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Immune Checkpoint Inhibitors in Gliomas

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Abstract
Purpose of Review Malignant gliomas result in disproportionately high morbidity and mortality compared with other primary tumors, and progression of disease is inevitable. Novel therapies to improve outcomes are needed and immune checkpoint inhibitors hold significant promise.
Recent Findings A limited body of preclinical evidence suggests that checkpoint inhibitors may be effective treatment for gliomas. Biomarkers to identify characteristics of gliomas responsive to these therapies will be essential. These may include mismatch repair deficiency and high mutational load that might be germline, somatic, or acquired after therapy. Evidence on the use of immune checkpoint inhibitors in gliomas is evolving. Clinical trials are underway and results are eagerly awaited.
Summary Understanding the role of immune checkpoint inhibitors in combination with other treatment modalities for gliomas is crucial to the improvement of outcomes. The design and conduct of future clinical trials need to account for increasingly complex treatment options.

Keywords Glioma · Glioblastoma · Anti-PD-1 · Anti-PD-L1 · Anti-CTLA-4 · Immune checkpoint inhibitors

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Introduction

Malignant gliomas account for approximately 70% of primary brain tumors in adults [1]. They comprise predominantly glioblastomas, anaplastic astrocytomas, and anaplastic oligodendrogliomas. Despite the standard first-line treatment—maximal safe surgical resection followed by radiotherapy and temozolomide—prognosis remains poor [2]. Morbidity and mortality are disproportionately high compared with that of other primary tumors, and progression of disease is inevitable. The nonspecific nature of conventional therapy often results in incapacitating damage to surrounding normal brain. Furthermore, malignant gliomas are markedly heterogeneous. This poses logistical challenges for targeted therapeutics but potential immunological targets. A hallmark of all cancers is evasion of the immune system [3]. Cancer cells escape attack from immune cells by mechanisms typically employed by the immune system to regulate itself. In glioblastoma, profound host immunosuppression can be mediated by a wide variety of mechanisms. Immune checkpoint inhibitors have also drawn increasing attention and enthusiasm since recent approvals for other advanced cancers, evidence of operational immune checkpoint expression in glioblastoma, and data from preclinical models.

What Are Immune Checkpoint Inhibitors?

Immune checkpoint inhibitors consist of antibodies that target negative immunologic regulators, such as cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), programmed cell death protein 1 (PD-1), and programmed cell death ligand 1 (PD-L1), to inactivate pathways that suppress T cell response against tumor cells [4]. PD-L1 binds to PD-1 expressed on T cells, B cells, dendritic cells, and natural killer T cells to suppress anticancer immunity. Thus, anti-PD-L1 and anti-PD-1 antibodies attempt to reverse the process whereby the tumor evades the immune system. Ipilimumab (anti-CTLA-4), nivolumab (anti-PD-1), and pembrolizumab (anti-PD-1) have been approved for melanoma and other malignant tumors. [5–10]. More than 20 other checkpoint inhibitors are at various stages of development (Table 1). This review explores the evidence to date on the use of checkpoint inhibitors in patients with gliomas.

Why Should Immune Checkpoint Inhibitors Work in Gliomas?

There is evidence that checkpoint inhibitors may be of value in tumors that express PD-L1, suggesting that this is an operational immune-suppressive pathway [11•]. Additionally, the presence of tumor-infiltrating lymphocytes (TIL) indicates that tumor-specific components of the adaptive immune system may be able to penetrate the central nervous system (CNS) to initiate an anticancer immune response. Immune surveillance in the CNS and the role of myeloid cells in the CNS is far more complex than previously thought [12], which has important implications for the potential efficacy of immune checkpoint inhibitors in the CNS microenvironment. The number of TIL has been associated with cancer patient outcomes. Some studies have suggested a positive correlation with better prognosis [13–15]. Others have shown no correlation [16] or a negative correlation [17]. These studies may have been significantly heterogeneous in tumor grade, histology, and treatment. Subsets of TIL may differ, as shown in a study where level of $CD8^+$ T cells and tumor grade were inversely correlated and the level of $CD4^+$ T cells and tumor grade were positively correlated [18•]. In a cohort of 264 gliomas, high levels of $CD4^+$ T cells combined with low levels of $CD8^+$ T cells were associated with a poorer overall prognosis. $FoxP3^+$ regulatory T cells (Tregs) were also found in glioblastomas and not in low-grade gliomas [18•]. This is in line with previous studies showing they were most frequently found in glioblastomas [19, 20]. One study, however, found no correlation with levels of Tregs and prognosis, suggesting gliomas may also mediate immunosuppression through other mechanisms [19]. The incidence of PD-L1 expression in glioblastoma is modest, with only 61% of tumors in one study having at least 1% of tumor cells expressing PD-L1 [11•], although 38% of tumors harbored at least 5% PD-L1 expression. Concordant expression of PD-1 on TIL and PD-L1 expression was to some extent associated with poorer outcomes. PD-1, PD-

