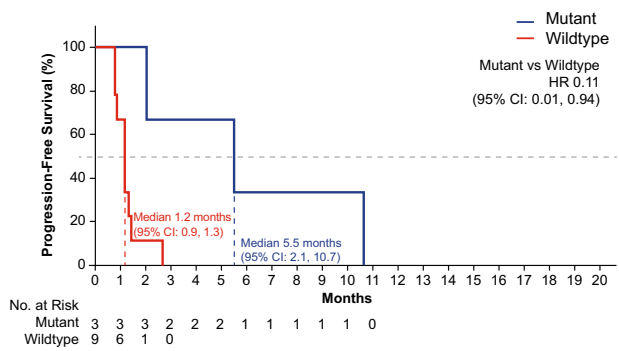


Fig. 2 Association between biomarkers and clinical outcome. **a** Quantitation of peripheral lymphocyte levels at baseline in populations based on systemic steroid use. The line in the middle of the box is plotted at the median. **b** Kaplan–Meier estimates of overall survival and progression-free survival in populations defined by baseline lymphocyte levels. **c** Kaplan–Meier estimate of overall survival in populations defined by *IDH1* mutation status. **d** Kaplan–Meier estimate of progression-free survival in populations defined by *IDH1* mutation status. *HR* hazard ratio, *LLN* lower limit of normal, *NE* not evaluable

toward improved outcome versus those who had lymphopenic levels. Higher CD4+ T-cell count has been associated with longer survival in GBM [43, 44], suggesting that this biomarker may be prognostic. Patients with *IDH1*-mutant tumors had improved outcomes versus those with *IDH1*-wild-type tumors, consistent with previous studies showing that *IDH1*-mutant gliomas represent a distinct disease entity [45]. Preclinical models have demonstrated that *IDH1* mutation in gliomas is associated with suppression of CD8+ T cell accumulation, contributing to the immunosuppressive tumor microenvironment [46]. A randomized controlled trial is required to determine whether peripheral lymphocyte levels and *IDH1* mutations are merely prognostic or also predictive of atezolizumab activity in GBM.

Tumors with high MSI have increased TMB and improved responses to PD-L1/PD-1 pathway inhibitors versus MSS tumors [47]. Mutations in DNA mismatch repair (MMR) genes developed in preclinical glioblastoma models treated with temozolomide [48] and in patients with low-grade gliomas that have progressed on temozolomide [49]. Sequencing of MMR genes did not show any evidence of MMR deficiency in patients enrolled in the GBM cohort of this study. In addition, mutations in DNA polymerase components are associated with a hypermutated phenotype. *POLE* is the catalytic subunit of the DNA polymerase complex, and *POLE* defects lead to loss of DNA replication fidelity. In a recent study, germline susceptibility variants in *POLE*, including L424V, were identified in hypermutated MSS colorectal cancers [34]. This mutation was also previously described in a patient with hypermutated GBM who displayed evidence of a clinical and immunologic response while on pembrolizumab [35]. *POLE* defects may lead to increased neoantigen burden and higher anti-tumor T-cell recognition [50].

In the patient cohort described here, all patients with recurrent GBM had MSS tumors. One patient who

experienced prolonged survival on atezolizumab had a *POLE* L424V-mutant tumor with high TMB. This patient's response was categorized as PD due to the emergence of new lesions; however, his long-term survival and the stabilization of these lesions suggest that this may have been pseudoprogression rather than true PD. Additional tissue was not obtained to confirm pseudoprogression. Evaluation of TMB and genomic alterations that confer a hypermutation phenotype, including *POLE* mutations, may help guide selection of patients with recurrent GBM who are more likely to receive benefit from atezolizumab.

