Title
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Journal
Clinical Cancer Research, 24(21)

ISSN
1078-0432

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Publication Date
2018-11-01

DOI
10.1158/1078-0432.ccr-17-2769

Peer reviewed

Published in final edited form as:

Clin Cancer Res. 2018 November 01; 24(21): 5198–5205. doi:10.1158/1078-0432.CCR-17-2769.

Immunotherapy of primary brain tumors: facts and hopes

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Abstract

The field of cancer immunotherapy has made exciting progress for some cancer types in recent years. However, recent failures of late phase clinical trials evaluating checkpoint blockade in glioblastoma (GBM) patients represent continued challenges for brain cancer immunotherapy. This is likely due to multiple factors, including but not limited to marked genetic and antigenic heterogeneity, relatively low mutational loads and paucity of GBM-infiltrating T cells. We review recent and ongoing studies targeting the checkpoint molecules as monotherapy or in combination with other modalities, and discuss the mechanisms underlying the unresponsiveness of GBM to single modality immunotherapy approaches. We also discuss other novel immunotherapy approaches that may promote T cell responses and overcome the “cold tumor” status of GBM, including oncolytic viruses and adoptive T cell therapy.

Introduction

Immunotherapy, in particular checkpoint blockade therapy, has been approved by the U.S. Food and Drug Administration (FDA) for multiple cancer types. However, early results from clinical trials in glioblastoma (GBM) have yet to demonstrate significant clinical benefits. This is likely due to multiple factors, including but not limited to marked genetic and antigenic heterogeneity, relatively low mutational loads and paucity of GBM-infiltrating T cells. In this regard, GBM is considered a type of “cold tumor.” In this concise review, we discuss the mechanisms underlying the unresponsiveness of GBM to single modality immunotherapy approaches thus far. We also discuss other novel immunotherapy approaches that may promote T cell responses and overcome the “cold tumor” status of GBM, including oncolytic viruses and CAR-T cell therapy.

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Conflict of interest statement: HO is an inventor of US utility patent application “H3.3 CTL peptides and uses thereof” (Case #: SF2015-163).

Promises and challenges of immune checkpoint blockade

Since 2011, there has been a revolutionary shift in the treatment of cancer owing to immune checkpoint blockade, including anti-programmed cell death (PD)1, anti-PD ligand (PD-L)1, and anti-cytotoxic T-lymphocyte-associated protein (CTLA)-4 targeted agents, which successfully demonstrated durable responses in a range of tumor types. For example, a phase 3 double-blinded randomized study of combination anti-PD-1 (nivolumab) and anti-CLTA-4 (ipilimumab) therapy in 945 patients with metastatic melanoma resulted in considerable improvement in overall survival at 3 years [CHECKMATE-067, NCT01844505; (1)], but with higher rates of severe adverse events (AEs) than those in single agent therapies. Subsequently, FDA approval for several agents in this class has been granted.

However, the application of these agents to primary brain tumors, such as GBM, has thus far yielded mixed results despite promising preclinical data (2). A phase 3, randomized, open-label trial of 369 patients comparing nivolumab to the anti-angiogenic monoclonal antibody (mAb) bevacizumab in patients with the first recurrence of GBM [cohort 2, CHECKMATE-143, NCT02017717; (3)] failed to demonstrate the benefit of nivolumab versus bevacizumab in the overall survival (OS). The OS was 9.8 months with nivolumab and 10.0 months with bevacizumab, and the 12-month OS was 42% in both arms. However, the median progression-free survival (PFS) was found to be 1.5 vs 3.5 months for nivolumab and bevacizumab, respectively. The overall response rate (ORR) was 8% (nivolumab) vs 23% (bevacizumab), with the median duration of response of 11.1 months (nivolumab) and 5.3 months (bevacizumab).

Exploratory cohorts from CHECKMATE-143 included nivolumab monotherapy compared to combination of nivolumab and ipilimumab (cohorts 1 and 1b) (4). At approximately 30 months follow-up, there were 3 partial responses, 20 patients with disease progression, and stable disease in 8 patients. Responses occurred in the non-randomized (allocated) cohort 1b, using a regimen of nivolumab 3 mg/kg plus ipilimumab 1 mg/kg for 4 doses every 3 weeks followed by nivolumab alone and in the randomized nivolumab monotherapy arm of cohort 1. The toxicities of immune and non-immune related AEs for either the combination or the monotherapy cohorts on CHECKMATE-143 are in line with other published reports of these agents, with higher rates of grade 3/4 SAEs following treatment of the combination arms. There were similar rates of AEs between bevacizumab and nivolumab in cohort 2.