Table 1 Immune checkpoint inhibitors in clinical trials

Target	Drug name	Class	Phase
CTLA-4	Ipilimumab (MDX-010, MDX-101)	Human IgG1/kappa	I/II/III/IV
	Tremelimumab (ticilimumab, CP-675206)	Human anti-CTLA4 IgG2 mab	I/II/III
PD-1	Nivolumab (ONO-4538, MDX-1106, BMS-936558)	Human IgG4/kappa	I/II/III/IV
	Pembrolizumab (lambrolizumab, MK-3475)	Humanized IgG4	I/II/III
	Pidilizumab (CT-011)	Humanized IgG1	I/II
	AMP-514 (MEDI0680)	Humanized IgG4	I/II
	REGN2810	Fully humanized IgG4	I
PD-L1	BMS936559 (MDX-1105)	Human IgG4	I
	Atezolizumab (MPDL3280A, RG7446)	Human IgG1	I/II/III
	Durvalumab (MEDI4736)	Humanized IgG1	I/II
	Avelumab (MSB0010718C)	Fully humanized IgG1	I/II/III
PD-L2	AMP-224	PD-L2-IgG2a fusion protein	I
LAG-3 (CD223)	IMP321	Chimeric IgG1	I/II
	BMS-986016		I/II
KIR	Lirilumab (IPH2101, BMS-986015)	Humanized IgG4	I/II
4-1BB (CD137)	Urelumab (BMS-663513)	Human IgG4	I
GITR (CD357)	TRX518	Humanized IgG1	I
CD27	Varlilumab (CDX-1127)	Human IgG1	I/II
OX40	MEDI6383	Human OX40 ligand fusion protein	I

L1, and TIL appear to be positively correlated with tumor grade in all gliomas, and levels of expression are higher in glioblastoma [21]. The data, collectively, have confounding features. First, it is unclear whether PD-1/PD-L1 expression correlates with treatment response, because clinical responses can be identified in cohorts that do not express PD-1 or PD-L1 [22]. Second, it is uncertain whether expression of PD-1 and/or PD-L1 on the immune cells should be considered [23]. Third, tissue testing of PD-L1 expression has been a challenge, with various antibodies and techniques with variable cut points for positivity having been established [24]. Nonetheless, the cumulative data indicate that immune checkpoint expression is operational in at least a subset of glioblastomas.

Preclinical animal models also seem to support the use of checkpoint inhibitors in gliomas. Anti-PD-1, anti-PD-L1, and anti-CTLA-4 therapies have been evaluated as single agents or in combination (Table 2). Each therapy alone produces small increments in long-term tumor-free survival. The combination of anti-CTLA-4 and anti-PD-1 has resulted in a 75% long-term response rate in an orthotopic, immunocompetent murine glioblastoma model [25]. In this animal study, there were increased numbers of activated CD8+ and natural killer cells with reductions in suppressive immune cells in the tumor microenvironment [25]. Combined blockade of CTLA-4, PD-1, and indoleamine dioxygenase (IDO) [26], and anti-CTLA-4 therapy alone [27] have also resulted in long-term survival in other murine models of glioblastoma, and combined blockade of CTLA-4 and IL-12 was shown to increase numbers of effector T cells and decrease Tregs [28]. Sequential vaccination with granulocyte-macrophage colony-stimulating factor (GM-CSF) followed by CTLA-4 blockade prolonged survival in mice with intracranial glioma [29]. Another study in mice, found an additive effect of anti-PD-1 therapy with stereotactic radiosurgery in terms of improved survival for the combined treatment, as compared to control, radiation alone, and anti-PD-1 antibody alone [30]. A key caveat of the model system used in many of these studies is that the GL261 glioma is moderately immunogeneic and expresses clonotypic, homogeneous, and rather robust levels of PD-L1, which is not the case in human gliomas [11•]. Therefore, the preclinical results may be overestimating the impact of immune checkpoint inhibitors. Intriguingly, much more robust preclinical vetting and justification for other therapeutic approaches have not generated the same volume of clinical trials [31].

