



Insights in the immunobiology of glioblastoma

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Abstract

Glioblastoma, a grade IV astrocytoma, is considered as the most malignant intracranial tumor, characterized by poor prognosis and therapy resistance. Tumor heterogeneity that often leads to distinct functional phenotypes contributes to glioblastoma (GB) indispensable growth and aggressiveness. The complex interaction of neoplastic cells with tumor microenvironment (TME) along with the presence of cancer stem-like cells (CSCs) largely confers to extrinsic and intrinsic GB heterogeneity. Recent data indicate that glioma cells secrete a variety of soluble immunoregulatory factors to attract different cell types to TME including astrocytes, endothelial cells, circulating stem cells, and a range of immune cells. These further induce a local production of cytokines, chemokines, and growth factors which upon crosstalk with extracellular matrix (ECM) components reprogram immune cells to inflammatory or anti-inflammatory phenotypes and manipulate host's immune response in favor of cancer growth and metastasis. Herein, we provide an overview of the immunobiologic factors that orchestrate the complex network of glioma cells and TME interactions in an effort to identify potential therapeutic targets for GB malignancy. Current therapeutic schemes and advances in targeting GB-TME crosstalk are further discussed.

Key messages

- Intrinsic and extrinsic tumor heterogeneity affects GB growth and aggressiveness.
 - GB cells secrete growth factors and chemoattractants to recruit immune cells to TME.
 - GAMs are a critical cell type in promoting GB growth.
 - GAMs change from pro-inflammatory, anti-tumor M1 phenotype to pro-tumorigenic M2.
 - Novel therapeutic agents target the crosstalk of neoplastic cells with TME.

Keywords Gliomas · Cytokines · Chemokines · GAMs · Immunotherapy · Vaccines

Introduction

Gliomas constitute the most common and malignant primary neoplasms of the central nervous system (CNS) with a mortality rate approaching 80% in the first year of diagnosis [1]. They derive from neuroepithelial stem cells and are categorized as diffuse astrocytic and oligodendroglial tumors, other astrocytic, ependymal tumors, and other gliomas [2].

Glioblastoma (GB) refers to grade IV diffuse astrocytoma and is considered as the most malignant intracranial tumor [2].

GBs are classified either as IDH wild-type tumors (representing about 90% of cases, mainly in patients over 55 years old), occurring de novo in the absence of a less malignant precursor, previously referred to as primary [3], or as IDH-mutant (representing about 10% of cases, mainly in younger patients) which have developed from a lower-grade diffuse glioma, characterized by a better prognosis and previously known as secondary. A third group encompasses glioblastoma tumors that IDH cannot be fully evaluated, and they are not otherwise specified (NOS) [2].

The median age at diagnosis of patients with IDH wild-type and IDH-mutant GBs is 62 and 44 years, respectively, whereas the median overall survival time after chemotherapy is 15 and 31 months, respectively, with men being at a higher risk [2].

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IDH wild-type GBs are further subdivided into epithelioid, giant-cell GB, and gliosarcoma [2]. Epithelioid GBs usually arise in children and younger adults and often contain a v-Raf murine sarcoma viral oncogene homolog B (*BRAF*) *V600E* mutation and hemizygous deletions of Teneurin transmembrane protein 3 (*ODZ3*) [4] but lack the other molecular features of conventional adult IDH wild-type GBs, such as epidermal growth factor receptor (*EGFR*) amplification. Finally, other patterns include glioblastoma with primitive neuronal component, previously referred to as GB with PNET-like component, which contains primitive cells with neuronal differentiation, often with a v-myc avian myelocytomatosis viral oncogene homolog (*MYC*) or v-myc avian myelocytomatosis viral oncogene neuroblastoma-derived homolog (*MYCN*) amplification; small-cell GB/astrocytoma, formerly characterized as uniform with frequent *EGFR* amplifications; and granular cell GB/astrocytoma [2].