Interpreting radiographic endpoints in patients with GBM has been challenging. Pseudoprogression, a transient worsening of enhancement on imaging, frequently occurs in patients treated with radiation and traditional cytotoxic chemotherapy [51]. With immunotherapeutic approaches, there is substantial concern that transient radiographic worsening can be seen before radiographic improvement. It is thought that the pathophysiology driving these radiographic changes differs from that observed with chemoradiotherapy. There is also concern that radiographic findings may be independent of survival outcomes in patients treated with immunotherapy. A contemporary assessment system was established to guide imaging interpretation, particularly within the context of clinical trials. The immunotherapy Response Assessment in Neuro-Oncology (iRANO) [52] is derived from RANO [29]. A key distinguishing feature of iRANO for the determination of PD (even in the setting of new lesions) is required confirmatory radiographic assessment in 3 months, among clinically stable patients who received treatment within 6 months.

In conclusion, this phase 1a study showed that atezolizumab was well tolerated in patients with GBM. Recent results have demonstrated lack of clinical efficacy with anti-PD-1 antibody monotherapy in biomarker-unselected patients with recurrent GBM [28]. The favorable safety profile supports combination studies with atezolizumab in this population, particularly in patients who may not require concomitant steroids. Potential options for combination studies with atezolizumab in recurrent GBM include radiation therapy, chemotherapy (temozolomide, lomustine), other immunotherapies, and bevacizumab. Each one targets a different part of the antitumor immune response and, in combination with atezolizumab, may help reinvigorate anticancer immunity in recurrent GBM.

Table 3 Exploratory biomarkers and overall survival

	<i>IDH1</i> status	<i>POLE</i> status	TMB, Mut/Mb	OS, months
Patient 1	Wild-type	L424V	183	17.7+
Patient 2	R132H	Wild-type	0.90	16.0
Patient 3	R132H	Wild-type	2.70	18.8+
Patient 4	R132H	Wild-type	4.50	2.5

All patients were microsatellite stable. *MGMT* promoter methylation was not part of the analysis panel

Mut/Mb mutations per megabase, *OS* overall survival, *TMB* tumor mutational burden

+ means censored

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Compliance with ethical standards

Conflict of interest RVL has received support from Roche-Genentech for meeting travel to present study results, honoraria for advisory boards for AstraZeneca, Abbvie, and Ziopharm and honoraria for medical editing for EBSCO publishing, Medlink Neurology, and American Physician Institute. JR has received honoraria for advisory boards from Novartis, Lilly, Orion, Servier, and Peptomyc, and has received research funding from Bayer and Novartis. JB has received honoraria for advisory boards from BMS. MF is an employee of, and has stock options with Roche-Genentech, and has stock options and a patent with Aduro BioTech. CO and LM are employees of, and have stock options with Roche-Genentech. SO is an employee of Roche-Genentech. WG, MT, ETW, KS and KB have no conflicts to disclose.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

