Journal for ImmunoTherapy of Cancer

T lymphocyte-targeted immune checkpoint modulation in glioma

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To cite: Kelly WJ, Giles AJ, Gilbert M. T lymphocyte-targeted immune checkpoint modulation in glioma. *Journal for ImmunoTherapy of Cancer* 2020;**8**:e000379. doi:10.1136/jitc-2019-000379

Accepted 05 January 2020

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ABSTRACT

Immunomodulatory therapies targeting inhibitory checkpoint molecules have revolutionized the treatment of solid tumor malignancies. Concerns about whether systemic administration of an immune checkpoint inhibitor could impact primary brain tumors were answered with the observation of definitive responses in pediatric patients harboring hypermutated gliomas. Although initial clinical results in patients with glioblastoma (GBM) were disappointing, recently published results have demonstrated a potential survival benefit in patients with recurrent GBM treated with neoadjuvant programmed cell death protein 1 blockade. While these findings necessitate verification in subsequent studies, they support the possibility of achieving clinical meaningful immune responses in malignant primary brain tumors including GBM, a disease in dire need of additional therapeutic options. There are several challenges involved in treating glioma with immune checkpoint modulators including the immunosuppressive nature of GBM itself with high inhibitory checkpoint expression, the immunoselective blood brain barrier impairing the ability for peripheral lymphocytes to traffic to the tumor microenvironment and the high prevalence of corticosteroid use which suppress lymphocyte activation. However, by simultaneously targeting multiple costimulatory and inhibitory pathways, it may be possible to achieve an effective antitumoral immune response. To this end, there are now several novel agents targeting more recently uncovered "second generation" checkpoint molecules. Given the multiplicity of drugs being considered for combination regimens, an increased understanding of the mechanisms of action and resistance combined with more robust preclinical and early clinical testing will be needed to be able to adequately test these agents. This review summarizes our current understanding of T lymphocyte-modulating checkpoint molecules as it pertains to glioma with the hope for a renewed focus on the most promising therapeutic strategies.

THE PROMISE OF IMMUNOMODULATORY CHECKPOINT THERAPIES

Immunomodulatory therapies targeting inhibitory checkpoint molecules have revolutionized the treatment of solid tumor malignancies.[1] Concerns about whether systemic administration of an immune checkpoint inhibitor could impact primary brain tumors were answered with the observation of definitive responses in pediatric patients harboring hypermutated gliomas.[2] Although initial clinical results in patients with glioblastoma (GBM) were disappointing, recently published results have demonstrated a potential survival benefit in patients with recurrent GBM treated with neoadjuvant programmed cell death protein 1 (PD-1) blockade.[3] While these findings necessitate verification in subsequent studies, they support the possibility of achieving clinical meaningful immune responses in malignant primary brain tumors including GBM, a disease in dire need of additional therapeutic options.

There are several challenges involved in treating glioma with immune checkpoint modulators. First is the immunosuppressive nature of GBM itself, with its high expression of inhibitory checkpoint molecules and cytokines such as tumor growth factor beta (TGF-β), vascular endothelial factor (VEGF), and interleukin 10 (IL-10).[4–9] Second, glioma tumors arise within the immunoselective blood brain barrier, thus impairing the ability for peripheral lymphocytes to traffic to the tumor microenvironment. However, recent studies in melanoma and non-small cell lung cancer have demonstrated that immune checkpoint inhibitors can indeed achieve intracranial response.[10–12] It is hypothesized that immune cells transverse the meninges through the fenestrated endothelial and tight-junction epithelial layers of the choroid plexis.[13] Alternatively, immune cells may directly migrate through meningeal blood vessels. In rat models, effector T lymphocytes have demonstrated the ability to transgress vascular walls into the cerebrospinal fluid (CSF).[14] Finally, immune modulation therapy in patients with glioma is complicated by the high prevalence of corticosteroid use which inhibits lymphocyte activation.[15 16]

By simultaneously targeting multiple costimulatory and inhibitory pathways, it may be possible to achieve an effective antitumoral immune response. To this end, there are now several novel agents targeting more recently uncovered "second generation" checkpoint molecules. This review summarizes our

current understanding of T lymphocyte-modulating checkpoint molecules as it pertains to glioma with the hope for a renewed focus on the most promising therapeutic strategies. Additionally, the current clinical trials investigating immune checkpoint inhibitors in glioma or GBM are referenced in tables 1 and 2.

TARGETING ACTIVATORS OF EFFECTOR T CELLS

Activating, also known as costimulatory, molecules promote effector T cell differentiation, proliferation, and activation. Effector T cells expressing the coreceptor CD8 recognize tumor peptides on antigen presenting cells (APC) through the interaction of the T-cell receptor (TCR) and major histocompatibility complex (MHC). Treatment with an antibody agonist targeting a costimulatory response can upregulate this signaling leading to enhanced cytotoxic T cell activity and ultimately tumor quiescence or regression. The costimulatory molecules with the most potential for further development are reviewed below.

4-1BB

4-1BB, also known as CD137, is a costimulatory receptor expressed on the surface of activated T cells as well as natural killer (NK) cells. 4-1BB plays a role in cytokine section, antiapoptotic signaling, NK cytotoxicity and promotion of T cell effector function.[17] After binding its natural ligand 4-1BBL, 4-1BB induces intracellular signaling through TNFR-associated factor 2.[18] Murine glioma models have shown in vivo antitumor activity from treatment with 4-1BB agonists. Radiotherapy combined with anti-4-1BB agonists can induce tumor eradication and prolong survival.[19] Furthermore, this antitumor response correlates with increased tumor-infiltrating lymphocyte (TIL) density and Interferon gamma (IFNy). The combination of a 4-1BB agonist and a CTLA-4 inhibitor, plus radiation treatment has also been shown to improve survival and TIL trafficking in an intracranial model.[20] Interestingly, depletion of CD4 T cells abrogated this effect to an even greater extent than CD8 depletion. Given this preclinical evidence for antitumoral efficacy and immune modulation, 4-1BB represents an attractive target for GBM and indeed is being explored in a clinical trial, NCT02658981, in patients with recurrent GBM.

GITR

Glucocorticoid-induced tumor necrosis factor (TNF)-related receptor (GITR) is highly expressed on T regulatory cells (Tregs) and induced by FOXP3 and NFkB signaling.[21] Tregs are immunosuppressive lymphocytes which act to inhibit recognition and clearance of tumor cells.[22] GITR ligand (TNFSF18) is expressed on APC. When GITR binds GITR ligand in concert with TCR stimulation, naïve T cells are activated, eventually leading to NF-kB mediated proliferation and cytokine production such as IL-2 and IFNy.[23–25] Murine studies have shown that treatment with GITR agonists result in improved survival, increased immune cell infiltrates and robust cytokine production by TILs, but structural and functional differences between murine and human GITR exist.[26 27] One study that evaluated the use of intracranial injections of a GITR agonist found improved overall survival (OS) and selective Treg depletion.[28] Systemic administration of these anti-GITR monoclonal antibody (mab) had limited effects on mouse survival. Another study combining anti-GITR mab with stereotactic radiosurgery showed increased effector CD4 infiltration, as well as elevated IFNy, IL2, and TNFa production but this did not translate into survival benefit.[29] The significance of the expression of GITR on Tregs is less clear. One study found no difference in the expression of GITR on peripheral blood cells between patients with GBM and healthy controls.[30] In contrast, another report suggests that tumor growth upregulates GITR on intratumoral Tregs.[31] There are multiple early phase trials evaluating GITR, but most are excluding patients with active central nervous system (CNS) metastasis and to date, there are no trials for patients with primary brain tumors. The GITR agonist BMS-986156 has recently demonstrated a favorable safety profile both alone and in combination with nivolumab. In a study of 66 patients, the most common side effect, occurring in 30%, was fever.[32] A phase I GBM trial, NCT03707457 combining an anti-GITR agent with PD-1 blockade is currently recruiting.