The strategy of combining immune checkpoint inhibition with anti-angiogenic therapy was shown to be safe in a small pilot study of pembrolizumab with or without bevacizumab of 6 patients with recurrent GBM [NCT02337491; (5)], with no dose-limiting toxicities. Final efficacy results PFS and OS have not been reported. Several other clinical trials are ongoing to explore immune checkpoint blockade in newly diagnosed GBM. Nivolumab in combination with radiation therapy alone (without concurrent temozolomide) is being explored in a phase 3, randomized, placebo-controlled trial for patients without O6-methylguanyl methyltransferase (MGMT) promoter methylation, a poor prognostic marker for GBM that also demonstrates decreased response to alkylating chemotherapy (such as temozolomide; CHECKMATE-498, NCT02617589; (6)). Nivolumab is also being studied in a phase 3, randomized, placebo-controlled trial for MGMT-promoter methylated GBM with

upfront concurrent radiation and chemotherapy [CHECKMATE-548, NCT02667587; (7)]. Neither trial has published results to date.

In addition, a phase 2 open label, non-randomized clinical trial uses anti-PD-L1 mAb durvalumab in multiple cohorts comprising newly diagnosed MGMT promoter unmethylated GBM patients (cohort A) and both bevacizumab-naïve and -refractory recurrent GBM patients (cohorts B, C, NCT02336165). Interim results of durvalumab monotherapy in cohort B reveal low treatment-related SAE rate of 10% and efficacy with PFS-6 of 20.0%, OS-6 of 59%, and ORR of 16.7% for partial response and ORR of 46.7% for stable disease (8). Results from the other cohorts of this trial are pending. A Cochrane systematic review of immune checkpoint blockade therapy for glioma published a protocol which may illuminate efficacy trends across multiple studies (9). While the abovementioned trials involve GBM, few published or presented studies have evaluated checkpoint inhibition for low grade glioma, meningioma, or other primary brain tumors.

There are several proposed causes for the lack of success of immune checkpoint inhibitors (ICI) for primary brain tumors thus far. As with many therapeutic agents, blood-brain barrier (BBB) penetration may be limited for large mAbs, such as ICI. Efficacy of CHECKMATE-143 may also have been affected by repression of immune responses from patients on corticosteroids or prior treatment with myelosuppressive chemotherapy. A consideration for the failure of these agents in GBM patients is the challenging definition of tumor progression or response with immunotherapy. For example, CHECKMATE-143 employed the RANO (Response Assessment in Neuro-Oncology) criteria under which MRI imaging changes, such as increased size of T2/FLAIR or T1-post gadolinium contrast-enhancing lesions would be deemed progressive disease. Clinical experience, however, demonstrates that treatment with ICI can result in an initial peri-tumoral inflammatory response and even new lesions but is followed by imaging improvement, causing a mischaracterization of progressive disease (referred to as pseudo-progression) if using RANO (10). These challenges have been addressed in the development of immunotherapy-specific response criteria, iRANO (11) which allow for such imaging changes to be observed within the first six months after starting immunotherapy (assuming clinical stability of the patient) for a three-month window to confirm progressive disease. iRANO is already being incorporated into recently developed clinical trials (e.g. NCT02658981 et al.).

The effectiveness of immune checkpoint blockade by tumors is hypothesized to require expression of PD-L1 on tumor cells and PD-1 on peritumoral cytotoxic T lymphocytes, both of which have been demonstrated to varying degrees in gliomas (12). Higher expression of both receptors has a negative prognostic impact on OS (13) and higher PD-L1 expression correlated with the mesenchymal expression subtype of GBM (12). Values of PD-L1 expression on GBM cells also depend on the diagnostic anti-PD-L1 antibody used for detection (14).