On the top of genetic and signaling defects, recent studies also demonstrate the important role of tumor heterogeneity in GB indispensable growth and aggressiveness that often leads to distinct functional phenotypes. The existence of different clones in the population of GB cells has been previously demonstrated, exhibiting differential therapy responses [5]. Specific cell niches inside the tumor known as cancer stem-like cells (CSCs) have been suggested to confer to intrinsic tumor heterogeneity. At the same time, interaction of neoplastic cells with the microenvironment leads to an extrinsic type of heterogeneity that enhances tumor infiltration and progression. Darmanis et al. used single-cell RNA sequencing in four GB patients to investigate neoplastic cells in the tumor core as well as those away from the primary tumor. They detected that different tumors shared some hallmark chromosomal abnormalities, while cancer cells in the same patient could also be categorized into subpopulations using their smaller-scale mutations. Infiltrating cells were also shown to have common characteristics in those patients [6]. Tumor-infiltrating macrophages and resident brain microglia were analyzed, showing that the first preferentially occupy the tumor core and the latter the peritumoral spaces. The tumor core microglial cells expressed anti-inflammatory and pro-angiogenic factors while the macrophages in the tumor periphery express more pro-inflammatory markers [6]. Another study demonstrated that the perivascular cluster of tumor cells carries the greatest potential for tumor progression and recurrence by being the most important niche for glioma stem cells [7].

Altogether, tumor microenvironment (TME) is composed of several cell types including astrocytes, endothelial cells, circulating stem cells, and a range of immune cells that are attracted by soluble factors secreted by glioma cells. Glioma-associated microglia/macrophages (GAMs), myeloid-derived suppressor cells (MDSCs), CD4⁺ and CD8⁺ T lymphocytes, T regulatory lymphocytes (Tregs), dendritic cells (DCs), and natural killer cells (NKs) have all been detected in TME

exhibiting a crucial role in GB proliferation, invasion, and resistance to treatment (Fig. 1). These cells and most importantly GAMs induce local production of cytokines and chemokines which upon crosstalk with extracellular matrix (ECM) components reprogram immune cells to inflammatory or anti-inflammatory phenotypes and manipulate host's immune response in favor of cancer growth and metastasis [1, 8, 9].

In this review, we discuss the important role of immune cells and soluble mediators in the regulation of TME in GB malignancy. We further appraise novel therapeutic interventions that may modulate TME in GB and improve treatment approaches.

Critical role of TME cellular heterogeneity in GB malignancy

Several types of lymphocytes infiltrate the TME including mostly CD4⁺ T helper, CD8⁺ T cytotoxic, and Tregs. CD4⁺ cells are more numerous than CD8⁺ in GB tissues and are associated with high tumor grade [8]. They express high levels of inhibitory co-receptors T cell immunoglobulin and mucin-domain containing-3 (TIM-3) and programmed cell death protein-1 (PD-1), characteristic of cell exhaustion. T cell exhaustion refers to a state of progressive T cell dysfunction with poor effector functions, prolonged antigen stimulation and expression of inhibitory receptors, and a distinct transcriptional state from that of functional memory or effector T cells. However, CD4⁺ cells retain the ability to secrete pro-inflammatory cytokines such as IFN γ which further promotes T cell migration into CNS [10]. In accordance, CD8⁺ lymphocytes observed in GB tissues are mainly inactive (CD25⁻) and suffer from cell exhaustion, possibly attributed to constant exposure to tumor antigens [11]. The immunosuppressive TME inhibits their activation by inducing them to express high levels of co-inhibitory receptors [11].

Exhausted T cells also exhibit metabolic changes. They are often characterized by a switch to a highly glycolytic metabolism through upregulation of glucose transporter 1 (GLUT1) expression, mediated by mammalian target of rapamycin (mTOR) signaling [12]. This switch may be a cause of exhaustion that leads to an antagonistic state between tumor and T cells over nutrients (such as glucose) which are scarce in TME. T cells that have gone through this metabolic change exhibit dysregulation of T cell receptor (TcR) signal transduction and are unable to combat tumors [12].

By contrast, Tregs (CD4⁺CD25⁺FoxP3⁺) are produced at higher rates in GB patients suppressing the function of antigen-presenting cells (APCs) and inhibiting the proliferation of cytokine-secreting T cells, thus contributing to immunosuppression [13, 14]. Forkhead box P3 (FOXP3) is the main transcription factor which controls the expression of