References

- Ostrom QT, Gittleman H, Xu J, Kromer C, Wolinsky Y, Kruchko C, Barnholtz-Sloan JS (2016) CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2009–2013. *Neuro Oncol* 18:v1–v75. <https://doi.org/10.1093/neuonc/nov207>
- Stupp R, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJ, Janzer RC, Ludwin SK, Allgeier A, Fisher B, Belanger K et al (2009) Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *Lancet Oncol* 10:459–466. [https://doi.org/10.1016/S1470-2045\(09\)70025-7](https://doi.org/10.1016/S1470-2045(09)70025-7)
- Lukas RV, Mrugala MM (2017) Pivotal therapeutic trials for infiltrating gliomas and how they affect clinical practice. *Neuro-Oncol Pract* 4(4):209–216. <https://doi.org/10.1093/nop/npw016>
- Stupp R, Taillibert S, Kanner A, Read W, Steinberg DM, Lhermitte B, Toms S, Idbaih A, Ahluwalia MS, Fink K et al (2017) Effect of tumor-treating fields plus maintenance temozolomide vs maintenance temozolomide alone on survival in patients with glioblastoma: a randomized clinical trial. *JAMA* 318:2306–2316. <https://doi.org/10.1001/jama.2017.18718>
- Weller M, van den Bent M, Hopkins K, Tonn JC, Stupp R, Falini A, Cohen-Jonathan-Moyal E, Frappaz D, Henriksson R, Balana C et al (2014) EANO guideline for the diagnosis and treatment of anaplastic gliomas and glioblastoma. *Lancet Oncol* 15:e395–e403. [https://doi.org/10.1016/S1470-2045\(14\)70011-7](https://doi.org/10.1016/S1470-2045(14)70011-7)
- Touat M, Idbaih A, Sanson A, Ligon KL (2017) Glioblastoma targeted therapy: updated approaches from recent biological insights. *Ann Oncol* 28(7):1457–1462. <https://doi.org/10.1093/annonc/mdx106>
- Wong ET, Gautam S, Malchow C, Lun M, Pan E, Brem S (2011) Bevacizumab for recurrent glioblastoma multiforme: a meta-analysis. *J Natl Compr Canc Netw* 9:403–407
- Stupp R, Wong ET, Kanner AA, Steinberg D, Engelhard H, Heidecke V, Kirson ED, Taillibert S, Liebermann F, Dbaly V et al (2012) NovoTTF-100A versus physician's choice chemotherapy in recurrent glioblastoma: a randomised phase III trial of a novel treatment modality. *Eur J Cancer* 48:2192–2202. <https://doi.org/10.1016/j.ejca.2012.04.011>
- Herbst RS, Soria JC, Kowanetz M, Fine GD, Hamid O, Gordon MS, Sosman JA, McDermott DF, Powderly JD, Gettinger SN et al (2014) Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature* 515:563–567. <https://doi.org/10.1038/nature14011>
- Chen DS, Irving BA, Hodi FS (2012) Molecular pathways: next-generation immunotherapy: inhibiting programmed death-ligand 1 and programmed death-1. *Clin Cancer Res* 18:6580–6587. <https://doi.org/10.1158/1078-0432.CCR-12-1362>
- Powles T, Eder JP, Fine GD, Braiteh FS, Loriot Y, Cruz C, Bellmunt J, Burris HA, Petrylak DP, Teng SL et al (2014) MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. *Nature* 515:558–562. <https://doi.org/10.1038/nature13904>
- Matsumoto K, Fukuyama S, Eguchi-Tsuda M, Nakano T, Matsumoto T, Matsumura M, Moriwaki A, Kan-o K, Wada Y, Yagita H et al (2008) B7-DC induced by IL-13 works as a feedback regulator in the effector phase of allergic asthma. *Biochem Biophys Res Commun* 365:170–175. <https://doi.org/10.1016/j.bbrc.2007.10.156>
- Akbari O, Stock P, Singh AK, Lombardi V, Lee WL, Freeman GJ, Sharpe AH, Umetsu DT, Dekruyff RH (2010) PD-L1 and PD-L2 modulate airway inflammation and iNKT-cell-dependent airway hyperreactivity in opposing directions. *Mucosal Immunol* 3:81–91. <https://doi.org/10.1038/mi.2009.112>
- Rosenberg JE, Hoffman-Censits J, Powles T, van der Heijden MS, Balar AV, Necchi A, Dawson N, O'Donnell PH, Balmanoukian A, Loriot Y et al (2016) Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial. *Lancet* 387:1909–1920. [https://doi.org/10.1016/S0140-6736\(16\)00561-4](https://doi.org/10.1016/S0140-6736(16)00561-4)
- Balar AV, Galsky MD, Rosenberg JE, Powles T, Petrylak DP, Bellmunt J, Loriot Y, Necchi A, Hoffman-Censits J, Perez-Gracia JL et al (2017) Atezolizumab as first-line treatment in cisplatin-ineligible patients with locally advanced and metastatic urothelial