GBM cells have a relatively low mutagenic burden (15), which is indicative of diminished responsiveness to ICI therapy (16). Rare exceptions are those tumors with deficiencies in *POLE* or mismatch repair genes (MMR, i.e. *MLH1, MSH2, MSH6, PMS2*) which have higher mutagenic burden (17). As a corollary to the tumor mutational burden, tumor

neoantigen expression (i.e. immunogenic epitopes derived from cancer-specific gene alterations) is also hypothesized to predict response to immunotherapy and may help direct treatment with specific agents (18). However, in an important study of these prospective biomarkers, even GBM patients with high tumor mutational burden were not enriched for cytotoxic T lymphocytes, PD-1-expressing T lymphocytes, or PD-L1-expressing tumor (19). Thus, it remains to be determined if there is a unique subtype of GBM or specific biomarker profile for which immune checkpoint inhibition is most effective.

Additionally, the tumor micro-environment (TME) of GBM may also contain immunosuppressive actors beyond PD-L1:PD-1 and CTLA-4. Other mechanisms, such as ones through the A2aR high-affinity adenosine receptor (on lymphocytes and tumor-associated macrophages) or PD-L2 (on macrophages lacking PD-L1 expression), may bypass ICI in glioma. The GBM TME has also been shown to contain regulatory T lymphocytes (Treg), while lacking substantial antigen-presenting cells (APC), all factors which abrogate effector T lymphocyte activity against tumors. The glioma TME demonstrates robust macrophage infiltration, including from phenotypically suppressive CD163+ M2 and undifferentiated M0 macrophages, particularly for mesenchymal gene expression GBM subtype (20). Among several biomarkers, STAT3 in particular has a role in driving immunosuppression and in tumor proliferation, survival, and angiogenesis in high grade glioma (21).

In contrast to primary brain tumors, treatment of brain metastases with ICI has shown clinical benefit. Although a detailed discussion of immunotherapy for central nervous system (CNS) metastases is beyond the scope of this review, comparing clinical results for such tumors can help elucidate the mechanism of their activity in primary brain tumors as well. For example, a dedicated clinical study that treated CNS metastases with combination nivolumab and ipilimumab followed by nivolumab alone demonstrated 19% complete responses and 56% ORR for un-irradiated intracranial disease [CHECKMATE-204, NCT02320058; (22)]. Severe treatment related AEs were 48% plus one death. Another open-label phase 2 clinical trial that examined combination nivolumab plus ipilimumab in un-irradiated brain metastases revealed similar results with 44% intracranial ORR and 68% severe AEs [ABC, NCT02374242, (23)] Interestingly, in the ABC trial, there were discordant responses for brain lesions to immune checkpoint therapy between patients treated with prior BRAF inhibitor treatment, 16% ORR compared to 53% for treatment-naïve patients.

Notably, patients demonstrating oligo-progressive disease of their melanoma brain metastases were allowed to receive stereotactic radiosurgery (SRS) in CHECKMATE-204. It has been hypothesized that tumor irradiation may improve the efficacy of ICI by i) triggering an type-I interferon-driven inflammatory response (24), ii) generating tumor neo-antigen uptake by APCs and MHC class I expression, and iii) eliminating phenotypically suppressive myeloid-derived suppressor cells (MDSCs) in the TME (25). Thus, the synergistic combination of radiation in both primary and metastatic brain tumors is being explored as a promising therapeutic direction to overcome immunologically cold tumors. For example, the use of ipilimumab alone adjuvant to SRS improved 1-year overall survival in patients with melanoma brain metastases compared to historical controls, 65% and 56%,

respectively (26). A planned trial for breast cancer metastatic to the brain will employ SRS and pembrolizumab and will prospectively observe both irradiated tumor responses along with Abscopal effects of non-irradiated metastases as well (NCT03449238). A dedicated phase II open label trial recently opened for combination nivolumab, ipilimumab, and salvage RT in melanoma with brain metastases at centers in Australia (ABC-X, NCT03340129), including an arm for combination of whole brain radiotherapy and immunotherapy in multiply-metastatic or leptomeningeal melanoma of the CNS. For high-grade glioma, a retrospective study of cranial re-irradiation up to 35 Gy plus anti-PD-1 treatment (with either pembrolizumab or nivolumab) reported 35% ORR and no increased cerebral edema with this combination (27), although no prospective data has been published.

Immune suppression in glioma

In addition to the presence of BBB, there are cellular and molecular mechanisms underlying the major challenges for developing effective immunotherapy strategies for gliomas.