Clinical Studies

Initially, a small number of glioblastoma patients were included in phase I studies of immune checkpoint inhibitors for solid tumors, such as those with pembrolizumab (KEYNOTE-028, NCT02054806) [32]. This example and another phase 1 study that included glioblastoma patients [33] suggested that, as in other cancers, only some patients benefit from these agents, indicating the need for reliable biomarkers to identify responders. A review of 22 patients (17 adults and 5 children) treated with pembrolizumab (median of three infusions in the adults) for recurrent primary CNS tumors showed progressive tumor growth during therapy. Two glioblastoma patients had tumor resection following treatment with pembrolizumab. PD-L1 staining of the tumor tissue was negative, with minimal tumor-lymphocytic response. Carter et al. [34] reported a case series of 20 patients treated with a combination of ipilimumab and bevacizumab, of whom three were treated after palliative radiotherapy, one after first-line chemoradiation, and 16 for recurrent disease. Approximately one third had a partial response, one third had stable disease, and one third had disease progression. A retrospective review of ten patients who received ipilimumab for recurrent glioblastoma found that progression-free survival and overall survival were similar to rates in historical controls treated with salvage chemotherapy but superior to rates in those who received no further treatment after first-line therapy [35]. In another case series of seven patients with recurrent high-grade glioma treated with ipilimumab, one patient progressed at 19.5 months but the others progressed before 6 months [36]. A further study of four additional patients with glioblastoma were treated with ipilimumab followed by pembrolizumab at progression, with concurrent bevacizumab and GM-CSF throughout. Two patients had a partial response on ipilimumab, one had progression on ipilimumab but stable disease on pembrolizumab, and the other had stable disease on both [37]. The use of nivolumab, with durable responses, has been reported in two pediatric sibling patients with glioblastoma with extraordinarily high mutational loads and DNA mismatch repair (MMR) defects [38•]. Some trials have explored the use of immune checkpoint inhibitors in patients with brain metastases [39, 40], but inherent differences in biology between metastases and primary CNS tumors make any extrapolation of efficacy difficult.

Many advanced stage clinical trials are now evaluating the use of checkpoint inhibitors, predominantly in patients with recurrent glioblastoma (Table 2, Fig. 1). CheckMate-143 (NCT02017717) is a randomized phase III open-label study examining the efficacy and safety of nivolumab alone versus bevacizumab [41]. The trial intends to evaluate the safety and tolerability of nivolumab administered alone or in combination with ipilimumab in patients having different lines of glioblastoma therapy. Preliminary results suggested that the adverse effect profile of nivolumab alone or in combination with ipilimumab was consistent with those of studies in other tumors [42]. However, follow-up data indicated the combination of nivolumab and ipilimumab had notable toxicity, with eight patients (80%) experiencing grade 3 or 4 adverse events,

Table 2 Current clinical trials of immune checkpoint inhibitors in patients with gliomas