- carcinoma: a single-arm, multicentre, phase 2 trial. *Lancet* 389:67–76
16. Rittmeyer A, Barlesi F, Waterkamp D, Park K, Ciardiello F, von Pawel J, Gadgeel SM, Hida T, Kowalski DM, Dols MC et al (2017) Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. *Lancet* 389:255–265
 17. Tecentriq (atezolizumab) (2018) [product insert]. Genentech, Inc., South San Francisco
 18. Berghoff AS, Kiesel B, Widhalm G, Rajky O, Ricken G, Wohrer A, Dieckmann K, Filipits M, Brandstetter A, Weller M et al (2015) Programmed death ligand 1 expression and tumor-infiltrating lymphocytes in glioblastoma. *Neuro Oncol* 17:1064–1075. <https://doi.org/10.1093/neuonc/nou307>
 19. Vlahovic G, Fecci PE, Reardon D, Sampson JH (2015) Programmed death ligand 1 (PD-L1) as an immunotherapy target in patients with glioblastoma. *Neuro Oncol* 17:1043–1045. <https://doi.org/10.1093/neuonc/nov071>
 20. Binder DC, Davis AA, Wainwright DA (2015) Immunotherapy for cancer in the central nervous system: current and future directions. *Oncoimmunology* 5:e1082027. <https://doi.org/10.1080/2162402X.2015.1082027>
 21. Balar AV, Weber JS (2017) PD-1 and PD-L1 antibodies in cancer: current status and future directions. *Cancer Immunol Immunother* 66:551–564. <https://doi.org/10.1007/s00262-017-1954-6>
 22. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, Skora AD, Luber BS, Azad NS, Laheru D et al (2015) PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med* 372:2509–2520. <https://doi.org/10.1056/NEJMoal1500596>
 23. McGranahan N, Furness AJ, Rosenthal R, Ramskov S, Lyngaa R, Saini SK, Jamal-Hanjani M, Wilson GA, Birkbak NJ, Hiley CT et al (2016) Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade. *Science* 351:1463–1469. <https://doi.org/10.1126/science.aaf1490>
 24. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, Lee W, Yuan J, Wong P, Ho TS et al (2015) Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science* 348:124–128. <https://doi.org/10.1126/science.aaa1348>
 25. Bouffet E, Larouche V, Campbell BB, Merico D, de Borja R, Aronson M, Durmo C, Krueger J, Cabric V, Ramaswamy V et al (2016) Immune checkpoint inhibition for hypermutant glioblastoma multiforme resulting from germline biallelic mismatch repair deficiency. *J Clin Oncol* 34:2206–2211. <https://doi.org/10.1200/JCO.2016.66.6552>
 26. Hodges TR, Ott M, Xiu J, Gatalica Z, Swensen J, Zhou S, Huse JT, de Groot J, Li S, Overwijk WW et al (2017) Mutational burden, immune checkpoint expression, and mismatch repair in glioma: implications for immune checkpoint immunotherapy. *Neuro Oncol*. <https://doi.org/10.1093/neuonc/nox026>
 27. Omuro A, Vlahovic G, Lim M, Sahebjam S, Baehring J, Cloughesy T, Voloschin A, Ramkissoon SH, Ligon KL, Latek R et al (2018) Nivolumab with or without ipilimumab in patients with recurrent glioblastoma: results from exploratory phase 1 cohorts of CheckMate 143. *Neuro Oncol* 20:674–686. <https://doi.org/10.1093/neuonc/nox208>
 28. Reardon DA, Omuro A, Brandes AA, Rieger J, Wick A, Sepulveda J, Phuphanich S, de Souza P, Ahluwalia MS, Lim M et al (2017) Randomized phase 3 study evaluating the efficacy and safety of nivolumab vs bevacizumab in patients with recurrent glioblastoma: CheckMate 143. *WFNOS OS10.3. Neuro-oncology* 19(suppl_3):iii21
 29. Wen PY, Macdonald DR, Reardon DA, Cloughesy TF, Sorensen AG, Galanis E, Degroot J, Wick W, Gilbert MR, Lassman AB et al (2010) Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group. *J Clin Oncol* 28:1963–1972. <https://doi.org/10.1200/JCO.2009.26.3541>