Microglia, macrophages and MDSC

Primary brain tumors, in particular GBM, possess an immunosuppressive phenotype, both locally in the CNS and systemically. About 30–50% of the GBM microenvironment is comprised of myeloid cells (28), namely, microglia, tumor-associated macrophages (TAM) and myeloid derived suppressive cells (MDSC). Increased populations of MDSCs are found in the serum and in peritumoral milieu of GBM patients (29). Paracrine network signaling between glioma cells and TAMs promote mutual coexistence, via secretion of chemokines and other factors (including CCL2, CSF-1, MCP-3, CXCL12, CX3CL1, GDNF, ATP and GM-CSF) and can attract myeloid cells (30,31). Additionally, gliomas secrete various immunomodulatory cytokines that suppress microglial activation and skew macrophages towards an immunosuppressive M2 phenotype (32–34). In GBM, increased CD163+, CD204 + M2-macrophages correlate with a poor clinical prognosis (35), whereas CD74+ M1 cells are associated with improved survival (36).

Single-cell RNA-sequencing allowed us to gain novel insights on blood-derived and microglial TAMs (37). Blood-derived TAMs are enriched in perivascular and necrotic regions, and express higher levels of genes associated with phagocytic activity, immune-suppression and oxidative metabolism than microglial TAMs. Furthermore, gene signature of blood-derived TAMs, but not microglial TAMs, correlates with significantly inferior survival in low-grade glioma. Importantly, TAMs frequently co-express canonical pro-inflammatory (M1) and alternatively activated (M2) genes in individual cells, suggesting that the nominal M1 vs. M2 dichotomy may have to be revised for glioma TAMs.

In regard to TAM-associated chemokines, CCL2 secretion by gliomas is correlated to histopathologic grade. The chemokine has been shown to recruit TAMs and abet the infiltration of Treg cells (38). In addition, it can promote tumor proliferation and angiogenesis through the CCL2/CCR2/IL-6 axis, leading to enhanced production of MMP2 that further augments tumor invasion (39). Secretion of IL-10 by M2-type myeloid cells also inhibits IFN-γ production, downregulates MHC class II on APCs and CD80/CD86, and

induces T cell anergy (40). Immunosuppression in GBM is partly dependent on upregulation of STAT3, which can reduce T cell proliferation, trigger T cell apoptosis and induce Tregs (41). Furthermore, GBM-derived GM-CSF plays a central role in IL-4Rα upregulation on MDSCs in glioma, while the production of arginase inhibits T cell proliferation and function (42).

Regulatory T cells

The presence of suppressive circulating and tumor-infiltrating Foxp3+CD25+CD4+ Tregs corresponds to decreased effector T cell responses, both peripherally as well as in tumors (43). Presence of Tregs positively correlates with tumor grade (44). These Tregs express high levels of glucocorticoid-induced TNFR-related protein (GITR) that suppresses the function of APCs via inhibitory cytokines (IL-10, TGF-β, et al) (45). Indoleamine 2,3 dioxygenase (IDO), an enzyme converting tryptophan to kynurenine, is a potent inhibitor of T cell proliferation and effector responses (46). IDO upregulation in glioma was associated with poor prognosis. Using a preclinical glioma model, IDO expressing tumors magnified recruitment of Tregs (47). Intratumoral Tregs exhibit increased expression of CTLA4 compared to blood-derived Tregs (48). Overcoming Treg-mediated suppression has been proposed with both cytotoxic approaches, such as with temozolomide or cyclophosphamide, as well immunotherapeutic approaches such as anti-CD25 antibody, or blockade of IDO, STAT3, CTLA4 and PD-L1 (43,49), but this paradigm will require further research into the timing and mechanism of such approaches (50).

VEGF and TGF-β mediated T-cell suppression

Microvascular proliferation and tumor-induced neoangiogenesis are a hallmark of GBM (51,52). Neoangiogenesis is related to high levels of secreted vascular endothelial growth factor (VEGF) that can promote tumor growth as well as disrupting the BBB leading to induction of interstitial pressure and cerebral edema (53). VEGF-mediated suppression of intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 on endothelium inhibits T-cell infiltration to GBM. VEGF leads to increased infiltration of macrophages that secrete inhibitory cytokines (such as TGF-β) contributing to the immunosuppressive tumor microenvironment. TGF-β further reduces ICAM expression, inhibiting perivascular T cell transmigration, such that blocking TGFbeta-1/2 improved T cell infiltration in preclinical studies (52,54).