ICOS

Inducible costimulatory (ICOS) is expressed by T cells following TCR crosslinking and CD28 costimulation.[5] Through its binding with the ICOS ligand (B7-H2), ICOS plays a role in a variety of immune processes including the regulation of T cell helper cells. The ICOS ligand protein and corresponding mRNA are expressed by gliomas and the neutralization of ICOS ligand subsequently reduces Th1 and Th2 cytokines.[33] Chimeric antigen receptor (CAR) T cells targeting ICOS and epidermal growth factor receptor variant III (EGFRvIII) have demonstrated cytotoxicity against glioma (U87) cells in vitro, although concerns about the relevance of the U87 model to human GBM remain.[34 35] These CAR T cells secreted IFNy as determined by cytokine release assay and suppressed tumor growth in a xenograph mouse model.

OX40

OX40 is a transmembrane glycoprotein expressed by Tregs and transiently expressed on T cells following TCR stimulation by viral antigen.[36 37] OX40 agonism inhibits Treg immunosuppression, thereby leading to effector T cell proliferation.[38] OX40 ligand is expressed on GBM tumor cells and high levels of OX40L mRNA are associated with prolonged progression-free survival in patients with GBM.[39] Murine glioma models have shown that OX40 agonists can induce tumor regression and increase TILs.[40] Similarly, combination immunotherapies have been investigated. One study combining anti-OX40 antibody plus dendritic cell (DC) vaccine plus local cranial

Table 1 Clinical trials in glioma or glioblastoma targeting activators of effector T cells

Target receptor	Agent	Clinical trial	Trial name	Phase	Study population	Initiated	Location(s)	Status	Target accrual
4-1BB	Urelumab	NCT02658981	Anti-LAG-3 or urelumab alone and in combination with nivolumab in treating patients with recurrent glioblastoma	I	Recurrent glioblastoma	8/2016	USA	Recruiting	100
GITR	MK-4166	NCT03707457	Biomarker-driven therapy using immune activators with nivolumab in patients with first recurrence of glioblastoma	I	Recurrent glioblastoma	3/2019	USA	Recruiting	30
CD27	Varlilumab	NCT02335918	A dose escalation and cohort expansion study of anti-CD27 (varlilumab) and anti-PD-1 (nivolumab) in advanced refractory solid tumors	I/II	Glioblastoma	1/2015	USA	Completed	175
CD27	Varlilumab	NCT03688178	DC migration study to evaluate TReg depletion in patients with GBM with and without varlilumab (DERIVe)	II	Glioblastoma	8/2019	USA	Not yet recruiting	112
CD27	Varlilumab	NCT02924038	A study of varlilumab and IMA950 vaccine plus poly-ICLC in patients with WHO Grade II LGG	I	LGG (WHO grade II)	1/2017	USA	Recruiting	30

DC, dendritic cell; GITR, glucocorticoid-induced TNF-related receptor; LAG-3, lymphocyte-activation gene 3; LGG, low-grade glioma.

Table 2 Clinical trials in glioma or glioblastoma targeting inhibitors of effector T cells

Target receptor	Agent	Clinical trial	Trial name	Phase	Study population	Initiated	Location(s)	Status	Target accrual
CTLA-4	Ipilimumab	NCT02017717	A study of the effectiveness and safety of nivolumab compared with bevacizumab and of nivolumab with or without ipilimumab in patients with glioblastoma (CheckMate 143)	III	GBM	1/2014	USA, Australia, Belgium, Denmark, France, Germany, Italy, Netherlands, Poland, Switzerland, UK	Active, not recruiting	626
CTLA-4	Ipilimumab	NCT03460782	An expanded access program of ipilimumab for patients with glioblastomas and gliomas	Expanded access	GBM	–	Albania, Bosnia, Herzegovina, Bulgaria, Croatia, Romania, Russian Federation, Serbia, Switzerland	Available	–
CTLA-4	Ipilimumab	NCT02311920	Ipilimumab and/or nivolumab in combination with temozolomide in treating patients with newly diagnosed glioblastoma or gliosarcoma	I	Newly diagnosed GBM	4/2015	USA	Active, not recruiting	32
CTLA-4	Ipilimumab	NCT03367715	Nivolumab, ipilimumab, and short-course radiotherapy in adults with newly diagnosed, MGMT unmethylated glioblastoma	II	Newly diagnosed GBM	2/2018	USA	Recruiting	24
CTLA-4	Ipilimumab	NCT02829931	Hypofractionated stereotactic irradiation with nivolumab, ipilimumab and bevacizumab in patients with recurrent high grade gliomas	I	Recurrent high-grade glioma	8/2016	USA	Recruiting	26
CTLA-4	Ipilimumab	NCT03233152	Intra-tumoral ipilimumab plus intravenous nivolumab following the resection of recurrent glioblastoma (GlitIpNi)	I	Recurrent GBM	11/2016	Belgium	Recruiting	6
CTLA-4	Ipilimumab	NCT03430791	Trial of combination TTF (Optune), nivolumab plus/minus ipilimumab for bevacizumab-naïve, recurrent glioblastoma	II	Recurrent GBM	8/2018	USA	Recruiting	60
CTLA-4	Ipilimumab	NCT03425292	A longitudinal assessment of tumor evolution in patients with brain cancer	I	Newly diagnosed GBM	3/2018	USA	Recruiting	45
CTLA-4	Ipilimumab	NCT03130959	An investigational immunotherapy study of nivolumab monotherapy and nivolumab in combination with ipilimumab in pediatric patients with high grade primary CNS malignancies (CheckMate 908)	I/II	High-grade primary glioma, diffuse intrinsic pontine glioma, medulloblastoma ependymoma	6/2017	USA, Australia, Brazil, Canada, France, Germany, Hong Kong, Israel, Netherlands, Norway, Poland, Russia, Spain, Sweden, UK	Active, not recruiting	170
CTLA-4	Ipilimumab	NCT03422094	Neoantigen-based personalized vaccine combined with immune checkpoint blockade therapy in patients with newly diagnosed, unmethylated glioblastoma	I	Newly diagnosed GBM	8/2018	USA	Recruiting	30
CTLA-4	Tremelimumab	NCT02794883	Tremelimumab and durvalumab in combination or alone in treating patient with recurrent malignant glioma	II	Malignant glioma, recurrent GBM	9/2016	USA	Active, not recruiting	36
PD-1	Pembrolizumab	NCT02313272	Hypofractionated stereotactic irradiation (HFSRT) with pembrolizumab and bevacizumab for recurrent high grade gliomas	I	Recurrent high-grade gliomas	5/2015	USA	Active, not recruiting	32
PD-1	Pembrolizumab	NCT02658279	Pembrolizumab (MK-3475) in patients with recurrent malignant glioma with a hypermutator phenotype	–	Recurrent malignant glioma	1/2016	USA	Recruiting	44
PD-1	Pembrolizumab	NCT02311582	MK-3475 in combination with MRI-guided laser ablation in recurrent malignant gliomas	I	Recurrent malignant glioma	8/2015	USA	Recruiting	58

