The Current Landscape of Immune Checkpoint Blockade in Glioblastoma

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KEYWORDS

• Glioblastoma (GBM) • Immunotherapy • Immune checkpoint inhibitor (ICI)
• Immune checkpoint blockade • Programmed cell death receptor 1 (PD-1)
• Programmed cell death ligand 1 (PD-L1) • Cytotoxic T- lymphocyte–associated protein 4 (CTLA-4)

KEY POINTS

- Immune checkpoint blockade has revolutionized the management of many solid malignancies.
- Similar positive results have not been duplicated in the treatment of glioblastoma with immune checkpoint blockade.
- There are ongoing studies to further evaluate the potential role of immune checkpoint blockade in the management of glioblastoma.
- Multimodal immunotherapy for glioblastoma is under active investigation, and results are expected to direct the future role of immunotherapy in glioblastoma.
- Identification of reliable biomarkers of response to immunotherapy treatment is essential to optimizing response in patients with glioblastoma.

BACKGROUND

Outcomes for patients with glioblastoma remains one of the poorest in oncology. Despite the emergence of multimodal therapies, prognosis is poor, with fewer than 50% of patients surviving for 1 year, and only 5% surviving beyond 5 years.[1] Immunotherapy focused on immune checkpoint blockade (ICB) has proven to be a successful approach in the management of patients with many different oncology indications. Despite tremendous interest in immunotherapies for high-grade gliomas, disease response has been low in clinical studies thus far. The theory of a largely immunosilent central nervous system (CNS) milieu as originally defined by Medawar's skin allograft transplantation studies[2] has been contested in more recent work documenting active lymphatics in the CNS[3,4] and is not consistent with known immune activation associated with CNS infections and neuroinflammatory conditions.

Multilayered immunosuppressive mechanisms deployed by glioblastoma cells complicate the quest to attack these tumors with immunotherapy. High-grade glioma cells express CD95 (Fas/apoptosis antigen 1) ligand, which induces apoptosis and T-cell suppression in the glioma microenvironment.[5,6] Similarly, the ligand for Programmed Death-1 (PD-L1) is upregulated in the glioblastoma microenvironment and has been shown to suppress T-cell recruitment and induces T-cell apoptosis.[7–9] Activation of the PD-1/PD-L1 pathway leads to a cascade of immunosuppressive mechanisms, including inhibition of tumor cell apoptosis, peripheral T effector cell exhaustion, and conversion of T effector cells to regulatory T cells (Tregs).[10] Multiple studies have shown that Treg cells also

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accumulate in the glioblastoma microenvironment to prevent the antiglioma immune response.[11–13] In addition, high-grade glioma cells secrete immunosuppressive factors, such as interleukin-10 (IL-10) and transforming growth factor-β (TGF-β).[14,15] Furthermore, the microenvironment of GBM tumors is characterized by a dominant population of myeloid cells, which can account for up to 40% of GBM tumors, and are programmed to exhibit a highly immunosuppressive phenotype.[16–18] Combined, these protective strategies form sophisticated escape routes from immune surveillance, thereby contributing to the development and progression of glioblastoma tumors. These immune escape mechanisms are discussed extensively in later sections.

Despite the emerging challenges associated with the immunosuppressed glioblastoma microenvironment, the efficacy of ICB in other cancers continues to fuel interest to further investigate these agents in neurooncology. Numerous immunotherapy approaches to glioblastoma are under evaluation, including immunomodulation with immune checkpoint inhibitors (ICIs; cytotoxic T-lymphocyte–associated antigen [anti-CTLA-4], anti–PD-1, anti–PD-L1 monoclonal antibodies); tumor antigen-specific and tumor-associated vaccines; adoptive T-cell therapies (chimeric antigen receptor T cells and bispecific T-cell engagers); and oncolytic virus therapies.[19]

THE BIOLOGICAL BASIS FOR IMMUNE CHECKPOINT INHIBITION

Evasion of the immune system by tumor cells is a major determinant of the proliferation and growth of malignant cells. At tumorigenesis, the immune system attempts to eliminate malignant cells beginning with presentation of tumor antigens by antigen-presenting cells (APC) to T cells. Antigen presentation triggers multiple sequential steps, including T-cell priming, clonal selection of antigen-specific T cells, activation and proliferation in secondary lymphoid tissues, trafficking of T cells to tumor sites, initiation of effector functions at target sites, and recruitment of other effector immune cells via cytokines and membrane ligand signaling.[20] Each step is coordinated by a balance between costimulatory/agonistic and antagonistic/inhibitory signals known as immune checkpoint proteins (checkpoints such as CTLA-4, PD-1, BTLA, VISTA, TIM-3, LAG3, and CD47; costimulatory molecules such as CD 28, CD137, OX40, and GITR) (Fig. 1). Inhibitory checkpoint proteins normally function physiologically as reins that dampen the amplitude and potency of T-cell–mediated responses.[21] This balance is essential for the prevention of autoimmunity. Nonetheless, tumor cells can exploit this normally protective mechanism by dysregulated expression of immune checkpoint proteins that can provide a mechanism of immune evasion.