Galectin-1 and T cell apoptosis

Emerging evidence suggests that Galectin-1, a glycan binding protein, is another factor in GBM immunosuppression. By interacting with beta-galactoside-expressing glycoproteins on T cell surface, galectin-1 on glioma cells or tumor endothelial cells can negatively regulate T cell survival, inhibit T cell proliferation, block effector cytokine production, and antagonize T-cell signaling. Furthermore, galectin-1 promotes accumulation and expansion Tregs, thwarting the effector T cell response (55).

Immunosuppression by mutations in isocitrate dehydrogenase (IDH) genes

Mutations of the isocitrate dehydrogenase (IDH) enzymes IDH1 and IDH2 are early and frequent (70–80%) genetic alterations in WHO grade II or III gliomas as well as in secondary GBM. These mutations results in the conversion of a-ketoglutarate to (R)-enantiomer of 2-hydroxyglutarate (R-2HG) (56), and coordinate genome-wide epigenetic changes (57). Our group recently reported that IDH mutations and R-2HG lead to a decrease in STAT1 (signal transducer and activator of transcription 1) and effector T-cell-attracting chemokines, such as CXCL10, thereby inhibiting accumulation of effector T-cells in gliomas (58). We also showed that an inhibitor of mutant IDH1, IDH-C35, reduces R-2HG, recovers STAT1 and CXCL10, and enhances glioma-infiltration of T-cells and the efficacy of vaccines against IDH-MUT gliomas in mice (58). IDH mutant gliomas demonstrate a significant reduction in total leukocyte population including macrophages, microglia, dendritic cells, T and B-cells (59) as well as lower PD-L1 expression (60). Together, these studies point to the significant impact of IDH mutations on the immunological environment of glioma.

In Table 1, we summarize the known mechanisms in glioma microenvironment that lead to immunosuppression.

Approaches to enhance effective T cell responses

Chimeric antigen receptor (CAR) T-cells have recently shown considerable success in treating hematological malignancies that are otherwise refractory to traditional chemotherapy (61). Application of this novel therapeutic approach to solid tumors is an on-going effort. For GBM, initial efforts have been encouraging, namely the results of a phase I studies of CAR-T cells, such as one targeting EGFRviii (62, reviewed in 63). Analysis of surgically resected tumor samples following administration of CAR-T cells showed that EGFRviii CAR-T were able to traffic to the active tumors, proliferate *in situ,* and exert direct EGFRviii activity that led to loss of EGFRviii-expression in tumors. T cell repertoire screening identified a marked increase in number and clonotypic diversity of tumor infiltrating T cells post CAR-T infusion, a secondary effect of EGFRviii-CAR-T trafficking possibly a result of epitope spreading. There was an increase in CD8+ effector T cells and other activated cells along with an increased expression of IFN-γ, Granzyme-b and CD25 in post-CAR-T infused tumors compared to pre-infusion-tumor tissue. However, the post-CAR-T-infused tumors had an increase in compensatory immune-suppressive molecules like IDO-1, PD-L1, TGF-β, IL-10 and Foxp3. Heterogeneity of EGFRviii expression and immune-suppressive mechanisms remain major barriers to the efficacy of this therapy, but may potentially be defeated by combinatorial approaches targeting the immune-suppressive environment. Nonetheless, EGFRviii-CAR-T treatment, induced an immunogenic tumor microenvironment without apparent neurotoxicity (62).

Therefore, development of effective and safe adoptive T cell transfer therapy may represent a promising modality to turn the “cold tumor” status of GBM TME into “hot”. Our team recently identified a novel neoantigen epitope encompassing the K27M mutation within the histone 3 variant H3.3, which is present in a majority of diffuse midline gliomas (64).

Furthermore, we have cloned cDNA for T-cell receptor (TCR) α- and β-chains derived from a high avidity H3.3.K27M-specific CTL clone (64), allowing us to develop novel vaccine- and TCR-transduced T-cell-based immunotherapy strategies in patients with H3.3K27M+ gliomas.

Oncolytic viruses as immunotherapy

An alternative strategy to overcome the "cold" microenvironment of brain tumors may be the therapeutic use of engineered viruses (reviewed in 65). An oncolytic virus can directly infect and kill tumor cells, while also engaging innate immunity and launching an enduring adaptive anti-tumor immune response (66). A key feature of such viruses is that entire inventory of tumor neo-antigens is available for uptake by APCs which become activated by viral infection. There are multiple vectors and strategies for oncolytic viruses currently being evaluated in clinical trials, several of which are presented in Table 2. Future directions may involve combination of oncolytic viral therapy with ICI to enhance the anti-tumor immune response.