Continued

Table 2 Continued

Target receptor	Agent	Clinical trial	Trial name	Phase	Study population	Initiated	Location(s)	Status	Target accrual
PD-1	Pembrolizumab	NCT02359565	Pembrolizumab in treating younger patients with recurrent, progressive, or refractory high-grade gliomas, diffuse intrinsic pontine gliomas, hypermutated brain tumors, ependymoma or medulloblastoma	I	Recurrent, progressive, or refractory high-grade gliomas, diffuse intrinsic pontine gliomas, hypermutated brain tumors, ependymoma or medulloblastoma	5/2015	USA	Recruiting	110
PD-1	Pembrolizumab	NCT03665545	Pembrolizumab in association with the IMA950/Poly-ICLC for relapsing glioblastoma (IMA950-160)	I/II	Relapsing GBM	10/2018	–	Recruiting	24
PD-1	Pembrolizumab	NCT03018288	Radiation therapy plus temozolomide and pembrolizumab with and without HSPPC-96 in newly diagnosed GBM	II	Newly diagnosed GBM	1/2017	USA	Recruiting	108
PD-1	Pembrolizumab	NCT02798406	Combination adenovirus +Pembrolizumabto trigger immune virus effects (CAPTIVE)	II	Recurrent GBM or gliosarcoma	6/2016	USA, Canada	Active, not recruiting	48
PD-1	Pembrolizumab	NCT02337686	Pharmacodynamic study of pembrolizumab in patients with recurrent glioblastoma	II	Recurrent GBM or gliosarcoma	4/2015	USA	Active, not recruiting	18
PD-1	Pembrolizumab	NCT02337491	Pembrolizumab± Bevacizumab for recurrent GBM	II	Recurrent GBM or gliosarcoma	2/2015	USA	Completed	80
PD-1	Pembrolizumab	NCT03722342	TTAC-001 and pembrolizumab phase Ib combination trial in recurrent glioblastoma	Ib	Recurrent GBM	12/2018 (Estimated)	South Korea	Recruiting	20
PD-1	Pembrolizumab	NCT02852655	A pilot surgical trial to evaluate early immunologic pharmacodynamic parameters for the PD-1 checkpoint inhibitor, pembrolizumab (MK-3475), in patients with surgically accessible recurrent/ progressive glioblastoma	–	Recurrent/ progressive GBM or gliosarcoma	9/2016	USA	Active, not yet recruiting	35
PD-1	Pembrolizumab	NCT03405792	Study testing the safety and efficacy of adjuvant temozolomide plus TTFields (Optune) plus pembrolizumab in patients with newly diagnosed glioblastoma (2-THE-TOP)	II	Newly diagnosed GBM	2/2018	USA	Recruiting	29
PD-1	Pembrolizumab	NCT02430363	Evaluation of the treatment effectiveness of glioblastoma/ gliosarcoma through the suppression of the PI3K/Akt pathway in compared with MK-3475	IIb	Relapsed GBM/ gliosarcoma	3/2013	USA, Belgium, Germany, Italy, Poland, Switzerland, Ukraine, UK	Unknown	58
PD-1	Pembrolizumab	NCT02530502	Radiation therapy with temozolomide and pembrolizumab in treating patients with newly diagnosed glioblastoma	I/II	Newly diagnosed GBM	10/2015	USA	Active, not recruiting	50
PD-1	Pembrolizumab	NCT03426891	Pembrolizumab and vorinostat combined with temozolomide for newly diagnosed glioblastoma	I	Newly diagnosed GBM or gliosarcoma	3/2018	USA	Recruiting	32
PD-1	Pembrolizumab	NCT03726515	CART-EGFRvIII+Pembrolizumab in GBM	I	Newly diagnosed GBM	3/2019	USA	Recruiting	7
PD-1	Pembrolizumab	NCT02287428	Personalized NeoAntigen cancer vaccine with RT plus pembrolizumab for patients with MGMT Unmethylated, newly diagnosed GBM	I	Newly diagnosed GBM	11/2014	USA	Active, not recruiting	46
PD-1	Pembrolizumab	NCT03347617	Ferumoxytol MRI in assessing response to pembrolizumab in patients with brain tumors from melanoma and glioblastoma	II	GBM	12/2017	USA	Recruiting	45
PD-1	Pembrolizumab	NCT04013672	Study of pembrolizumab plus SurVaxM for glioblastoma at first recurrence	II	Recurrent GBM	9/2019 (estimated)	USA	Not yet recruiting	51
PD-1	Pembrolizumab	NCT03899857	Pembrolizumab for newly diagnosed glioblastoma (PERGOLA)	II	Newly diagnosed GBM	5/2019 (estimated)	Switzerland	Not yet recruiting	56

Continued

Table 2 Continued

Target receptor	Agent	Clinical trial	Trial name	Phase	Study population	Initiated	Location(s)	Status	Target accrual
PD-1	Pembrolizumab	NCT03797326	Efficacy and safety of pembrolizumab (MK-3475) plus lenvatinib (E7080/MK-7902) in previously treated participants with select solid tumors (MK-7902–005/E7080-G00-224/LEAP-005)	II	GBM	2/2019	Australia, Canada, Chile, France, Germany, Israel, Korea, Spain, UK, USA	Recruiting	180
PD-1	Nivolumab	NCT02550249	Neoadjuvant nivolumab in glioblastoma (Neo-nivo)	II	GBM	6/2016	Spain	Completed	29
PD-1	Nivolumab	NCT03452579	Nivolumab plus standard dose bevacizumab vs nivolumab plus low dose bevacizumab in GBM	II	Recurrent GBM	5/2018	USA	Recruiting	90
PD-1	Nivolumab	NCT03636477	A study of Ad-RTS-hIL-12 with veledimex in combination with nivolumab in subjects with glioblastoma; a substudy to ATI001-102	I	Recurrent or progressive GBM	6/2018	USA	Recruiting	18
PD-1	Nivolumab	NCT03014804	Autologous dendritic cells pulsed with tumor lysate antigen vaccine and nivolumab in treating patients with recurrent glioblastoma	II	Recurrent GBM	1/2019 (estimated)	USA	Withdrawn	30
PD-1	Nivolumab	NCT02667587	An investigational immunotherapy study of temozolomide plus radiation therapy with nivolumab or placebo, for newly diagnosed patients with glioblastoma (CheckMate548)	III	Newly diagnosed GBM	5/2016	USA, Australia, Austria, Belgium, Canada, Denmark, France, Germany, Israel, Italy, Japan, Netherlands Norway, Poland, Russia, Spain, Sweden, Switzerland, UK	Recruiting	693
PD-1	Nivolumab	NCT02617589	An investigational immunotherapy study of nivolumab compared with temozolomide, each given with radiation therapy, for newly diagnosed patients with glioblastoma (CheckMate 498)	III	Newly diagnosed GBM	2/2016	USA, Australia, Austria, Belgium, Canada, Denmark, France, Germany, Israel, Italy, Japan, Netherlands Norway, Poland, Russia, Spain, Sweden, Switzerland, UK	Recruiting	550
PD-1	Nivolumab	NCT02327078	A study of the safety, tolerability, and efficacy of epacadostat administered in combination with nivolumab in select advanced cancers	I/II	GBM	11/2014	USA, UK	Active not recruiting	309
PD-1	Nivolumab	NCT02529072	Nivolumab with DC vaccines for recurrent brain tumors (AVERT)	I	Recurrent glioma or astrocytoma	1/2016	USA	Active, not recruiting	7
PD-1	Nivolumab	NCT03718767	Nivolumab in people with IDH-mutant gliomas with and without hypermutator phenotype	II	Glioma	3/2019	USA	Recruiting	95
PD-1	Nivolumab	NCT03576612	GMCI, nivolumab, and radiation therapy in treating patients with newly diagnosed high-grade gliomas (GMCI)	I	High-grade glioma	2/2018	USA	Recruiting	36
PD-1	Nivolumab	NCT03557359	Nivolumab for recurrent or progressive IDH mutant gliomas	II	Gliomas	6/2018	USA	Recruiting	37
PD-1	Nivolumab	NCT03718767	Nivolumab in people with IDH-mutant gliomas with and without hypermutator phenotype	II	Gliomas	3/2019	USA	Recruiting	95
PD-1	Nivolumab	NCT02526017	Study of cabiralizumab in combination with nivolumab in patients with selected advanced cancers (FPA008-003)	I	Glioma	9/2015	USA	Active, not recruiting	295