The authors focus here on the classical inhibitory immune checkpoint molecules for which multiple therapeutic targets have been developed: CTLA-4 and the PD-1 pathway.

Cytotoxic T-Lymphocyte–Associated Antigen-4

CTLA-4 was identified as a major immune modulator after the discovery that CD28 costimulation plays a critical role in the activation of T cells.[22,23] Presentation of antigen proteins alone is insufficient to trigger T-cell activation because costimulatory signals in addition to T-cell receptor (TCR) engagement of foreign peptide antigen–major histocompatibility complexes (MHC) are needed to trigger T-cell activation, priming, and clonal expansion. The primary costimulatory signal is the interaction of CD28 expressed on T cells with B7-1 (CD80) and B7-2 (CD86) expressed on the surface of specialized APC.[24] B7-1 and B7-2 provide positive costimulatory signals through CD28 (Fig. 2). CTLA-4 is a homolog of CD28 that binds both B7-1 and B7-2 with greater affinity than CD28.[25,26] CTLA-4 is upregulated following TCR-tumor peptide bound MHC complex (pMHC) binding. Its expression by T cells is most active 2 to 3 days following TCR engagement.[27,28] CTLA-4

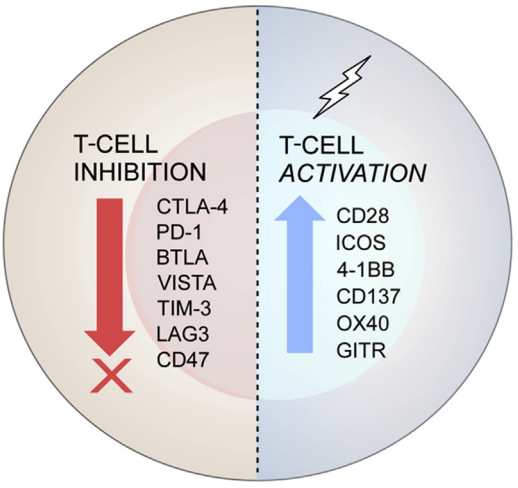

Fig. 1. Costimulatory immune modulatory proteins expressed by T cells to enhance T-cell activation (*green*). Coinhibitory immune modulatory proteins expressed following the interaction of the antigen pMHC complex with the TCR to produce attenuation of T-cell activity (*red*).

hinders TCR signaling by competing with the costimulatory CD28 molecule for the B7 ligands B7-1 and B7-2 (see Fig. 2). Because CTLA-4 has higher avidity and affinity for CD80 and CD86, it outcompetes CD28 in binding both ligands.[29,30] CTLA-4 binding limits CD28 downstream signaling (primarily mediated by PI3K and AKT), thereby dampening the stimulation of T cells.[31,32] In addition, CTLA-4 downregulates the expression of B7 ligands on APC through modulating cytokines, such as IL-10 or TGF-β, or via transendocytosis.[33] Thus, CTLA-4 primarily functions to attenuate T-cell activity at sites of T-cell priming in lymphatic tissues. Because of its central role in regulating T-cell activation, inhibition by CTLA4 is normally critical for self-tolerance and avoidance of autoimmunity.

Once the role of CTLA-4 as a negative regulator of T-cell responses was established, the possibility that blockade of immune inhibition engineered by CTLA-4 and B7-1/B7-2 interactions might augment T-cell responses to tumor cells and enhance antitumor immunity was explored. Leach and colleagues[34] provided early evidence that CTLA-4 blockade using antibodies enhanced antitumor immune responses in vivo. In mice transfected with colon carcinoma cells who were treated with anti-CTLA-4 or anti-CD28 injections, anti–CTLA-4–treated mice showed significant reduction in tumor growth.

Currently, ipilimumab is the only human CTLA-4-blocking antibody currently approved by the Food and Drug Administration (FDA).[35,36] Other CTLA-4 targeting agents, such as tremelimumab (a fully human monoclonal antibody against CTLA-4), are also under investigation in multiple cancer types.[37]

Programmed Death-1 and Programmed Death Ligand 1/2 Pathway

PD-1 or CD279 is another inhibitory molecule secreted during T-cell priming and activation. It is a cell surface receptor encoded by the *Pdcd1* gene. PD-1 is expressed by T lymphocytes, B cells, dendritic cells, macrophages, and natural killer cells. Its immunosuppressive activity is multifold. In chronic inflammatory states (eg, chronic infections and malignancies), persistent PD-1 expression causes T cells to enter into a state of metabolic exhaustion.[38,39] Like CTLA-4, PD-1 also counteracts the stimulatory signal induced by TCR engagement with CD28 via its ligands.[40] PD-L1 engages with PD-1 to provide inhibitory signals to suppress activated $CD4^+$, $CD8^+$ cells and to induce T-cell apoptosis.[41] When PD-1 engages with its ligands PD-L1 (B7-H1) and PD-L2 (B7-H2), dephosphorylation of protein tyrosine phosphatases (PTPs), such as SHP2, occurs.[42] PTP dephosphorylation leads to antagonism of positive signals typically mediated by TCR and CD28, and the inhibition of downstream signaling pathways (Fig. 3). The result is decreased T-cell activation, proliferation, survival, and cytokine production.[40] Although PD-L1 is expressed primarily by tumor cells and myeloid cells (such as macrophages), PD-L2 is nearly exclusively expressed some myeloid cells. This myeloid activity is essential to the inhibition of immunity, as myeloid cell expression of PD-L1/2 contributes to the inhibition of T cells in the tumor microenvironment.[43]