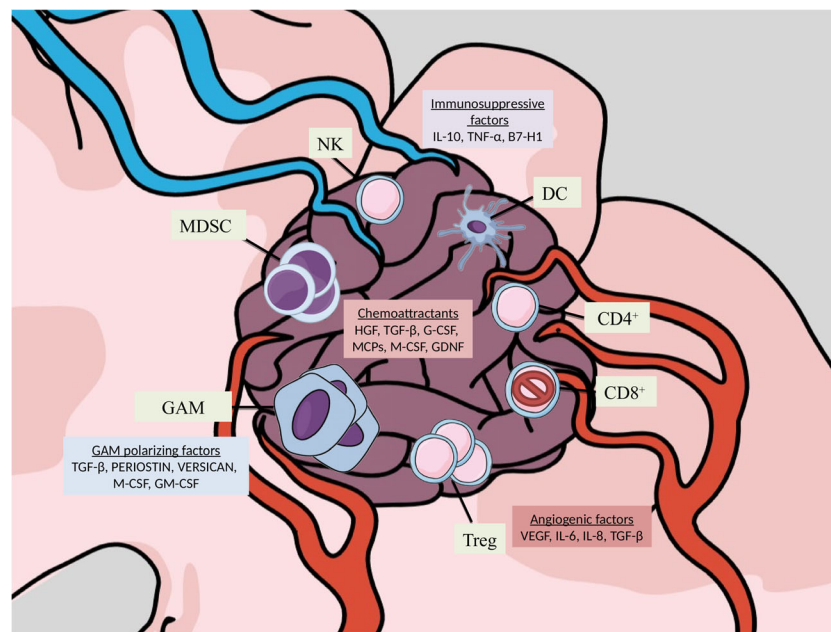


Fig. 1 Cell types and factors involved in glioma and tumor microenvironment (TME) crosstalk. Glioma cells secrete various chemoattractants to recruit different cell populations in TME including glioma associated microglia/macrophages (GAMs), myeloid-derived suppressor cells (MDSCs), CD4⁺ and CD8⁺ T lymphocytes, T regulatory lymphocytes (Tregs), dendritic cells (DCs), and natural killer cells (NKs) which play a crucial role in GB proliferation, invasion, and resistance to treatment. Furthermore, they produce polarizing factors to stimulate the

transition of GAMs to M2-like anti-inflammatory, pro-tumorigenic phenotype along with additional immunosuppressive and angiogenic factors to enhance glioma cell migration and invasion. HGF, hepatocyte growth factor; TGF- β , transforming growth factor- β ; MCP, monocyte chemoattractant protein; GDNF, glial-derived neurotrophic factor; IL-10, interleukin-10; TNF- α , tumor necrosis- α ; B7-H1, B7-homolog 1; GM-CSF, granulocyte-macrophage colony-stimulating factor; VEGF, vascular endothelial growth factor

cytokine genes, including IL-10 and transforming growth factor- β (TGF- β), and regulates the immunosuppressive activity of Tregs [13]. The majority of T cell populations in glioma tissues have been identified as Tregs based on FOXP3⁺ staining [14] while increased Treg levels have been correlated with increased tumor grade [15]. However, contradictory data exist regarding the prognostic importance of increased Treg levels in GB patients and need further investigation [8].

Clinical studies showed an extensive recruitment of microglia and peripheral macrophages known as GAMs in TME that increased with glioma progression and tumor grade [8]. These cells can adapt a “plastic” phenotype based on environmental cues and switch between the M1 pro-inflammatory, anti-tumor state to M2 (M2a,b,c) cytoprotective and immunosuppressive state. In brain tumors, GAMs with the M2-like phenotype, M2c were activated by the anti-inflammatory cytokines (IL-10, TGF- β) produced by tumor cells and were involved in tumor growth [8]. In GB, GAMs can be polarized by tumor cell-secreted molecules such as macrophage colony-stimulating factor (M-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) into the anti-inflammatory phenotype M2c and enhance tumor proliferation [16]. GAMs may also develop an M2-like phenotype-inducing loop by expressing both IL-10 and its

receptor (IL-10R) while they also express a vast array of molecules such as TGF- β , IL-6, IL-1 β , EGF that suppress immune cell response to tumor cells and enhance their proliferative, invasive, and migratory functions [17].

Another cell population that promotes immunosuppression in GB is tumor-infiltrating dendritic cells (TIDCs) that inhibit T cell immunity and participate in glioma progression [18]. Xin Yu et al. demonstrated that anti-T cell immunoreceptor with Ig and ITIM domains (TIGIT), a membrane protein expressed on T cells, exerted its effect only in the presence of dendritic cells and had an inhibitory effect on the activation of T cells when it was bound to the poliovirus receptor (PVR) [19]. At the same time, increased PD-1 expression prevents T cell activation by blocking the co-stimulatory signal of APCs. Studies on the immunosuppressive properties of DCs have shown that immature DCs contribute to the immune tolerance observed in cancer, suggesting a link between immature DCs and promotion of Treg cell development [8]. Dhodapkar et al. demonstrated suppression of antigen-specific immune response as well as of antigen-specific T cell immunity upon injection of immature antigen-pulsed DCs in humans [20]. Furthermore, an increase in antigen-specific IL-10 secretion by the CD8⁺ T cell population was also observed [20].