Checkpoint inhibitor	Clinicaltrials.gov registry number	Status	Phase	Tumor type	Treatment groups
Nivolumab	NCT02648633	Recruiting	I	Recurrent glioblastoma	Stereotactic radiosurgery with nivolumab and valproate
Nivolumab	NCT02829931	Recruiting	I	Recurrent high-grade gliomas	Nivolumab combined with hypofractionated stereotactic irradiation
Nivolumab	NCT02529072 (AVERT)	Recruiting	I	Recurrent high-grade gliomas	Nivolumab alone or with dendritic cell vaccine before and after surgical resection for recurrence
Nivolumab	NCT02667587 (CheckMate-548)	Recruiting	II	Newly diagnosed MGMT-methylated glioblastoma	Temozolomide plus radiotherapy combined with nivolumab or placebo
Nivolumab	NCT02617589 (CheckMate-498)	Recruiting	III	Newly diagnosed unmethylated MGMT glioblastoma	Nivolumab or temozolomide in combination with radiotherapy
Pembrolizumab	NCT02658279	Recruiting	Proof of concept, pilot	Recurrent malignant glioma with hypermutator phenotype	Pembrolizumab alone
Pembrolizumab	NCT02359565	Suspended		Recurrent, progressive or refractory high-grade gliomas, or diffuse intrinsic pontine glioma in children	Pembrolizumab alone
Pembrolizumab	NCT02852655	Not yet open	Pilot	Surgically accessible recurrent or progressive glioblastoma	Post-surgery pembrolizumab with or without pre-surgery pembrolizumab
Pembrolizumab	NCT02313272	Recruiting	I	Recurrent high-grade glioma	Hypofractionated stereotactic irradiation with pembrolizumab and bevacizumab
Pembrolizumab	NCT02530502	Recruiting	I/II	Newly diagnosed glioblastoma	Pembrolizumab with radiotherapy and temozolomide (phase I), radiotherapy and temozolomide with or without pembrolizumab (phase II)
Pembrolizumab	NCT02430363	Enrolling by invitation	I/II	Glioblastoma, gliosarcoma	Pembrolizumab or PI3K/Akt pathway inhibitors
Pembrolizumab	NCT02311582	Recruiting	I/II	Recurrent malignant glioma	Pembrolizumab alone or combined with MRI-guided laser ablation
Pembrolizumab	NCT02337686	Recruiting	II	Recurrent glioblastoma	Pembrolizumab before and after surgery
Pembrolizumab	NCT02798406 (CAPTIVE, KEYNOTE-192)	Recruiting	II	Recurrent glioblastoma or gliosarcoma	Genetically modified oncolytic adenovirus (DNC-2401) injection followed by pembrolizumab
Pembrolizumab	NCT02337491	Completed recruitment	II	Recurrent glioblastoma	Pembrolizumab with or without bevacizumab
Pidilizumab	NCT01952769	Recruiting	I/II	Diffuse intrinsic pontine glioma	Pidilizumab with radiotherapy (phase I), radiotherapy followed by pidilizumab with cyclophosphamide (phase II)
Durvalumab	NCT02336165	Recruiting	II	Newly diagnosed unmethylated MGMT glioblastoma, recurrent glioblastoma	Durvalumab with radiotherapy in newly diagnosed unmethylated MGMT glioblastoma, durvalumab alone or in combination with bevacizumab in recurrent glioblastoma
Durvalumab	NCT02866747 (STERIMGLI)	Not yet open	I/II	Recurrent glioblastoma	Hypofractionated stereotactic radiotherapy alone or with durvalumab
REGN2810	NCT02383212	Recruiting	I	Glioblastoma	REGN2810 with stereotactic radiosurgery
Combinations					
Nivolumab, ipilimumab	NCT02311920	Suspended	I	Newly diagnosed glioblastoma or gliosarcoma	Ipilimumab, nivolumab, or both in combination with temozolomide
Nivolumab, ipilimumab	NCT02017717 (CheckMate-143)	Recruiting	I/III	Newly diagnosed and recurrent glioblastoma	Nivolumab alone or in combination with ipilimumab in different lines of glioblastoma therapy (phase I), nivolumab or bevacizumab in recurrent glioblastoma (phase III)
Nivolumab, varlilumab	NCT02335918	Recruiting	I/II	Recurrent or progressive glioblastoma	Nivolumab and varlilumab in combination (glioblastoma patients eligible in phase II only)
Nivolumab, BMS-986016, urelumab	NCT02658981	Not yet open	I	Recurrent glioblastoma or gliosarcoma	BMS-986016 alone, urelumab alone, BMS-986016 and nivolumab in combination, urelumab and nivolumab in combination
Tremelimumab, durvalumab	NCT02794883	Not yet open	II	Recurrent glioblastoma	Tremelimumab and durvalumab, alone and in combination