Altogether, the PD-1 and PD-L1/2 pathway triggers immunosuppressive mechanisms, including cytokines that lead to the inhibition of tumor cell apoptosis, peripheral T effector cell anergy, and conversion of T effector cells to Tregs.[44–46]

PD-L1 staining has been reported in glioblastoma tissues to varying extents and expression differs in molecular glioblastoma subtypes. Berghoff and colleagues[7] reported prominent expression of PD-L1 by glioma cells in most of their human glioblastoma samples. They reported low PD-L1 expression in proneural glioblastoma subtypes; meanwhile, high PD-L1 expression was observed in the mesenchymal glioblastoma

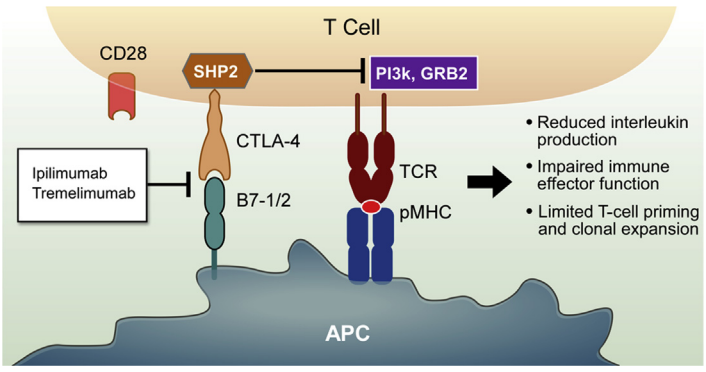

Fig. 2. CTLA-4 inhibits TCR signaling, thereby limiting interleukin production, T-cell priming, and survival. Anti-CTLA antibodies act to block this pathway.

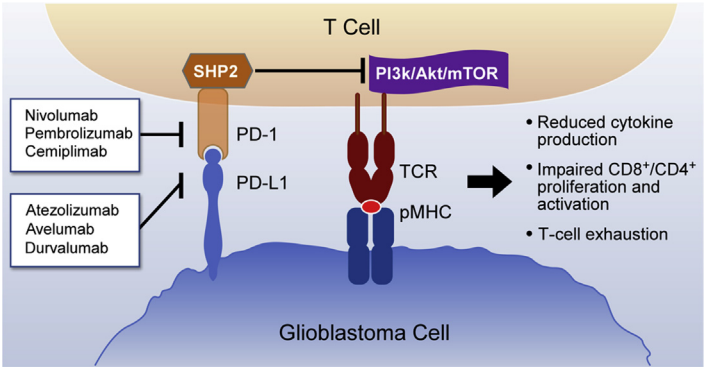

Fig. 3. PD-1/PD-L1 interaction inhibits TCR signaling to impair T-cell proliferation and to induce T-cell exhaustion. Anti–PD-1 and anti–PD-L1 antibodies act to block this pathway.

subtypes. Similarly, Heiland and colleagues[47] showed that PD-L1 expression was elevated in high-grade gliomas compared with lower-grade gliomas. They also reported increased PD-L1 expression in mesenchymal glioma types. PD-L1 presence in the glioblastoma microenvironment was associated with activation of the MAPK pathway. Finally, Nduom and colleagues[48] showed that most GBM tumors (61%) demonstrated PD-L1 expression as defined by detection among $\geq$1% of cells.

Therapeutic antibodies against PD-L1 (atezolizumab, avelumab, and durvalumab) and PD-1 (nivolumab, pembrolizumab, and cemiplimab) have been developed. These agents have demonstrated varying levels of efficacy in different cancer types (**Table 1**).

Beyond the Cytotoxic T-Lymphocyte–Associated Antigen-4, Programmed Death-1/Programmed Death Ligand-1 Pathways

T cells recruited into the glioblastoma tumor environment tend to overexpress PD-1, CTLA-4, and other inhibitory regulators (TIM-3, LAG-3, CD160, 2B4, TIGIT, CD39, and BTLA).[49,50] The immunosuppressive glioblastoma microenvironment is further enhanced by the expression of PD-L1 by microglia. PD-L1 expression by microglial cells is amplified when in close proximity to GBM cells, which in turn may promote apoptosis of cytotoxic T cells, thereby sparing glioma cells from T-cell–mediated killing.[51]

Tumor-infiltrating myeloid cells (TIM) further enhance immune resistance in the tumor microenvironment. Myeloid cells derived from healthy tissues typically express immunostimulatory cytokines to stimulate the proliferation and antitumor function of T cells and natural killer cells. In contrast, tumor-associated macrophages (TAMs) have poor antigen-presenting capability and produce factors that suppress T-cell proliferation and activity. Mantovani and Sica[52] showed that exposure to IL-4 and IL-10 in tumors may induce TAMs to develop an immunosuppressive polarized type II phenotype, and these are referred to as M2 macrophages.