Continued

Table 2 Continued

Target receptor	Agent	Clinical trial	Trial name	Phase	Study population	Initiated	Location(s)	Status	Target accrual
PD-1	Nivolumab	NCT03173950	Immune checkpoint inhibitor nivolumab in people with select rare CNS cancers	II	Ependymoma, medulloblastoma, pineoblastoma, choroid plexus carcinoma, chordoma, gliomatosis, brainstem glioma, midline glioma, ATRT, meningioma, gliosarcoma, primary brain sarcoma	7/2017	USA	Recruiting	180
PD-1	Nivolumab	NCT03925246	Efficacy of nivolumab for recurrent IDH mutated high-grade gliomas (REVOLUMAB)	II	High-grade glioma, brain cancer	5/2019	Pending	Recruiting	39
PD-1	Nivolumab	NCT02550249	Neoadjuvant nivolumab in glioblastoma (Neo-nivo)	II	GBM	6/2015	Spain	Completed	29
PD-1	Nivolumab	NCT03743662	Nivolumab with radiation therapy and bevacizumab for recurrent MGMT methylated glioblastoma	II	Recurrent GBM	11/2018	USA	Recruiting	94
PD-1	Nivolumab	NCT03557359	Nivolumab for recurrent or progressive IDH mutant gliomas	II	Recurrent or progressive glioma	6/2018	USA	Recruiting	37
PD-1	Nivolumab	NCT03890952	Translational study of nivolumab in combination with bevacizumab for recurrent glioblastoma	II	Recurrent adult brain tumor	10/2018	Denmark	Recruiting	40
PD-1	MDV9300	NCT01952769	Anti PD1 antibody in diffuse intrinsic pontine glioma	I/II	Diffuse intrinsic pontine GBM	2/2014	Israel	Unknown	50
PD-1	Cemiplimab	NCT03690869	REGN2810 in pediatric patients with relapsed, refractory solid, or CNS tumors and safety and efficacy of REGN2810 in combination with radiotherapy in pediatric patient with newly diagnosed or recurrent glioma	I/II	Relapsed or refractory CNS tumor, diffuse intrinsic pontine glioma, high-grade glioma	8/2018	USA	Recruiting	150
PD-1	Cemiplimab	NCT03491683	INO-5401 and INO-9012 delivered by electroporation (EP) in combination with cemiplimab (REGN2810) in newly diagnosed glioblastoma	I/II	Newly diagnosed GBM	5/2018	USA	Active, not recruiting	52
PD-1	TIL	NCT03347097	TIL adoptive therapy for patients with glioblastoma multiforme	I	GBM	1/2017	China	Recruiting	40
PD-L1	Atezolizumab	NCT03174197	Atezolizumab (aPDL1)+temozolomide and radiation for newly diagnosed GBM	I/II	Newly diagnosed GBM	6/2017	USA	Recruiting	60
PD-L1	Atezolizumab	NCT03673787	A trial of ipatasertib in combination with atezolizumab (IceCAP)	I	Advanced GBM	8/2018	UK	Recruiting	51
PD-L1	Atezolizumab	NCT03158389	NCT Neuro Master Match – N^2M^2 (NOA-20) (N^2M^2)	I/II	Newly diagnosed GBM	5/2018	Germany	Recruiting	350
PD-L1	Durvalumab	NCT02866747	A study evaluating the association of hypofractionated stereotactic radiation therapy and durvalumab for patients with recurrent glioblastoma (STERIMGLI)	I/II	Recurrent GBM	1/2017	France	Recruiting	62
PD-L1	Durvalumab	NCT02336165	Phase 2 study of MEDI4736 in patients with glioblastoma	II	GBM	2/2015	USA, Australia	Active, not recruiting	159
PD-L1	Durvalumab	NCT03991832	Study of olaparib and durvalumab in IDH-mutated solid tumors	II	Glioma	9/2019 (estimated)	Canada	Not yet recruiting	78
PD-L1	Avelumab	NCT03341806	Avelumab with laster interstitial therapy for recurrent glioblastoma	I	Recurrent GBM	6/2018	USA	Recruiting	30

Continued

Table 2 Continued

Target receptor	Agent	Clinical trial	Trial name	Phase	Study population	Initiated	Location(s)	Status	Target accrual
PD-L1	Avelumab	NCT03750071	VXM01 plus avelumab combination study in progressive glioblastoma	I/II	Recurrent GBM	11/2018	France, Germany, Netherlands	Recruiting	30
PD-L1	Avelumab	NCT03291314	Clinical trial on the combination of avelumab and axitinib for the treatment of patients with recurrent glioblastoma (GliAvAx)	II	Recurrent GBM	5/2017	Belgium	Completed	52
PD-L1	Avelumab	NCT03893903	AMPLIFYing NEOepitope-specific VACcine responses in progressive diffuse glioma (AMPLIFY-NEOVAC)	I	Malignant glioma	10/2018	Germany	Recruiting	60
PD-L1	Avelumab	NCT02968940	Avelumab with hypofractionated radiation therapy in adults with IDH mutant glioblastoma	II	GBM	3/2017	USA	Active, not recruiting	43
PD-L1	Avelumab	NCT03047473	Avelumab in patients with newly diagnosed glioblastoma	II	GBM	10/2017	Canada	Recruiting	30
PD-L1	Chimeric switch receptor modified T cells	NCT02937844	Pilot study of autologous chimeric switch receptor modified T cells in recurrent glioblastoma multiforme	I	Recurrent GBM	6/2016	China	Unknown	20
LAG-3	BMS-986016	NCT03493932	Cytokine microdialysis for real-time immune monitoring in patients with glioblastoma undergoing checkpoint blockade	I	Solitary recurrent GBM	9/2018	USA	Recruiting	15
TIGIT	OMP-313M32	NCT03119428	A study of OMP-313M32 in subjects with locally advanced or metastatic solid tumors	I	Locally advanced, recurrent, or metastatic solid tumors	5/2017	USA	Terminated	30
TIM-3	MBG453	NCT03961971	Trial of anti-TIM-3 in combination with anti-PD-1 and SRS in recurrent GBM	I	Recurrent GBM	8/2019 (estimated)	USA	Not Yet Recruiting	15

Tables 1 and 2 were sourced from ClinicalTrials.gov. Search terms included "glioma OR glioblastoma" in conjunction with "4-1BB", "GITR", "ICOS", "OX40", "CD27", "B7-H3", CTLA-4", "PD-1", "PD-L1", "LAG-3", "TIGIT", "TIM-3", and "A2AR". Clinical trials were included if available eligibility information did not exclude glioma or glioblastoma. Due to the breadth of institutes involved the accuracy of individual trials could not be verified beyond the information publically available at ClinicalTrials.gov.