MGMT O-6-methylguanine-DNA methyltransferase

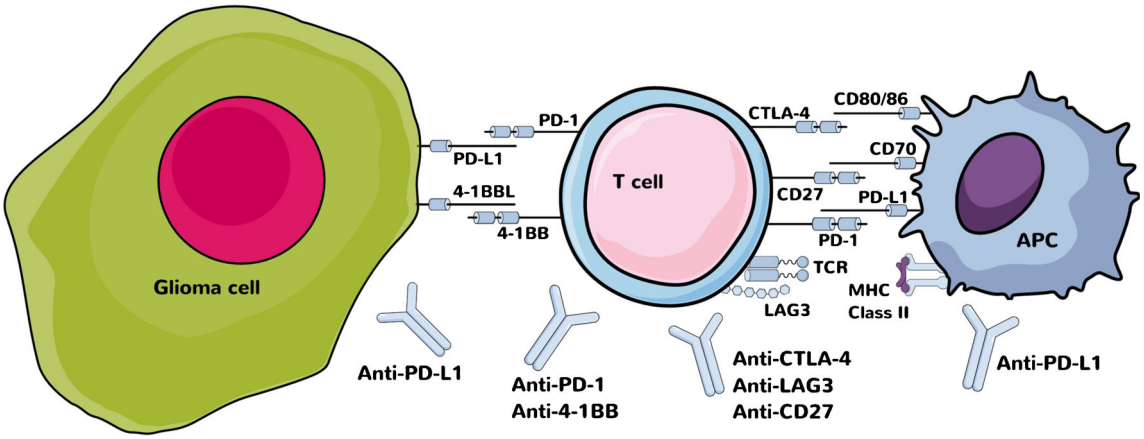

Fig. 1 Immune checkpoint inhibitors currently in clinical trials for gliomas

which lead to discontinuation of treatment in five patients (50%). Overall survival rates at 12 months was 40% for the nivolumab-alone arm, 30% for nivolumab 1 mg/kg plus ipilimumab 3 mg/kg arm, and 25% for nivolumab 3 mg/kg plus ipilimumab 1 mg/kg arm [43]. This is in comparison to other recent trials of newer treatment approaches including the use of bevacizumab plus lomustine with a median overall survival of 9.1 months [44] and bevacizumab plus rindopepimut with a median overall survival of 12 months [45]. A multicenter randomized phase II recurrent glioblastoma trial of pembrolizumab with or without bevacizumab (NCT023337491) has reported data on the safety lead-in, which indicated that this combination can be administered without dose-limiting or unexpected toxicity [46]. Of six patients, one had a partial response, two had stable disease, and three had progressive disease. Median overall survival was 6.8 months, with two patients remaining alive at time of reporting (at 327 and 328 days). Another multicenter phase II trial is evaluating durvalumab in five different cohorts of patients (NCT02336165) [47]. Durvalumab is given in combination with radiotherapy for newly diagnosed glioblastoma or with bevacizumab for recurrent glioblastoma. There were no dose-limiting toxicities at time of reporting, although the study is still recruiting.

Studies assessing nivolumab include the phase III trial (CheckMate-498) of nivolumab or temozolomide in combination with radiotherapy followed by nivolumab or temozolomide in newly diagnosed glioblastoma with unmethylated O-6-methylguanine-DNA methyltransferase (MGMT) status (NCT02617589) [48]. The study aims to randomize approximately 550 patients. A companion phase II trial (CheckMate-548, NCT02667587) is investigating nivolumab added to standard radiotherapy and temozolomide followed by adjuvant temozolomide with nivolumab in newly diagnosed glioblastomas that have a methylated *MGMT* promotor. Ipilimumab, nivolumab, and a combination of both in conjunction with temozolomide are also being studied in newly diagnosed glioblastoma or gliosarcoma (NCT02311920) [49]. Several phase I trials in recurrent glioblastoma are evaluating nivolumab in combination with stereotactic radiosurgery, hypofractionated stereotactic irradiation, or dendritic cell vaccines.

Pembrolizumab is also being examined in other trials including with re-irradiation in recurrent glioblastoma. A current proof of concept pilot study is evaluating the therapeutic impact of pembrolizumab in recurrent glioblastomas containing the hypermutator phenotype (NCT02658279). Preliminary data from a phase I trial of pembrolizumab and bevacizumab with hypofractionated stereotactic irradiation in recurrent high-grade glioma (NCT02313272) has found no dose-limiting toxicity, and the three patients evaluable for response at the time of reporting had durable disease control [50]. Further trials are evaluating pembrolizumab for glioblastoma at various stages. They include combination treatment with radiotherapy and temozolomide for newly diagnosed glioblastoma and preoperative and postoperative treatment for recurrent glioblastoma. Pembrolizumab is also being examined with other novel therapies such as PI3K/Akt pathway inhibitors, genetically modified oncolytic adenovirus (DNC-2401) injection, magnetic resonance imaging-guided laser ablation, and hypofractionated stereotactic irradiation.