Neurotoxicity associated with CAR-T therapy

While none of the GBM CAR-T trials demonstrated significant neurotoxicity to date, in CD19-directed CAR-T therapy studies for pediatric ALL (acute lymphoblastic leukemia), severe neurotoxicity and treatment-related deaths were observed (67). This neurotoxicity was unrelated to presence or degree of intra-CNS disease and was shown to be associated with cytokine-release syndrome (CRS), specifically peak IL-15 secretion, BBB permeability, and endothelial activation (68–70). The rates of severe CRS were abrogated in a pilot study by combined pre-treatment with tocilizumab (anti-IL-6 mAb) and dexamethasone without affecting OS or ORR (71). Another direction that has been used to avoid CRS-related neurotoxicity includes local delivery of CAR-T cells, thus limiting systemic immune effects (72–74).

Concluding remarks

ICI represent a novel therapeutic approach to mitigate the immunosuppressive nature of glioma. Although results of early clinical trials failed to demonstrate clear efficacy for anti-PD1-directed therapy, encouraging initial results for these agents in brain metastases, either alone, in combination with anti-CTLA-4, or in the setting of radiation therapy, suggest a direction for further exploration. We reviewed various mechanisms that leads to immunosuppression in GBM. On the other hand, induction of roust inflammatory responses by intravenous infusion of EGFRviii CAR-T therapy suggests that it is possible to turn their "cold" environment to "hot" without inducing neurotoxicity. Targeting shared tumor neoantigens as T cell therapy could help achieve effective anti-tumor immunity. Intraventicular delivery of T cells versus intravenous delivery of T cells could reduce the possibly reduce neurotoxicity and enhance efficacy of treatment. Focusing on parameters like immune suppression can help achieve better efficacy by combinatorial treatment approaches targeting immune-suppression.

Acknowledgments

Funding: NIH/NINDS 1R35 NS105068 (Okada)

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Table 1

Mechanisms of immunosuppression in gliomas

Type	Mechanism	Examples	References
Tumor-cell intrinsic	Glioma-mutations and effect on tumor microenvironment	**IDH1-R132H mutation:** Downregulates effector molecules like IFNγ, Granzyme-b, CXCL9, CXCL10 thereby reduces total $CD8^+$ T-cell numbers in the tumors	(58,60)
		NF1 loss: Increases M2-like macrophages/microglia in tumors	(20)
		N-Myc amplification: Decreases IFNγ, CXCL10 resulting in poor infiltration of T-cells to tumors	(75)
		Mesenchymal subtype of tumors: Enhances M2 macrophages/microglia, reduces responses to radiation, increases PD-L1 on tumors	(20)
		Absence tumor hypermutation: Decreases T-cells in tumors	(15,16)
	Glioma-associated downregulation of HLA and antigen presentation	**Loss of Heterozygosity (LOH) in HLA:** Associates with shorter survival and decrease in intra-tumoral $CD8^+$ T-cells	(76)
		Tapasin: Closely associates with HLA-loss, levels correlate with survival	(77)
	Glioma expression of immune-checkpoint receptors	**PD-L1:** Higher expression correlates with worst prognosis. Suppresses CTL proliferation and function	(13)
		CTLA4: Modulates T-cell activation to an immune-suppressive state	(78)
	Glioma-specific receptors suppressing T-cell proliferation/function	**GLUT1:** Increases expression on glioma cells, enhances glucose intake, reduces T-cell proliferation by competition in glucose uptake	(78,79)
		Galectin-1: Inhibits T-cell proliferation and effector responses. Increases MDSC and immune-suppressive macrophages in tumor microenvironment	(55)
		STAT3: GBM Cancer initiating cells inhibits CTL proliferation and function, induces Tregs, triggers T-cell apoptosis through STAT3	(41)
	Glioma-induced immune-modulatory molecules	**TGF-b:** Polarizes T-cells, macrophages, microglia to immune-suppressive states. Inhibits effector responses in T-cells, downregulates MHC-II on glioma cells and myeloid cells, promotes Treg activity.	(80)
		VEGF: Causes downregulation of ICAM-1 and VCAM-1, inhibits T-cell transmigration through GBM vessels	(52)
	Glioma-induced T cell apoptosis	**CD70:** Mediates T-cell apoptosis upon interaction with CD27	(81)
		Gangliosides: Mediates T-cell apoptosis	(81)
Tumor-cell extrinsic	Suppression in CTL responses by immune-suppressive TAMs, Microglia and MDSC	**IL-6:** Suppresses effector cell responses. Activates STAT3 to further inhibit T-cell proliferation and function. Increases infiltration of suppressive TAMs and Microglia through IL-6-CCL2-CCR2 loop	(41,82)