TAMs markedly infiltrate the tumor microenvironment. Macrophage differentiation, growth, and infiltration are regulated by several growth factors, including colony stimulating factor-1 (CSF-1). Treatments such as radiation, chemotherapy, and immunotherapies induce CSF-1 secretion from tumor cells, which promotes the influx of myeloid cells into the tumor microenvironment.[43] Overexpression of CSF-1 and chemokine (C-C motif) ligand 2 (CCL2), regulatory molecules for macrophages, has been associated with poor prognosis in multiple solid malignancies,[53] including glial tumors. Ding and colleagues[54] showed that M2-type macrophages were present in all glioma grades with higher expression levels associated with higher-grade gliomas. Flow cytometry studies demonstrate that tumor-infiltrating monocytes/macrophages from patients with GBM exhibit increased expression of PD-L1 (B7-H1).[55] Although PD-L1 was previously understood to be secreted by glioma cells, studies suggest that TIMs may in fact be the major source of PD-L1 in the glioma microenvironment.[43,56] Therefore, TIMs form a critical component of glioma immunosuppression that is influenced by many factors, including PD-1/PD-L1 pathway.

PRECLINICAL STUDIES

In a study of mice injected with malignant glioma cells (SMA560), CTLA-4 blockade using a monoclonal antibody to CTLA-4 produced long-term survival in some treated mice. In addition, $CD4^+$ T-cell activity was restored and Treg-mediated immunosuppression was ameliorated.[57] In a subsequent study, combining anti–CTLA-4 blockade with granulocyte-macrophage colony-stimulating factor (GM-CSF) expressing whole glioma cell vaccination was shown to successfully increase

Table 1
Selected Food and Drug Administration–approved immune checkpoint blockade agents for solid malignancies[a]

Tumor Type	ICB Agent	Immune Checkpoint Target	Year of FDA Approval
Melanoma			
Melanoma (unresectable or metastatic)	Ipilimumab	CTLA-4	2011
Melanoma (progressed following treatment with ipilimumab)	Nivolumab	PD-1	2014
Melanoma (unresectable or metastatic)	Pembrolizumab	PD-1	2014
Melanoma (BRAF wild type)	Ipilimumab + nivolumab	CTLA-4 + PD-1	2015
Melanoma (adjuvant)	Ipilimumab	CTLA-4	2015
Melanoma (any BRAF status)	Ipilimumab + nivolumab	CTLA-4 + PD-1	2016
Lung/pleural malignancies			
Non–small cell lung cancer	Nivolumab	PD-1	2015
Non–small cell lung cancer	Pembrolizumab	PD-1	2015
Non–small cell lung cancer	Atezolizumab	PD-L1	2016
Non–small cell lung cancer	Durvalumab	PD-L1	2018
Small cell lung cancer (extensive)	Atezolizumab	PD-L1	2019
Mesothelioma	Nivolumab + ipilimumab	PD-1 + CTLA-4	2020
Genitourinary carcinomas			
Renal cell carcinoma	Nivolumab	PD-1	2015
Urothelial carcinoma	Atezolizumab	PD-L1	2016
Urothelial carcinoma	Avelumab	PD-L1	2017
Urothelial carcinoma	Durvalumab	PD-L1	2017
Urothelial carcinoma	Nivolumab	PD-1	2017
Urothelial carcinoma	Pembrolizumab	PD-1	2017
Renal cell carcinoma	Ipilimumab + nivolumab	CTLA-4 + PD-1	2018
Gastrointestinal tract/hepatobiliary tumors			
MSI-high, MMR-deficient metastatic colorectal cancer	Nivolumab	PD-1	2017
Microsatellite instability (MSI)-high or Mismatch repair (MMR)-deficient solid tumors of any histology	Pembrolizumab	PD-1	2017
Hepatocellular carcinoma	Nivolumab	PD-1	2017
Gastric and gastroesophageal carcinoma	Pembrolizumab	PD-1	2017
Other cutaneous cancers			
Merkel cell carcinoma	Avelumab	PD-L1	2017
Merkel cell carcinoma	Pembrolizumab	PD-1	2018
Advanced cutaneous squamous cell carcinoma	Cemiplimab	PD-1	2018

[a] Summary of FDA approval for selected immune checkpoint blockade therapies granted as of October 2020.