 30. Vennapusa B, Baker B, Kowanetz M, Boone J, Menzl I, Bruey JM, Fine G, Mariathasan S, McCaffery I, Mocchi S et al (2018) Development of a PD-L1 Complementary Diagnostic Immunohistochemistry Assay (SP142) for Atezolizumab. *Appl Immunohistochem Mol Morphol*. <https://doi.org/10.1097/PAL.0000000000000594>
 31. Chalmers ZR, Connelly CF, Fabrizio D, Gay L, Ali SM, Ennis R, Schrock A, Campbell B, Shlien A, Chmielecki J et al (2017) Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. *Genome Med*. <https://doi.org/10.1186/s13073-017-0424-2>
 32. Grant R, Brown PD (2016) Fatigue randomized controlled trials—how tired is “too tired” in patients undergoing glioma treatment? *Neuro Oncol* 18:759–760. <https://doi.org/10.1093/neuonc/now052>
 33. Szopa W, Burley TA, Kramer-Marek G, Kaspera W (2017) Diagnostic and therapeutic biomarkers in glioblastoma: current status and future perspectives. *Biomed Res Int* 2017:8013575. <https://doi.org/10.1155/2017/8013575>
 34. Palles C, Cazier JB, Howarth KM, Domingo E, Jones AM, Broderick P, Kemp Z, Spain SL, Guarino E, Salguero I et al (2013) Germline mutations affecting the proofreading domains of POLE and POLD1 predispose to colorectal adenomas and carcinomas. *Nat Genet* 45:136–144. <https://doi.org/10.1038/ng.2503>
 35. Johanns TM, Miller CA, Dorward IG, Tsien C, Chang E, Perry A, Uppaluri R, Ferguson C, Schmidt RE, Dahiya S et al (2016) Immunogenomics of hypermutated glioblastoma: a patient with germline POLE deficiency treated with checkpoint blockade immunotherapy. *Cancer Discov* 6:1230–1236
 36. Chamberlain MC, Johnston SK (2010) Salvage therapy with single agent bevacizumab for recurrent glioblastoma. *J Neurooncol* 96:259–269. <https://doi.org/10.1007/s11060-009-9957-6>
 37. Reardon DA, Desjardins A, Peters KB, Gururangan S, Sampson JH, McLendon RE, Herndon JE 2nd, Bulusu A, Threatt S, Friedman AH et al (2012) Phase II study of carboplatin, irinotecan, and bevacizumab for bevacizumab naive, recurrent glioblastoma. *J Neurooncol* 107:155–164. <https://doi.org/10.1007/s11060-011-0722-2>
 38. Huang Y, Goel S, Duda DG, Fukumura D, Jain RK (2013) Vascular normalization as an emerging strategy to enhance cancer immunotherapy. *Cancer Res* 73:2943–2948. <https://doi.org/10.1158/0008-5472.CAN-12-4354>
 39. Nduom EK, Wei J, Yaghi NK, Huang N, Kong LY, Gabrusiewicz K, Ling X, Zhou S, Ivan C, Chen JQ et al (2016) PD-L1 expression and prognostic impact in glioblastoma. *Neuro Oncol* 18:195–205. <https://doi.org/10.1093/neuonc/nov172>
 40. Hirsch FR, McElhinny A, Stanforth D, Ranger-Moore J, Jansson M, Kulangara K, Richardson W, Towne P, Hanks D, Vennapusa B et al (2017) PD-L1 immunohistochemistry assays for lung cancer: results from phase 1 of the blueprint PD-L1 IHC assay comparison project. *J Thorac Oncol* 12:208–222
 41. Seki M, Ushiyama C, Seta N, Abe K, Fukazawa T, Asakawa J, Takasaki Y, Hashimoto H (1998) Apoptosis of lymphocytes induced by glucocorticoids and relationship to therapeutic efficacy in patients with systemic lupus erythematosus. *Arthritis Rheum* 41:823–830. [https://doi.org/10.1002/1529-0131\(199805\)41:53.0.CO;2-%23](https://doi.org/10.1002/1529-0131(199805)41:53.0.CO;2-%23)
 42. Olnes MJ, Kotliarov Y, Biancotto A, Cheung F, Chen J, Shi R, Zhou H, Wang E, Tsang JS, Nussenblatt R et al (2016) Effects of systemically administered hydrocortisone on the human immune system. *Sci Rep* 6:23002. <https://doi.org/10.1038/srep23002>
 43. Grossman SA, Ye X, Lesser G, Sloan A, Carraway H, Desideri S, Piantadosi S, NABTT CNS Consortium (2011)