Type	Mechanism	Examples	References
		IL-10: Inhibits IFNγ, TNFα and T-cell function, promotes Tregs, downregulates CD80, CD86, MHC-II in myeloid cells causing $CD8^+$ T-cell anergy	(40)
		FasL: Induces T-cell apoptosis	(83)
		IL-4Ra: Promotes MDSC in glioma microenvironment, produces immune-suppressive arginase, inhibits T-cell proliferation and function	(42)
		CCL2: Induces Treg, increases infiltration of TAMs, microglia and MDSC that produce CTL inhibitory factors	(38)
		PGE2: Induces regulatory DC, leads to differentiation and accumulation of suppressive MDSC, reduces Th1 cytokine secretion	(45)
	Regulatory T cell mediated suppression of CTLs	**GITR:** Induces Treg expansion, inhibits CTL function, leads to secretion of IL-10 and suppresses APC function	(45)
		IDO1: Increase in IDO levels in glioma associates with poor prognosis. Inhibits T-cell proliferation and function, induces Treg recruitment to tumors, reduces CTL infiltration to tumors	(84)
	Hypoxia	Causes abnormal glioma vasculature, increases VEGF secretion, downregulates ICAM and VCAM molecules thereby inhibiting $CD8^+$ T- cell infiltration to tumors, activates Tregs via STAT3, increases immune-suppressive mechanisms by promoting M2-type myeloid cells in tumors	(85)
Immune-privilege of CNS	Differential homing patterns in CNS versus periphery	T-cell homing to CNS compared to periphery is a two-step process. First step involves crossing the post-capillary venular endothelium and second step involves crossing the glia limitans	(86)
		Lack of resident T-cells or professional APCs in brain parenchyma. Decrease in MHC-II expression on APCs inhibits antigen presentation in parenchyma of CNS	(86)
		Afferent perivascular drainage pathway for ISF from CNS to regional cervical lymph nodes prevents cellular trafficking. Immune cells traffic through CSF's lymphatic drainage	(86,87)

Table 2

Viral therapy for primary brain tumors

Name	Virus	Features	Advantages	Caveats	Trial, References
PVS-RIPO	Poliovirus	Oncolytic, CD155 is receptor for attachment, modified with rhinovirus IRES to prevent replication in anterior horn motor neurons	Induces IFN-response and activation of DCs and tumor Ag-specific effector T lymphocytes	Delivered surgically via convection-enhanced delivery (CED) Limited activity in immunocompetent persons	NCT01491893 (88)
DNX-2401	Adenovirus Delta-24-RGD	Oncolytic	20% OS at 36 mo, 12% CR in recurrent high-grade glioma	Direct intratumoral injection at time of resection or through surgically placed catheter.	NCT00805376 (89)
Toca-511	Replicating retroviral vector	Non-oncolytic, requires gene integration and cell division to produce cytosine deaminase	20% long-term OS (without tumor recurrence in survivors) May be administered intravenously	Requires oral administration of 5-FC for conversion to cytotoxic agent 5-FU	NCT02414165 (90)
INXN-2001	Adenovirus	Vector Ad-RTS-hIL-12 has IL-12 expression under control of an inducible promoter	Induced IFNgamma expression and increased CD8+ effector T lymphocytes	Requires oral compound (veledimex) to activate viral expression of IL-12	NCT02026271; NCT03330197 (91,92)
MV-NIS, MV-CEA	Measles virus	Oncolytic, CD46 is MV receptor, based on vaccine strain	Engineered to produce thyroidal sodium iodide symporter or CEA (carcino-embryonic antigen)	Administered directly intratumorally or via lumbar puncture	NCT02962167, NCT00390299 (93)