A2AR, adenosine A2 receptor; ATRT, atypical teratoid-rhabdoid tumor; CNS, central nervous system; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; GBM, glioblastoma; GITR, glucocorticoid-induced TNF-related receptor; ICOS, inducible costimulatory; IDH, isocitrate dehydrogenase; LAG-3, lymphocyte-activation gene 3; MGMT, methylguanine-DNA methyltransferase; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; TIGIT, T-cell immunoreceptor with Ig and ITIM domains; TIL, tumor-infiltrating T lymphocyte; TIM-3, T-cell immunoglobulin and mucin-domain containing-3.

radiation demonstrated tumor regression, TIL infiltration, and improved survival compared with mice treated with only two of the three modalities.[41] Another study combining granulocyte macrophage colony stimulating factor-expressing cells and an OX40 agonist, showing that the combined therapy promoted Th1 responses while also decreasing Th2 response.[42] Of note, coexpression of PD-1, TIM-3, and lymphocyte-activation gene 3 (LAG-3) was also reduced.

CD27

CD27, a member of the TNF receptor family, is expressed on naïve CD4 and CD8 T cells and upregulated with T cell activation. On activation by binding CD70, a surface antigen expressed on meningioma and glioma cells,[43] CD27 promotes proliferation by facilitating entry into the cell cycle as well as promoting effector T cell differentiation.[44] In vitro studies have shown that glioma cells respond to CD70 signaling. Cell lines engineered to produce soluble CD70 increase proliferation and IFN secretion of cocultured lymphocytes.[45] Treating in vivo murine glioma models with both a CD70 specific CAR T therapy[46] and a CD70 antibody-drug conjugate produced tumor regression.[47] However, other studies have suggested that the CD27 pathway may actually suppress T lymphocytes. One study found that glioma cells expressing CD70 could induce T cell apoptosis while simultaneously inhibiting glioma growth.[48] This finding may be explained by lytic NK activity or a mechanistic difference in the apoptosis-regulating Siva pathway between human and mice cells. Furthermore, CD70 overexpression has been shown to increase recruitment of immunosuppressive tumor-associated macrophages.[49] NCT02924038 is currently examining the CD27 agonist varlilumab in patients with low-grade glioma. Another study, NCT02335918, is looking at the combination of varlilumab with PD-1 blockade among a variety of solid tumors including GBM. Finally, the clinical trial NCT03688178 is investigating varilumab in combination with a DC vaccine and is scheduled to begin soon pending new testing requirements from the FDA.

TARGETING INHIBITORS OF EFFECTOR T CELLS

Inhibitory checkpoint molecules downregulate T cell differentiation, proliferation, and activation. Currently, most immune checkpoint inhibitors are antibodies that target these inhibitory molecules. By blocking the interaction of the inhibitory molecules with their ligand immune checkpoint inhibitors, these checkpoint inhibitors prevent suppression of effector T cells and, in turn, allow for cytotoxic activity. The inhibitory molecules with the most potential for use in clinical trials are reviewed below.

CTLA-4

Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) is constitutively expressed on activated Tregs.[50 51] Additionally, CTLA-4 expression can be induced on cytotoxic T cells after their stimulation. CTLA-4 competes with the costimulatory molecule CD28 for the CD80 and CD86 ligands (B7-1 and B7-2, respectively), ultimately leading to T cell inactivation if CTLA-4 engagement predominates. Prognostically, CTLA-4 expression on CD4 and CD8 T cells may positively correlate with survival in patients with GBM.[52] Several murine glioma models have shown antitumor efficacy and improved survival using species-specific mab blockade of CTLA-4. Although long-term tumor-free survival in mice is only modest with single agent anti-CTLA-4 therapy, combinational studies with anti-PD-1 or anti-PD-L1 have demonstrated cure rates as high as 75%.[53] CTLA-4 antibody blockade has also been shown to enhance whole tumor-cell vaccine efficacy and improve survival.[54 55] In mice treated with a herpes simplex oncolytic virus, the combination of anti-CTLA-4 and anti PD-1 therapy was found to improve survival.[56] Furthermore, combined with intratumoral administration of IL-12, CTLA-4 inhibitors were shown to cause a reduction in tumor burden, decreasing Tregs and increasing effector T cells.[57] Several early phase studies are examining CTLA-4 inhibitors in GBM. CheckMate 143, a phase I study of 40 patients with recurrent GBM treated with the PD-1 inhibitor nivolumab, alone or in combination with the CTLA inhibitor ipilimumab, showed a survival of only 7 to 10 months and response rates of 0%–11%.[58] Additionally, nivolumab did not improve OS when compared with bevacizumab in this population.[59] There remain several trials studying ipilimumab in conjunction with nivolumab. NCT03367715 is examining nivolumab and ipilimumab in conjunction with short-course radiotherapy. Similarly, NCT02829931 and NCT03425292 combine CTLA-4, PD-1 and bevacizumab with radiation therapy. NCT03233152 is investigating intratumoral administration of ipilimumab in conjunction with nivolumab after resection of recurrent GBM. NCT03430791 uses nivolumab and ipilimumab with tumor-treating fields. NCT03422094 combines dual checkpoint blockade with a personalized neoantigen-based vaccine (NeoVax). The pediatric study, CheckMate 908, is examining ipilimumab and nivolumab in childhood CNS malignancies. NCT02794883 is a study of the CTLA-4 inhibitor tremelimumab plus the PD-1 inhibitor durvalumab in recurrent glioma. Additionally, an expanded access program of ipilimumab is available in countries worldwide although it is currently not available in the USA and many European nations.

PD-1

PD-1, is expressed on activated T cells. When PD-1 binds its ligand, programmed death ligand 1 (PD-L1), TCR signaling is downregulated, which in turn decreases T cell proliferation and activation. PD-1 has also been implicated in the activation of epithelial-mesenchymal transition[60] and prevention of Treg expansion in glioma.[61] Estimates of PD-L1 expression on human GBM have ranged between 19% and 88%, with some variation ascribed to the technique and antibody use.[62–64] Prognostically, PD-1 expression on TILS and PD-L1 expression on tumor cells