- Immunosuppression in patients with high-grade gliomas treated with radiation and temozolomide. *Clin Cancer Res* 17:5473–5480. <https://doi.org/10.1158/1078-0432.CCR-11-0774>
44. Wong ET, Lok E, Gautam S, Swanson KD (2015) Dexamethasone exerts profound immunologic interference on treatment efficacy for recurrent glioblastoma. *Br J Cancer* 113:232–241. <https://doi.org/10.1038/bjc.2015.238>
 45. Waitkus MS, Diplas BH, Yan H (2016) Isocitrate dehydrogenase mutations in gliomas. *Neuro Oncol* 18:16–26. <https://doi.org/10.1093/neuonc/nov136>
 46. Kohanbash G, Carrera DA, Shrivastav S, Ahn BJ, Jahan N, Mazor T, Chheda ZS, Downey KM, Watchmaker PB, Beppler C et al (2017) Isocitrate dehydrogenase mutations suppress STAT1 and CD8+ T cell accumulation in gliomas. *J Clin Invest* 127:1425–1437
 47. Dudley JC, Lin MT, Le DT, Eshleman JR (2016) Microsatellite instability as a biomarker for PD-1 blockade. *Clin Cancer Res* 22:813–820. <https://doi.org/10.1158/1078-0432.CCR-15-1678>
 48. Yip S, Miao J, Cahill DP, Iafrate AJ, Aldape K, Nutt CL, Louis DN (2009) MSH6 mutations arise in glioblastomas during temozolomide therapy and mediate temozolomide resistance. *Clin Cancer Res* 15:4622–4629. <https://doi.org/10.1158/1078-0432.CCR-08-3012>
 49. van Thuijl HF, Mazor T, Johnson BE, Fouse SD, Aihara K, Hong C, Malmstrom A, Hallbeck M, Heimans JJ, Kloezeman JJ et al (2015) Evolution of DNA repair defects during malignant progression of low-grade gliomas after temozolomide treatment. *Acta Neuropathol* 129:597–607. <https://doi.org/10.1007/s00401-015-1403-6>
 50. Howitt BE, Shukla SA, Sholl LM, Ritterhouse LL, Watkins JC, Rodig S, Stover E, Strickland KC, D’Andrea AD, Wu CJ et al (2015) Association of polymerase e-mutated and microsatellite-unstable endometrial cancers with neoantigen load, number of tumor-infiltrating lymphocytes, and expression of PD-1 and PD-L1. *JAMA Oncol* 1:1319–1323. <https://doi.org/10.1001/jamaoncol.2015.2151>
 51. Brandes AA, Franceschi E, Tosoni A, Blatt V, Pession A, Tallini G, Bertorelle R, Bartolini S, Calucci F, Andreoli A et al (2008) MGMT promoter methylation status can predict the incidence and outcome of pseudoprogression after concomitant radiochemotherapy in newly diagnosed glioblastoma patients. *J Clin Oncol* 26:2192–2197. <https://doi.org/10.1200/JCO.2007.14.8163>
 52. Okada H, Weller M, Huang R, Finocchiaro G, Gilbert MR, Wick W, Ellingson BM, Hashimoto N, Pollack IF, Brandes AA et al (2015) Immunotherapy response assessment in neuro-oncology: a report of the RANO working group. *Lancet Oncol* 16:e534–e542. [https://doi.org/10.1016/S1470-2045\(15\)00088-1](https://doi.org/10.1016/S1470-2045(15)00088-1)