survival in glioma-injected mice.[58] Another murine experiment showed that mice harboring an intracranial GL-261 glial tumor had improved survival when treated with single-agent or combination monoclonal antibodies against PD-1, PD-L1, and CTLA with the greatest benefit reported in mice treated with the combination of PD-1 plus CTLA-4 blockade.[59]

Additional studies investigated combining ICB with standard therapies, such as radiation. Zeng and colleagues treated mice inoculated with GL-261 glioma tumors with anti–PD-1 antibody only or radiation plus anti–PD-1 antibody. They reported no significant survival benefit in the antibody-alone arm (27 days), but longer survival was noted in the radiation plus anti–PD-1 antibody arm (53 days).[60] They also reported increased glioma infiltration by $CD8^+$ effector cells and downregulation of Tregs.[60] These results suggested that immune checkpoint blockade could work synergistically with radiation to create a proinflammatory tumor environment against glioma cells.

Although these preclinical studies promised significant efficacy of immune checkpoint blockade in gliomas, this promise has not been achieved in clinical studies. One reason for the disconnect between preclinical and clinical experiences is that GL-261, the most widely used syngeneic mouse orthotopic glioma cell line used in murine ICI experiments,[57,59,61] exhibits robust immunogenicity and capacity to propagate an immune response. This murine tumor has been shown to possess a striking tumor mutational burden,[62] whereas human malignant glioma tumors typically exhibit a low tumor mutational burden (TMB).[63] The low mutational load in human GBM tumors has been demonstrated to be a prognosticator of poor response to ICB therapies.[64] Genoud and colleagues[65] introduced a murine glioma model (SB28) with a low mutational burden and poor immunogenic activity, which is more comparable to human glioma tumors. Murine GBM models that are less intrinsically immunogenic may be more informative to guide immunotherapy drug development for GBM patients.

CLINICAL STUDIES

Several anti–CTLA-4, anti–PD-1, and anti–PD-L1 antibodies have been tested in the context of brain tumors and in other solid tumors, including a number that have received approval by the FDA (see **Table 1**). Ipilimumab, a fully humanized monoclonal antibody that inactivates CTLA4, was first approved by the FDA for unresectable, advanced (stage III or IV) melanoma in 2011.[66] Multiple studies have also demonstrated activity of ipilimumab against brain metastases, including an open-label phase 2 trial, which reported a 24% response rate to ipilimumab among corticosteroid-naïve patients with brain metastases treated with ipilimumab.[67]

Clinical trials investigating blockade of the PD-1/PD-L1 pathway have shown efficacy in the treatment of many solid cancers. PD-1/PD-L1 pathway inhibition has also shown encouraging activity for some patients with brain metastases.[68]

Programmed Death-1/Programmed Death Ligand-1 Blockade in Glioblastoma

Nivolumab

Results of the first randomized phase 3 trial to evaluate immune checkpoint inhibition in patients with glioblastoma (CheckMate-143) were recently reported.[69] In this open-label trial, patients with first recurrence of glioblastoma after standard chemoradiation therapy were randomized 1:1 to 3 mg/kg of nivolumab (n = 184) or 10 mg/kg of bevacizumab (n = 185) every 2 weeks. Median overall survival (OS) was similar in both groups at 9.8 months (95% confidence interval [CI], 8.2–11.8 months) with nivolumab versus 10.0 months (95% CI, 9.0–11.8 months) with bevacizumab (hazard ratio [HR], 1.04; 95% CI, 0.83–1.30; $P = .76$). However, a planned subgroup analysis demonstrated that patients with MGMT methylated tumors and no baseline steroid use showed improved survival with nivolumab with a median OS of 17 months (n = 31) versus 10.1 months for bevacizumab (n = 25). Mean progression-free survival (PFS) disfavored nivolumab (1.5 months for nivolumab and 3.5 months for bevacizumab; $P < .001$). Similarly, the objective response rate (ORR) in evaluable patients in the nivolumab (n = 153) arm versus bevacizumab (n = 156) arm was 7.8% and 23%, respectively. Notably in those who achieved response, the effect was more durable for nivolumab (11.1 months) versus bevacizumab (5.3 months). Steroid use seemed to confound the clinical benefit picture, as patients in the nivolumab cohort who were on steroids at baseline had a median OS of 7 months compared with 12.6 months among patients without baseline steroid use. Similar rates of grade 3 or 4 treatment-related adverse effects (TRAEs) were observed in both arms: nivolumab (18.1%) and bevacizumab (15.2%).[69]

CheckMate-498[70] is an open-label, randomized phase 3 study that compares the OS of nivolumab or temozolomide (TMZ), each in combination with radiotherapy and then after radiotherapy, in patients with newly diagnosed MGMT-unmethylated GBM. Data from CheckMate-498 remain unpublished at this time. However, in May 2019, Bristol-Myers Squibb (BMS) announced that CheckMate-498 did not meet its primary endpoint of OS on final analysis.[71]

CheckMate-548 is a placebo-controlled, blinded, randomized phase 3 study evaluating nivolumab combined with concurrent standard chemoradiation versus standard of care in patients with newly diagnosed MGMT-methylated glioblastoma. BMS announced that the trial did not meet one of its primary endpoints, PFS, in September

2019.[72] Outcome for the primary endpoint of median OS has not been released.