both correlate with glioma grade.[65] Zeng *et al*, described improved survival in murine models with the combination of PD-1 inhibitor plus radiotherapy.[66] Combining anti-PD-1 with temozolomide, the standard of care for treatment of GBM, produces decreased tumor growth, increased TILs and improved survival when compared with monotherapy.[67–69] PD-1 blockade combined with oncolytic viral therapy has also been shown to increase survival in mice.[70 71] DC vaccines in combination with PD-1 inhibitors likewise demonstrate improved murine survival.[72] Finally, the combination of a PD-1 inhibitor and toll-like receptor (TLR-3) agonist therapy increased DC activation and T cell proliferation.[73] A phase I study of 16 patients with recurrent GBM treated with atezolizumab reported a favorable safety profile but a disappointing median survival of only 4.2 months.[74] Similarly, the phase I trial KEYNOTE-028 described 26 patients with recurrent PD-L1+GBM treated with pembrolizumab, observing an OS of 14.4 months and objective response rate of only 4%.[75] However, a pilot trial of 35 patients with recurrent GBM recently found neoadjuvant pembrolizumab treatment arm to have an OS of 13.7 months, a statistically significant difference to the 7.5 month survival of those receiving adjuvant (post-surgical) pembrolizumab.[3] While the OS is in keeping with historical expectations, the molecular findings suggest that treatment effect may actually be responsible for the discrepancies between the two cohort arms. In this study, neoadjuvant PD-1 blockade upregulated T-cell-γ and IFN-γ signals and downregulated cell-cycle-related transcripts. Focal expression of PD-L1 on tumor cells was also inducible by neoadjuvant treatment. TCR sequencing demonstrated enhanced clonal expansion in this cohort as well. A companion paper detailing 30 patients with GBM treated with neoadjuvant nivolumab likewise showed increases in chemokine transcripts, immune infiltration and TCR clonal diversity.[76] Two patients on this study remained alive over 28 months later. Another study performed genomic and transcriptomic analysis of 66 patients with GBM treated with PD-1 inhibitors, highlighting alterations of PTEN in non-responders and the MAPK pathway in responders.[77]

There are numerous early phase trials examining PD-1 and PD-L1 targeting agents in gliomas, often in conjunction with radiation or bevacizumab. In addition to these, Checkmate 548 and 498 are phase III clinical trials examining checkpoint inhibitor therapy in MGMT methylated and unmethylated populations, respectively. A recent press release from CheckMate498 indicates that the study did not meet its primary endpoint of OS.[78] NCT03491683 combines a PD-1 inhibitor with an IL-12 and antigen-stimulation strategy delivered by intramuscular injection and electroporation. Another interesting trial, NCT03347097, is examining the use of pluripotent immune killer cells constructed from transgenic-modified TILs to highly express PD-1. Combinational approaches with tyrosine kinase inhibitors (TKI) are also under investigation. GLIAVAX, a phase II trial of 54 patients with recurrent GBM treated with avelumab and axitinib demonstrated favorable tolerability but did not meet the primary endpoint goal with a 6-month progression-free survival (PFS-6) of only 18%.[79] The addition of pembrolizumab to bevacizumab, a VEGF inhibitor, also did not improve PFS-6.[80] Other TKI combinations being studied with PD-1 blockade include TTAC-001 (VEGFR-2/KDR) and lenvatinib (VEGFR1/2/3). Small molecule inhibitors such as vorinostate (histone deacetylase inhibitor), ipatasertib (AKT), olaparib (PARP), and epacadostat (indoleamine 2,3-dixoygenase-1) are also being under investigation in GBM. The NCT Neuro Master Match employs molecular characterization and bioinformatic evaluation to stratify patients toward multiple therapeutic arms including atezolizumab. Vaccine combinations currently under investigation include the IMA950 (multipeptide vaccine), HSPPC-96 (heat shock protein peptide complex), DNX-2401 (genetically modified oncolytic adenovirus), SurVaxM (survivin tumor-specific antigen), DCVax-L (autologous DC pulsed with tumor lysate antigen), VXM01 (VEGFR-2 DNA vaccine), NeoVax, and a IDH1 R132H-specific vaccine. Additionally, there are also CAR T strategies targeting EGFRvIII and PD-1. NCT02359565 and NCT03173950 are examining PD-1 inhibitors among rare or pediatric CNS tumors populations. Finally, NCT02311582 and NCT03341806 are studies focused on the effects of PD-1 inhibition in conjunction with MRI-guided laser ablation and laser interstitial thermal therapy, respectively.

LAG-3

LAG-3 is an immunoglobulin expressed on NK, DC, and B cells that plays an inhibitory role in T cell proliferation and cytokine secretion.[81] In glioma samples analyzed by flow cytometry, 30% of CD8 TIL express LAG-3.[82] Much lower expression (1.25%) was observed in CD4 TILs. One phase I study, NCT03493932, is examining LAG-3 inhibitor therapy after cytokine microdialysis and tumor resection in patients with recurrent GBM.

TIGIT

T cell immunoreceptor with Ig and ITIM domains (TIGIT) is a transmembrane protein which binds to CD155 and inhibits NK cell activity.[83] TIGIT expression is also found on CD8 T cells, CD4 T cells, Tregs, and NK cells. Pediatric glial tumors contain CD4 and CD8 T cells which express TIGIT as well as TIM3, OX40, and 4-1BB.[84] Combination treatment with TIGIT and PD-1 blockade was shown to increase cytotoxic CD8 cells, reduce Tregs and improved survival in a murine glioma model.[85] PD-1 and TIGIT coblockage increased IFNy- and TNFa-producing CD8 (and CD4) T cells as compared with monotherapy groups. Tregs were also decreased but with no significant difference observed between combination and monotherapy groups. Reimplantation of tumor in the surviving animals demonstrated immune memory with no deaths at 90 days compared with a median survival of 21 days in a control group. There are currently no trials of TIGIT agents specifically in glioma or GBM. However,

NCT03119428 is examining the anti-TIGIT agent OMP-313M32 in locally advanced, recurrent or metastatic solid tumor malignancies.

TIM-3

T-cell immunoglobulin and mucin-domain containing-3 (TIM-3) is present on T cells, DC, macrophages, NK, and tumor cells. On binding galectin-9 and phosphatidylserine, TIM-3 induces T cell apoptosis, increasing phagocytosis and upregulating proinflammatory cytokine secretion.[86] TIM-3 also binds carcinoembryonic antigen cell adhesion molecule 1 (CAECAM1) on activated T cells thereby inhibiting them.[86] TIM-3 is expressed in higher grade glioma and negatively correlates with T-cell-mediated immune responses.[87] Peripheral T lymphocyte expression of TIM-3 is also increased and correlates with worsened grade.[88 89] Furthermore, this expression may be even higher in intratumoral effector lymphocytes.[90] Combined antibody blockade of TIM-3 and its CAECAM ligand have been shown to prolong survival in GBM mouse models.[91] This therapy increased the ratio of CD4/CD8 to Tregs among brain-infiltrating lymphocytes (BIL) and selective depletion of these CD4 and CD8 cell extinguished the survival effect. IFNy and TGF-β were upregulated and decreased, respectively, reflecting shift in the cytokine milieu. Triple therapy with PD-1 blockade, TIM-3 blockade, and radiation has also been demonstrated to improved survival compared with dual or monotherapy.[92] Combination therapy also increased the CD8 effector/Treg ratio, IFNγ-producing CD4 cells and IFNγ-producing CD8 cells. Interestingly, BIL coexpression of PD-1 and TIM-3 were noted to increase with time. This suggests that the natural history of glioma may upregulate TIM-3, representing an immunosuppressive adaptation that can be countered with inhibitors. Following reimplantation, none of these long-term survivors established tumors, thus demonstrating that the mice had achieved immunological memory. A clinical trial, NCT03961971, combining the anti-TIM-3 inhibitor MBG453 with the PD-1 inhibitor spartalizumab and stereotactic radiosurgery is scheduled to begin August 2019.