Trials of immune checkpoint inhibitors in treatment combinations are also currently under way or due to open, including a phase II trial of nivolumab and varlilumab in recurrent or progressive glioblastoma, a phase I trial of nivolumab, BMS-986016, and urelumab in recurrent glioblastoma or gliosarcoma, and a phase II trial of tremelimumab and durvalumab in recurrent glioblastoma. A phase I/II trial of pidilizumab, an anti-PD-1 antibody, alone is also open, in patients with diffuse intrinsic pontine glioma and relapsed high-grade glioma (NCT01952769) [51]. Glioblastomas, are also represented in open phase I/II trials of durvalumab (NCT01693562), pembrolizumab (NCT02054806), ipilimumab plus imatinib (NCT01738139), nivolumab plus epacadostat (NCT02327078), nivolumab plus FPA008 (NCT02526017), and durvalumab plus AMP-514 (NCT02118337).

In Search of a Biomarker of Response to Immune Checkpoint Inhibitors

When immune checkpoint inhibitors were first introduced into clinical trials, it was assumed that tumor expression of PD-1 and/or PD-L1 might select clinical responders. Immunohistochemical expression of PD-L1 was shown to potentially correlate with tumor response [52]. However, patients who do not express PD-L1 can have significant responses to checkpoint inhibitor therapy [4]. The exact role of PD-L1, including technical considerations such as the percentage of PD-L1 expression required in tumor cells to indicate positivity, are yet to be completely defined. Furthermore, several different assays are currently used in clinical trials to determine PD-L1 expression [53]. Some anti-PD-L1 antibodies have been questioned as having a false high-positivity rate and in turn having caused unjustified enthusiasm, and the most reliable antibody has not been determined [11•].

Methods of detecting PD-L1 in plasma of patients with glioma have also been developed. In one study, 52.9% of patients with high-grade glioma were reported to have detectable levels [54]. PD-L1 has also been detected in ultrasonic aspiration brain tissue from surgery [55]. TILs or $CD8^+$ T cell infiltrates have been proposed as a surrogate for the presence of antigen, immune activation, and trafficking to the tumor microenvironment. They are positively correlated with response to therapy in other tumors, particularly melanoma [56] and lung cancer, [57] but their potential role in gliomas is not as clear.

Because PD-1/PD-L1 expression levels have not been a reliable biomarker for drug response, the focus has shifted to mutational load and now most recently to MMR and microsatellite instability (MSI). A higher mutational load in tumors may result in more tumor antigens, including neoantigens [58], with an associated increase in immunogenicity [59]. A higher mutational load has been associated with longer survival and long-term benefit of immunotherapy [60] in a variety of cancers [61]. Techniques and cut points for defining mutational load are not harmonized, so other more easily measured indices have been proposed, such as determining mutations in the exonuclease domain of polymerase E (POLE) which leads to hypermutations and neoantigen load [62]. However, this has not yet been investigated in glioblastoma patients. Microsatellite instability as a consequence of mutations in MMR genes leads to high mutational burden in the tumor cells. Cells with abnormal MMR function facilitate insertions or deletions that could be frameshift mutations [63, 64]. As a result of the high mutational load in MSI tumors, many tumor-specific antigens are created. In turn, some of these neoantigens will be processed, presented on major histocompatibility complex molecules, and recognized as foreign by T cells. There is mounting evidence that implicates the efficacy of PD-1 blockade to defective MMR (dMMR)/MSI-high tumors. In a phase I trial of nivolumab in 39 patients with refractory solid tumors, one colorectal cancer patient with dMMR had a durable complete response persisting for over 21 months [65]. A phase II trial of pembrolizumab in 41 patients with progressive metastatic carcinoma with or without dMMR found that MMR status predicted clinical benefit [66•]. At the time of analysis, the hazard ratio for disease progression or death between dMMR tumors and MMR-proficient tumors was 0.04 (95% CI, 0.01–0.21, $P < 0.001$). Individuals with germline Lynch syndrome MMR defects have long been recognized to be at increased risk of CNS tumors [67, 68]. Two cases of biallelic dMMR in glioblastoma have responded to nivolumab [38•], and another case of glioblastoma in a patient with germline POLE deficiency responded to pembrolizumab with histological confirmation of lymphocytic infiltration [69]. However, germline POLE mutations are exceedingly rare and the functional capacity of the lymphocytic infiltration was not established. There have been no studies in CNS gliomas, including spontaneously arising gliomas without a predisposing germline alteration that have addressed the associations of mutational load, dMMR, and MSI.