Pembrolizumab

Keynote-028 is a basket study of pembrolizumab for various tumor types that included an arm for recurrent glioblastoma (n = 26). Patients received pembrolizumab 10 mg/kg every 2 weeks for up to 24 months. The primary end point was ORR per RECIST v. 1.1 guidelines. Among GBM patients, there was 1 partial response (n = 25; ORR, 4.0%, 95% CI, 0.1–20.4); 12 patients (48.0%) had stable disease. Median PFS was 2.8 months (95% CI, 1.9–9.1), and median OS was 14.4 months (95% CI, 10.3–not reached). TRAEs were reported in 19 (73.1%) patients, most commonly fatigue and rash (n = 6 each, 23.1%). Four (15.4%) patients experienced grade 3 or 4 TRAEs (lymphopenia, type 2 diabetes mellitus, arthritis, and syncope). None of the patients died or discontinued pembrolizumab because of a treatment-related adverse event.[73]

Another phase 2 study (NCT02337491) investigated the use of pembrolizumab (200 mg intravenously [IV] every 3 weeks) with bevacizumab (cohort A) or without (cohort B) bevacizumab (10 mg/kg IV every 2 weeks), in bevacizumab-naïve patients at first or second recurrence of glioblastoma.[74] The primary endpoint was PFS at 6 (PFS-6) months per RANO guidelines for each cohort. PFS-6 was 26% (95% CI, 16.3–41.5) in cohort A and 6.7% (95% CI, 1.6–25.4) in cohort B. Median OS was 8.8 months (95% CI, 7.7–14.2) in cohort A and 10.3 months (95% CI, 8.5–12.5) in cohort B. There were no grade 4 or 5 TRAEs reported. Grade 2 or 3 TRAEs occurred in $\geq$10% patients, including cohort A, hypertension (50%), fatigue (18%), headache (16%), infection (14%), and proteinuria (14%); cohort B, headache (30%) and fatigue (17%).

Atezolizumab

Atezolizumab is a humanized monoclonal antibody directed against PD-L1. It is approved in the treatment of patients with advanced metastatic urothelial carcinoma after the failure of platinum-based chemotherapy and the treatment of patients with metastatic non–small cell lung carcinoma.[75,76] There are multiple ongoing phase 1/2 studies evaluating atezolizumab for GBM, including combinations with (1) standard chemoradiation (NCT03174197); (2) D2C7-IT, a dual-specific EGFRwt/EGFRvIII monoclonal antibody (NCT04160494); and (3) ipatasertib, a selective inhibitor of AKT isoforms 1/2/3 (NCT03673787). The results of these trials have not been released.

Durvalumab

Durvalumab is a humanized monoclonal antibody directed against PD-L1 approved for the treatment of patients with advanced urothelial carcinoma.[77] Preliminary results of a phase 2 study of durvalumab (NCT02336165) that includes 1 arm of newly GBM diagnosed patients and 4 arms of recurrent GBM patients showed overall acceptable tolerability of the drug. The study is ongoing, and finalized data on efficacy are expected in the near future.[78]

Avelumab

Avelumab is a PD-L1 inhibitor approved for the treatment of metastatic Merkel-cell carcinoma, metastatic urothelial carcinoma, and advanced renal cell carcinoma.[79] NCT03341806 is an ongoing phase 1 study evaluating avelumab combined with laser interstitial thermal therapy (LITT) in patients with recurrent glioblastoma.

Cytotoxic T-Lymphocyte–Associated Antigen-4 Blockade in Glioblastoma

In an exploratory phase 1 cohort of CheckMate-143, the tolerability and efficacy of nivolumab and ipilimumab in recurrent glioblastoma were tested. In a small cohort, the addition of ipilimumab to nivolumab did not appear to improve OS, and nivolumab monotherapy was better tolerated when compared with combination therapy.[80] In a case series of 20 patients with recurrent glioblastoma treated with ipilimumab and bevacizumab, 31% of patients showed a partial response, 31% had stable disease, and 38% had disease progression.[81] There are limited studies testing ipilimumab or CTLA-4 blockade in glioblastoma. The Ipi-Glio trial testing adjuvant ipilimumab with temozolomide versus temozolomide alone was recently announced.[82] NCT02794883 is an active phase 2 trial designed to test tremelimumab (anti–CTLA-4 monoclonal antibody) and durvalumab (anti–PD-L1 antibody) as monotherapies and combination therapies among patients with recurrent malignant glioma.

MULTIMODAL IMMUNOTHERAPIES IN GLIOBLASTOMA

Given the heterogeneity of glioblastoma tumors and the multiple immune escape mechanisms deployed by these tumors, combinatorial treatment approaches will likely be required to achieve meaningful therapeutic benefit. Current strategies include combining immune checkpoint inhibitors, cytotoxic therapy, including radiation therapy, antiangiogenesis agents, targeted therapies, and other immunotherapy modalities (**Table 2**).