A2AR

Adenosine A2 receptor (A2AR) is a G protein-coupled receptor. Tissue breakdown and hypoxia promote extracellular adenosine production which in turn leads to anti-inflammatory effects mediated by these G protein-coupled receptors.[93] Increased cyclic AMP upregulates immunosuppressive cytokines, increases PD-1, induces T cell anergy and promotes Treg differentiation. Taken together, these events create a more immunosuppressive tumor microenvironment. Inhibition of A2AR is, therefore, a potential method to stimulate the immune system. Several purine derivatives related to this have been identified as having in vivo activity against glioma. The surface ectoenzyme CD39 catabolizes proinflammatory ATP into AMP, and glioma cells have been shown to induce CD4 T cell suppression via CD39, an effect which is preventable with the introduction of A2AR antagonists.[94]

B7-H3

B7-H3, an immunomodulatory protein expressed on both lymphocytes and tumor cells, appears to have a host of complex effects on T lymphocytes. Initial reports of human B7-H3 identified a role in upregulating T cell proliferation, and it was noted that cytotoxicity with antibody blockade of B7-H3, or its potential ligand TLT-2, in turn suppresses T cell activation.[95 96] However, more recent experiments of murine B7-H3 conversely suggest that B7-H3 may actually have suppressive effects on cytotoxic T cells by downregulating proinflammatory Th1 cells.[97 98] B7-H3 expression in glioma correlates with higher grade and worsened prognosis.[99 100] One study in murine glioma models found that B7-H3 gene silencing of B7-H3 leads to a less invasive phenotype.[101] Together, these suggest that B7-H3 upregulation promotes tumor growth and invasion which, if true, could be through immune or non-immune related mechanisms.

VISTA

V-domain Ig suppressor of T cell activation (VISTA), also known as PD-1H, is an inhibitory molecule expressed on hematopoietic cells and tumor cells. On binding an unknown receptor VISTA suppresses CD4 and CD8 T cell activation and promotes Treg proliferation.[102 103] In murine glioma models (GL261), VISTA knockout was shown to prolong survival and synergism with radiation was observed.[104] VISTA is not currently being studied in human trials.

B7-H4

There are a host of other immune checkpoint molecules which have been shown to influence the tumor microenviroment but remain early in development. B7-H4 (B7S1) is an inhibitory molecule expressed on APC which inhibits T cells.[105 106] Additionally, B7-H4 is expressed by glioma stem-like cells (U251).[107] B7-H4 expression has been shown to be associated with prognosis in GBM where it appears to mediate cross talk between glioma and macrophages via the IL-6/STAT3 signaling pathway.[108] Other checkpoint molecules such as B and T lymphocyte attenuator (BTLA) have yet to be sufficiently described in glioma.

ALTERNATIVE MECHANISMS OF IMMUNE MODULATION FOR T LYMPHOCYTE ACTIVATION

To date, the success of targeting immune modulatory molecules has been by using monoclonal antibodies that block either ligand or receptor. However, multiple other mechanisms of targeting these interactions are also under development.

In contrast to monoclonal antibodies, small molecule inhibitors contain only sufficient chemical structures needed to antagonize target molecules. Small molecule

inhibitors would conceivably possess greater molecular stability, less immunogenicity and improved penetration to tumor sites than monoclonal antibodies. The ability to more easily penetrate the BBB makes these small molecule inhibitors particularly attractive for the treatment of glioma. The PD-1/PD-L1 binding site has been characterized as hydrophobic and spatially flat.[109] To this end, various compounds have been developed that specifically block the PD-1/PD-L1 interaction.[110] These compounds consist of a core scaffold of either three aromatic rings or two and a benzodioxan ring with additional variant moieties. They have been shown to bind PD-L1 and block the interaction of PD-1 as verified by the restoration of 15N resonance signal on radiolabeled PD-1.[111] The EC_{50} of the least toxic compounds BMS-1001 and BMS-1116 was 33.4 and 40.5 uM, respectively. These compounds were shown to induce TCR stimulation, as measured by a luciferase reporter gene. Crystallography demonstrated that BMS-1166 showed four protein bonds with a dimer complex of PD-L1. Furthermore, four decompensation fragments, representing the core aromatic and benzodioxan structures, displayed PD-L1 affinity.

Typical monoclonal antibodies are ~150 kDa in weight and consist of two heavy and two light protein chains, as well as Fab and single-chain variable fragments. Nanobodies, also called single-domain antibodies, are recombinant antibody fragments containing a monomeric antigen-binding domain. Nanobodies are only ~15 kDa, more hydrophilic, more stable and less sterically hindered then their full antibody counterparts, drastically improving their ability to penetrate into tumor and molecular sites.[112–114] Furthermore, these nanobodies can be humanized to further decrease their immunogenicity.[115] Various delivery systems for immune checkpoint modulators, including platelet, viral and bacterial vectors are also under active investigation.[116] Caplacizumab, a nanobody targeting von Willebrand factor, recently received the first ever FDA approval for a nanobody-directed therapy.[117] KN035, an anti-PD-L1 IgV-type nanobody has been shown to bind chiefly through a 21 amino acid segment that includes the Ile54, Tyr56 and Arg113 residues which participate in the PD-1 interaction.[118] Notably, KN035 affinity for PD-L1 is ~1000-fold stronger than PD-1's affinity for PD-L1 (IC_{50}=5.25 nM).[119]

CAR T cells, T cells genetically engineered with MHC-independent recognition of tumor-associated antigens (TAA), have shown increasing benefit in the treatment of hematological malignancy.[120 121] CAR T cells with anti-PD-L1 targeting have demonstrated cytotoxic activity in mice bearing melanoma and colon tumors.[122] In addition, CAR T cells have been developed with a switch receptor construct composed of extracellular PD-1 domains coupled to transmembrane and cytoplasmic CD28 signaling domains.[123] These CAR T cells have been shown to induce tumor regression in murine models of prostate cancer. CAR T cells have also been engineered to secrete PD-L1 antibody. In orthotropic renal cell cancer models, these CAR T cells were capable of inhibiting tumor growth via T-cell exhaustion and NK cell recruitment.[124]

Using vaccine therapy to induce T lymphocyte activation is another approach. Tumor vaccines are intended to provoke an adaptive immune response to TAA whereby these TAA are presented by MHC I/II on APC for recognition by naïve T cells which then proliferate and differentiate into cytotoxic T lymphocytes with specificity for tumors expressing said TAA.[125] Several vaccine types exist including peptide-based, heat-shock protein and DC vaccines.

Peptide vaccines consist of TAA extracted from tumor tissue or synthesized from known epitopes. Some of the epitopes frequently employed in GBM-targeting peptide vaccines include melanoma-associated antigen 1 (MAGE-1), human epidermal growth factor receptor 2 (HER2) and gp100.[126] They are frequently administered with an immunostimulatory adjuvant such as a TLR agonist. There have been several studies targeting the epidermal growth factor receptor variant III, a transmembrane tyrosine kinase receptor variant which is expressed in 24%–67% of GBM.[127 128] Phase II studies (ACTIVATE, ReACT) of EGFRVIII specific peptide initially showed promising OS in the newly diagnosed and recurrent settings, respectively.[129 130] However, ACTIV, a phase III of 745 patients with newly diagnosed EGFRvIII+GBM treated with CDX-110 and GM-CSF showed no OS advantage over control.[131] Another approach has been to use intratumor delivery of recombinant polio-rhinovirus chimera, PVSRIPO. PVSRIPO binds with high affinity to poliovirus receptor (CD155) which is highly expressed on malignant glioma.[132] A phase I trial of 61 patients with recurrent GBM showed a median OS of 12.5 months.[133]

Heat-shock vaccines combine TAA with chaperone heat shock proteins (HSP). HSP are involved in post-translational protein folding as well as modulation of the immune response.[134–136] HSP complex with TAA and then internalized into APC via receptor-mediated endocytosis, via CD91 and LOX-1, for presentation to CD8+ T cells by MHC I.[137–139] Additionally, HSP can trigger an innate immune response through TLR.[140] Phase II results of 41 patients with recurrent GBM treated with a 96 kD HSP protein complex, HSPPC-96, showed a median OS of 42.6 weeks.[141] NCT03018288 is an ongoing randomized, double-blind phase II trial of surgery, chemoradiation, and pembrolizumab with and without HSPPC-96 in newly diagnosed MGMT unmethylated GBM.