Several lines of evidence indicate that DNA repair defects are important in a subset of glioblastomas. In addition to the relatively rare cases of inherited MMR defects in glioblastoma, this may include a small percentage of newly diagnosed tumors and a larger proportion of tumors during and after standard therapy with radiation and alkylating agents. Most glioblastomas with DNA repair defects of various types demonstrate a 'hypermutator' phenotype that may make these tumors particularly sensitive to immune checkpoint blockade, on the basis of emerging data from initial studies in glioblastoma and other solid tumors [70]. This "hypermutator" phenotype has been described in glioblastoma specimens with MSH6 mutations [71]. The first cancer studied by the Cancer Genome Atlas (TCGA) was glioblastoma, with the finding that hypermutated samples harbored mutations in at least one of the MMR genes MLH1, MSH2, MSH6, or PMS2 [72]. The incidence of MSI-low in patients with sporadic glioblastoma is 8.5%. MSI-low was identified in 5.5% of newly diagnosed tumors and in 25% of patients with recurrence [72]. MSI-high was not identified in this population of 129 subjects. MMR protein expression was lost in only one subject with MSI-low, although the level of expression might have been affected. Of the recurrent tumors with acquired mutations in MSH6, there was, in particular, an increase in rates of C:G > T:A mutations. That all of these patients also received alkylating agents (most commonly temozolomide) as part of their initial treatment and the resulting mutation pattern is indicative of alkylator-induced mutations in the setting of MMR defects [72, 73]. MSH6 mutations, particularly, may cause hypermutation in the glioma cell genome, which may accelerate tumor progression [74]. Decrease in MSH6 expression or mutation might also be a consequence of temozolomide treatment as well as a mechanism of resistance to it. Another subset of glioblastoma tumors with a potential

hypermutator phenotype are lower-grade gliomas that recur after treatment with alkylator therapy [75]. In one study, 60% (6 of 10) of these tumors treated with temozolomide had significantly high mutation rates (32–91 mutations/Mb), and 97% of the mutations were C > T/G > A. As in the tumors with somatic MSH6 mutations, this pattern of mutations was associated with acquired MMR defects and consistent with induced mutations from alkylator exposure. Another study also demonstrated a link in five out of six cases, between MMR deficiency and temozolomide therapy with MGMT methylation status in low-grade gliomas between diagnosis and recurrence [76]. Frequent alterations in the MMR system have also been found in malignant astrocytomas [77]. Although only 5% of tumors were MSI-high, lack of MSH6 expression correlated with longer overall survival when patients were treated with radiotherapy alone.

Taken together, these observations suggest that a small number of newly diagnosed and a much larger proportion of recurrent glioblastomas have inherent or acquired MMR defects and/or a hypermutator phenotype. Depending on the tumor, these defects and the hypermutator phenotype may be present at diagnosis, emerge during initial treatment with radiotherapy and temozolomide, or develop at recurrence. The high numbers of neoantigens in these tumors may make them more susceptible to checkpoint blockade. Numerous other biomarkers are under investigation in a range of cancers [78]. This includes biomarkers in the tumor itself, such as IDO and diversity of T cell repertoire, and in blood, such as circulating lymphocytes, neutrophils, eosinophils, and monocytes, Tregs, soluble CD25, and various cytokines and chemokines. Ultimately, rather than single markers, integrated gene expression profiles may be crucial in selecting and predicting which patients will benefit from immunotherapy [79].

Discussion

There is limited evidence to date suggesting that immune checkpoint inhibitors may have activity in malignant gliomas. Results from the numerous clinical trials currently in progress are eagerly awaited. However, the unique characteristics of gliomas mean that a deeper understanding of the interaction between immune checkpoint inhibitors and local CNS myeloid cells is required [80]. The growing body of evidence from preclinical studies is also needed, as is the use of novel animal and humanized models, particularly to better evaluate immune checkpoint inhibitors and combination immunotherapy [81].