Combination with Oncolytic Viruses

There are multiple clinical trials underway that are currently evaluating oncolytic viruses combined

Table 2
Selected active, recruiting clinical trials of immune checkpoint blockade in high-grade gliomas[a]

Clinical Trial Number	Intervention	Combinational Strategy	Phase	Disease	Primary Outcome Measure
NCT03367715	Nivolumab + Ipilimumab + Short-course radiotherapy	Anti-PD-1 + Anti–CTLA-4 +Radiation therapy	2	Newly diagnosed, MGMT unmethylated glioblastoma	OS
NCT04396860	Ipilimumab + Nivolumab + radiation vs standard chemoradiation	Anti–CTLA-4 + Anti–PD-1 + Radiation therapy	2/3	Newly diagnosed, MGMT unmethylated glioblastoma	PFS OS
NCT04013672	Pembrolizumab + SurVaxM	Anti-PD-1 + Tumor-specific antigen vaccine	2	Recurrent glioblastoma	PFS
NCT03018288	Radiation + temozolomide + pembrolizumab ± Heat shock protein peptide-complex (HSPPC-96)	Anti-PD-1 + Chemoradiation + Tumor-derived peptide vaccine	2	Newly diagnosed glioblastoma	OS
NCT04479241	PVSRIPO and pembrolizumab	Anti-PD-1 + Oncolytic virus	1	Recurrent glioblastoma	Safety and tolerability
NCT03341806	Avelumab + LITT	Anti-PD-L1 + Laser interstitial thermotherapy	1	Recurrent glioblastoma	ORR Dose-limiting toxicity
NCT04160494	Atezolizumab + D2C7-IT	Anti-PD-L1 + EGFRwt/vIII immunotoxin	1	Recurrent (World Health Organization) grade IV malignant glioma	Tolerability
NCT02866747	Durvalumab + Hypofractionated stereotactic radiotherapy	Anti-PD-L1 + Radiation therapy	1/2	Recurrent glioblastoma	OS Dose-limiting toxicity

[a] Selected active, recruiting clinical trials as of October 2020.

with checkpoint inhibitors. Oncolytic viruses induce immunogenic cell death leading to increased tumor antigen release. Coadministration of anti–PD-1 or anti–CTLA 4 antibody will decrease compensatory enhanced expression of inhibitory immune checkpoints in this setting, thereby allowing the development of a robust immune response. The CAPTIVE trial (NCT02798406) is an ongoing phase 2 study investigating the oncolytic adenovirus DNX-2401 and pembrolizumab in patients with recurrent glioblastoma.[83]

Combination with Vaccines

Integrating tumor vaccines with immune checkpoint inhibitors has been shown to improve long-term survival in murine glioma studies.[58,84] NCT02287428 is an ongoing phase 1 study evaluating a personalized neoantigen cancer vaccine derived from glioma-specific protein-coding mutations in combination with pembrolizumab and radiation therapy. An autologous tumor lysate-loaded dendritic cell vaccine is being tested in combination with ICB in a phase 1 clinical trial among patients with recurrent glioblastoma (NCT04201873).

CHALLENGES OF IMMUNE CHECKPOINT INHIBITION THERAPY

Biomarkers that may predict response to ICB are emerging for many cancers, but their utility for GBM patients is not well understood. High-tumor mutational variance has been associated with an increased rate of immunogenic neoantigens that could trigger a robust immune response.[85] However, glioblastoma tumors exhibit a relatively small tumor mutational variance compared with other solid tumors.[86] Temozolomide, a cornerstone of glioblastoma treatment, is myelosuppressive and has been shown to increase the proportion of exhausted T cells in mice.[86] T-cell exhaustion can reduce the response to checkpoint blockade, suggesting that baseline T-cell exhaustion may be a negative biomarker of response. Immunophenotyping of the glioblastoma microenvironment has shown a paucity of immune-effector cells.[87] TAM and Tregs are immunosuppressive cells that are prevalent in glioblastoma tissues.[88,89]

Furthermore, molecular genetic abnormalities of GBM tumors may lead to immunomodulation of the GBM TME and may contribute to differentiating ICB responders from nonresponders. In their retrospective analysis of 66 patients with recurrent GBM who received anti–PD-1 therapy, Zhao and colleagues[90] identified distinct molecular genetic signatures in 17 patients who were responders (14 months OS) versus 49 patients who were nonresponders (10 months OS). Genomic and transcriptomic analysis of both cohorts revealed *PTEN* mutations to be associated with immunosuppressive gene signatures that were more enriched in nonresponders, whereas there was enrichment of MAPK pathway alterations (PTPN11, BRAF) in responders. Interestingly, studies in melanoma have shown that loss of PTEN function in tumor cells correlates with decreased T-cell recruitment, decreased T-cell–mediated cell death, and poorer outcomes with PD-1 inhibitor therapy.[91] Furthermore, Zhao and colleagues[90] also demonstrated that failure of immune checkpoint therapy may occur because of posttreatment genetic immune-editing. In their analysis of the clonal alterations of mutations after treatment, they found 3 missense mutations (MYPN R409H, UBQLN3 R159W, CYP27B1 G194E) that were present before anti–PD-1 therapy but were not detectable after immune checkpoint therapy. These results suggest that immune checkpoint therapy may lead to immune editing and loss of immunogenic mutations as a mechanism of treatment resistance.