DC vaccines use professional APC which participate in the innate immune response.[142] On internalization of antigen, DC migrate to lymphoid tissue where they stimulate CD4 and CD8 T cells. The VICTORI study, a trial of 20 patients with newly diagnosed grade III/IV glioma treated with autologous DC vaccine (DC pulsed with EGFRvIII peptide conjugated to keyhole limpet haemocyanin) showed an OS of 22.8 months from diagnosis.[143] A significant increase in antigen-specific T cell proliferation was observed in postvaccination peripheral blood. Another phase I, ICT-107, treated 20 patients with GBM

(and 1 brainstem glioma) with a multiepitope-pulsed DC vaccine (HER2, TRP-2, gp100, MAG-1, IL13Ra2, AIM-2) observing a median OS of 38.4 months with expression of target epitopes (MAGE-1, AIM-2) correlating with improved survival.[126] Other trials with smaller patient cohorts have shown long-term response and correlative immune response.[144] Finally, a comparison of 6 patients with malignant glioma treated with glioma-associated antigen compared with 28 patients treated with autologous tumor lysate-pulsed DC vaccine showed elevated NK activity in the glioma-associated antigen cohort.[145] Additionally, decreased T reg ratio and activated NK cells correlated with prolonged survival in these patients.

CONCLUSION

The development of PD-1, CTLA-4 and newer, "second generation" checkpoint modulators represents a potential opportunity to develop novel therapies for the treatment of primary brain tumors, particularly GBM. Unfortunately, there are currently limited data on immune checkpoint inhibitors in other types of glioma such as oligodendroglioma or astrocytoma. As these diseases tend to have a more indolent, although still malignant course, it is feasible that immune surveillance aided by checkpoint modulation could fair better than the more proliferative and aggressive GBM. A series of 10 patients with recurrent or refractory pediatric brain tumors including pineoblastoma, medulloblastoma, ependymoma and CNS embryonal showed transient partial responses in patients with PD-L1 expression and higher tumor mutation burden.[146] A study of the immune checkpoint inhibitor nivolumab is ongoing in patients with select rare CNS cancers (NCT03173950). Additionally, responses to immune checkpoint blockade have been reported, although rarely, in other primary CNS malignancies such as meningioma and primary CNS lymphoma[147–149]

Initial studies using single agent immune modulators in GBM have been mostly unsuccessful, thereby providing a stronger rationale for the consideration of combinational regimens. However, as outlined in this review, there are many candidate agents, which when considered for combination regimens, yield an impractical number of dual agent treatment regimens. Therefore, increased understanding of the mechanisms of action and resistance combined with more robust preclinical and early clinical testing will be needed to be able to adequately test the most promising therapies.

Among the activating immune checkpoint molecules, the ones with the most robust preclinical data are 4-1BB and GITR. Agonists against both these targets have demonstrated antitumoral activity and alteration of the cellular milieu in murine glioma models. They have both also proven to be synergistic with radiotherapy in mice, a particularly attractive feature as radiotherapy remains the mainstay of treatment for newly diagnosed GBM. GITR is also of interest in that, as its name suggests, it is upregulated by glucocorticoids.[150] Given the high prevalence of steroid use in patients with GBM, GITR may be overexpressed on the TILs of these patients. Although corticosteroids blunt the immune response of checkpoint modulators, upregulation of GITR with the addition of a robust GITR agonist may have an antitumor effect even in the presence of steroids. Furthermore, intracranial administration of GITR may be necessary to provoke an immune response. Given the available preclinical data, further early phase clinical trials to demonstrate safety and efficacy of these agonists in glioma are needed.

Other activating immune molecules including ICOS, OX40, and CD27 need further study in glioma mice models, specifically intracranial models, to demonstrate that agents targeting these molecules produce tumor regression, immune modulation, and improved survival. Preclinical work suggests combining OX40 agonists with vaccine therapies would likely be of most benefit. Cellular and antibody therapies directed against CD27 and its ligand CD70 have shown tumor inhibition in mice. However, contradictory preclinical findings have called into question whether CD27 and B7-H3 signaling plays a protumoral or antitumoral role in the immune system and more work is needed to elucidate if the antitumoral effect initially observed is indeed T lymphocyte mediated.

The inhibitory immune checkpoint molecules PD-1 and CTLA-4 are under active investigation with a recent study suggesting efficacy from PD-1 inhibitors when given preoperatively. This is consistent with the efficacy observed in early phase studies of Merkel cell, bladder, and triple negative breast cancer, suggesting that neoadjuvant treatment is a viable immunomodulatory approach.[151] Other modulatory strategies may also have utility. TIM-3 antibody blockade improves survival and, like 4-1BB and GITR, appears to have synergistic benefit with radiation in mice models. TIM-3 studies have also shown immunomodulation with increased immune cell infiltration, activation, and memory. Similarly, TIGIT, in combination with PD-1 blockade has demonstrated improved survival as well as increased cytotoxicity and decreased regulatory T cells. Both TIM-3 and TIGIT may be good candidates for phase I/II trials in patients with glioma. In contrast, the physiological mechanisms regarding LAG-3, A2AR, VISTA, and B7-H4 in glioma remain unclear and both targets would likely benefit from further preclinical studies to better define efficacy as well as their immunomodulatory effects. Similarly, conflicting findings exist for B7-H3, mandating additional research to elucidate the effect of B7-H3 blockade on different T cell subpopulations and associated tumor response before its consideration for clinical trial testing.

The function of immunomodulatory molecules such as those described here can be activated or inhibited by mab binding and this has to date proved to be the most successful approach. However, several other mechanisms for regulation exist, each with their own advantages and disadvantages. Small molecule inhibitors such as BMS-1166 could reach to molecular sites that larger antibodies cannot access due to steric hindrance. Nanobodies,

like KN035 can bind PD-L1 with great affinity. Furthermore, delivery systems such as viral vectors may improve BBB infiltration of nanobodies. CAR T cells hold great potential due to the range of genetic modifications (chimeric switch-receptors, antibody secretion) currently under development. Finally, vaccine therapy can prime cytotoxic and helper T cells for a more robust immune response.

In conclusion, the development of several novel immune checkpoint molecules represents a potential mechanism to overcome the immunosuppressive environment of GBM and achieve meaningful benefit. Of these "second generation" checkpoint molecules, the ones with the greatest potential and most preclinical data in glioma, are 4-1BB, GITR, TIM-3, and TIGIT. All of which should be considered for early phase clinical trials in glioma.

Acknowledgements We would like to acknowledge Chun Zhang Yang for his guidance regarding the U87 model.

Contributors WJK drafted the initial manuscript. AJG assisted with design and scientific review. MG provided concept and interpretation of scientific data. All authors contributed to manuscript editing, proofread and have approved the final manuscript.

Funding The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Patient consent for publication Not required.

Provenance and peer review Not commissioned; externally peer reviewed.

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