Dosing and Schedule

The optimal dosing and dosage schedule of checkpoint inhibitors are also yet to be clearly defined, including for melanoma, for which we have the greatest experience so far [4]. Immune checkpoint inhibitors differ substantially from traditional cytotoxic agents in that the dosage is not linearly associated with efficacy and toxicity. This has significant implication for the design and analysis of phase I trials in particular, which must incorporate complex information on pharmacodynamic and pharmacokinetic characteristics [82]. Additionally, there are currently no guidelines that indicate when one should cease therapy with PD-L1/PD-L1 antibodies, except in cases of grade ≥3 toxicity. Some patients with melanoma have durable responses long after cessation of therapy. It is not clear that maintenance therapy with PD-1/PDL-1 antibodies after response is necessary [83]. This clearly has huge implications, not only financially, but in terms of toxicity and quality of life.

Combination Approaches

The emergence of checkpoint inhibitors raises the possibility of combination therapy with both established and novel therapies. This includes dual checkpoint blockade, other immunotherapies such as vaccines, chemotherapy, targeted therapy, and radiotherapy. Especially for CNS tumors, determining the timing and sequence of checkpoint inhibitors with radiotherapy and surgery is significant. It has been proposed that radiotherapy may enhance the systemic efficacy of checkpoint inhibitors via an abscopal effect [84], and outcomes of trials examining this hypothesis are awaited. As treatment options become increasingly complex, understanding the role of checkpoint inhibitors is crucial.

Imaging Response Criteria

The use of checkpoint inhibitors raises important considerations with regard to radiological assessment of response. This is particularly crucial in cases of early-progression imaging findings, to distinguish patients who may still derive a clinical benefit from those who are truly resistant to therapy, as response can manifest after an initial increase in tumor burden or the appearance of new lesions [32]. The Immunotherapy Response Assessment for Neuro-Oncology (iRANO) criteria represent specific guidelines for the interpretation of imaging in patients with neuro-oncological tumors treated with checkpoint inhibitors [85]. This includes separate recommendations for low- and high-grade gliomas and brain metastases, and how to evaluate imaging in patients on corticosteroids.

Toxicities

Potential toxicity of checkpoint inhibitors in gliomas is also of concern. Nonspecific immunologic activation, termed immune-related adverse events, particularly involves the

dermatologic, gastrointestinal, hepatic, and endocrine systems. Although preliminary evidence suggests an adverse event profile similar to those of other solid tumors [42], there is potential for greater incidence of CNS-specific toxicity such as encephalitis. This has not occurred with previous immunotherapeutic approaches such as peptide and dendritic cell vaccine therapy in glioblastoma [86]. The use of anti-CTLA-4 therapy in a mouse model did not result in significant experimental allergic encephalomyelitis [27]. Nevertheless, it will be crucial to understanding the toxicity profile of immune checkpoint inhibitors in gliomas, particularly if used in combination with other treatment modalities. It will be important to have appropriate management algorithms in place for any adverse events [87]. Ongoing refinements to and improvements in the method of reporting immune-related adverse events in clinical trials will be critical [88].

Other Immune Checkpoint Inhibitors

There are rapidly moving clinical trials assessing other classes of checkpoint inhibitors (Table 1), and also new classes of antibodies that have dual targets, for example, PD-L1 and TGF-beta (MSB001135930). Other checkpoint inhibitors include anti-LAG-3 or urelumab tested alone and in combination with nivolumab in patients with recurrent glioblastoma.

Conclusion

The use of immune checkpoint inhibitors in gliomas holds promise, with encouraging early data, and numerous clinical trials are in progress. This is despite relatively limited preclinical evidence. Determining which subset of patients are likely to benefit is the key to the most effective use of these agents, and to avoid unnecessary toxicity. Identifying appropriate biomarkers for patient selection is crucial. Understanding the role of immune checkpoint inhibitors in combination with other treatment modalities for gliomas is vital to improving outcomes. With novel checkpoint inhibitors continually under development, the design and conduct of future clinical trials need to account for the increasing complexity of treatment options.

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Compliance with Ethical Standards

Conflict of Interest Aaron C. Tan declares that he has no conflict of interest.

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Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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