In addition, questions about the optimal timing of ICB for patients with glioblastoma remain unanswered. Data suggest that cytoreductive surgery may increase efficacy by reducing residual tumor burden to be attacked by the mounted anticancer immune response. However, the best opportunity window (ie, before resection of recurrent tumor or after resection of recurrent tumor) remains unclear. Cloughesy and colleagues[92] conducted a randomized trial comparing 2 arms of pembrolizumab: before (neoadjuvant) and after (adjuvant) surgery versus adjuvant therapy only in 35 patients with recurrent, surgically resectable glioblastoma. Patients who received the neoadjuvant pembrolizumab arm (n = 16) had significantly improved OS (13.7 vs 7.5 months, $P = .04$) and PFS (3.3 vs 2.4 months, $P = .03$) compared with the adjuvant pembrolizumab (n = 19) only. The investigators also reported an increase in transcription of genes related to T-cell expansion and interferon-γ responsiveness in patients treated with neoadjuvant pembrolizumab. These findings support the hypothesis that neoadjuvant use of immune checkpoint inhibitors may enhance immune responses and improve efficacy of ICB.

Clinically, corticosteroids for the management of symptomatic cerebral edema among patients with glioblastoma present another potential challenge to immunotherapy. Corticosteroids, which are routinely used to decrease cerebral edema in patients with brain tumors, are

immunosuppressive. Data on the impact of steroids on ICB in solid malignancies have been mixed, with some studies showing reduced efficacy[93,94] and other studies reporting no significant effect.[95,96]

Immune-related adverse events (irAEs) are the primary toxicities of ICIs. These side effects are generally more severe when combined CTLA-4 and PD-1/PD-L1 inhibition therapies are used and less prevalent with PD-1/PD-L1 monotherapy. They can affect multiple organs simultaneously and may become life-threatening if not addressed. Some of the most commonly reported irAEs include colitis, pneumonitis, hepatitis, myocarditis, hypophysitis, and encephalitis.[97] For these reasons, patients receiving ICIs are regularly monitored for treatment-related complications.

FUTURE DIRECTIONS

Identification of reliable biomarkers of response to immune checkpoint blockade and other immunotherapies is critical to the success of these approaches in patients with glioblastoma. The spectrum of possible predictive biomarkers is wide, and the expression of these biomarkers is highly variable with conflicting data on the strength of their association with survival.[98,99] Studies investigating other mechanisms of immune evasion, such as downregulated expression of MHC class I and II molecules[100,101] in the glioma environment, should also be considered. Given the number of molecules involved in the T-cell activation pathway (inhibitory molecules, such as BTLA, VISTA, TIM-3, LAG3, and CD47; co-stimulatory molecules, such as CD137, OX40, and GITR), the potential for combinatorial immunotherapy strategies in cancer and glioma treatment is promising.

SUMMARY

The management of hematologic and solid malignancies with immunotherapies, such as immune blockade, has yielded remarkably favorable results. In contrast, clinical studies investigating ICB in patients with glioblastoma have yielded disappointing results thus far. Nevertheless, several exciting immunotherapeutic approaches, including combinatorial regimens, are being investigated to overcome challenges associated with the dominantly immunosuppressive tumor microenvironment in order to generate effective antiglioma responses. Results of ongoing clinical trials are expected to clarify the future role of immune checkpoint blockade and other immunotherapies in the management of glioblastoma.

CLINICS CARE POINTS

- Immune checkpoint blockade in patients with glioblastoma has yielded disappointing results thus far.
- Results of ongoing clinical trials are expected to clarify the future role of immune checkpoint blockade and other immunotherapies in the management of glioblastoma.
- Corticosteroids are frequently prescribed to glioblastoma patients to treat symptomatic cerebral edema. However, these agents have immunosuppressive effects that may limit efficacy of immunotherapy approaches in patients with glioblastoma.
- Immune checkpoint inhibitors may cause immune-related adverse events, which range from mild to marked in severity that require proactive monitoring to mitigate as well as specialized care.

DISCLOSURE

O.O. Akintola has nothing to disclose. D.A. Reardon is an advisor to Abbvie; Advantagene; Agenus; Amgen; Bayer; Bristol-Myers Squibb; Celldex; DelMar; EMD Serono; Genentech/Roche; Imvax; Inovio; Medicenna Biopharma, Inc; Merck; Merck KGaA; Monteris; Novocure; Oncorus; Oxigene; Regeneron; Stemline; Sumitono Dainippon Pharma; Taiho Oncology, Inc.

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