In concert, increased MDSCs present in TME exhibit an overall immunosuppressive effect over NK, CD4⁺, and CD8⁺

cell functions [21]. The polymononuclear MDSCs (CD15⁺) that are predominantly found in GB suppress CD8⁺ T cells by producing reactive oxygen species (ROS) and secreting immunosuppressive cytokines as well as by increasing the production of Tregs [22]. Moreover, the number of CD4⁺ effector memory T cells was associated with the number of polymononuclear MDSCs within the tumor area. These CD4⁺ effector memory T cells demonstrated upregulated expression of PD-1 and showed signs of exhaustion, implicating MDSCs in their suppression [22].

Finally, NK cells compose a small part of tumor-infiltrating cells in TME but are rendered inactive due to interactions of class I human leukocyte antigens (HLAs) present in glioma cells with killer-cell immunoglobulin-like receptors (KIR) that block their effective recognition and subsequent killing [8]. Furthermore, increased TGF- β levels produced by glioma have been shown to downregulate the natural killer group 2 member D (NKG2D) receptor on the surface of NK cells [23] and escape immune surveillance [24].

Role of soluble immunoregulatory factors in TME of GB

Several growth factors, cytokines and chemoattractants, have been detected to stimulate migration and polarization of immune cells in TME to allow generation of distinct cellular phenotypes that support tumor growth (Fig. 1).

Chemoattracting/recruitment factors

Neoplastic cells secrete multiple chemokines that attract non-neoplastic cells to the TME. The expression of hepatocyte growth factor (HGF)/scatter factor (SF) and its binding receptor c-Met has been detected in GAMs and in neoplastic cells of the GB where it induces their proliferation and invasion [25]. Secretion of HGF/SF by glioma cells has been demonstrated to promote the migration of both microglial cells and bone marrow-derived macrophages [25].

Monocyte chemoattractant protein (MCP) family has also been involved in chemotaxis and migration of immune cells from the periphery [26]. MCP-1 and MCP-3 are capable of attracting immune cells through binding to CCR2 and CCR1 as well as CCR2 and CCR3 receptors, respectively [27]. MCP-3 has been reported to be a potent stimulant for the migration of macrophages, monocytes, and NK and T cells as well as DCs [28]. Furthermore, the glial-derived neurotrophic factor (GDNF) which is expressed in neurons and glial cells and overexpressed in high-grade glioma cell lines [29] is involved in the chemotaxis of microglia and promotes GB migration in an autocrine manner [30].

Other known chemokines and receptors that are expressed by GAMs and are involved in chemoattraction include

CX3CL1/CX3CR1, C-X-C motif chemokine ligand 12 (CXCL12)/C-X-C chemokine receptor type 4 and 7 (CXCR4/CXCR7), CCL2/CCR2, and CCL5/CCR5 [8, 9].

GAM-polarizing factors

GAMs have been observed to undergo a change in their phenotype from M1 to pro-tumorigenic M2 type as a result of several polarizing factors. Among them, TGF- β 2 acts in synergy with prostaglandin E2 to promote the M2 polarization of GAMs and other immune cells [31] through inhibition of MHC I and II expression on glioma and microglial surface [32]. Additionally, TGF- β 2 secretion by GAMs causes the upregulation of its own receptors in tumor cells, thus promoting tumor growth [33].

The downstream effector of TGF- β signaling, periostin, is an ECM protein also implicated in the attraction and activation of GAMs in GB [33]. In mice xenografts, knockdown of *periostin* was shown to drastically reduce the number of M2-like GAMs leading to elevation of the M1 polarized GAMs and inhibition of tumor growth [34]. In addition, TGF- β 1 has been detected to stimulate the synthesis of the chondroitin-sulfate proteoglycan versican by glioma cells which further acts on GAMs to promote inflammatory cytokine production and support glioma invasion [8].

Finally, macrophage and granulocyte-macrophage colony-stimulating factors (M-CSF, GM-CSF) have been shown to exhibit GAM polarizing effects but their results still remain contradictory in gliomas [8].

ECM degradation mediators

The crosstalk of GAMs and neoplastic cells further stimulates cytoskeletal and ECM rearrangements in TME that enhance their migratory and invasive potential. During M1/M2 polarization of GAMs, lactadherin and osteopontin released from neoplastic cells promote changes in actin filament contraction and microtubule rearrangements, further enhancing their migratory capacity [9]. GAM-derived TGF- β 2 has been demonstrated to increase matrix metalloproteinase-2 (MMP-2) expression that promotes ECM deposition and facilitates invasion of gliomas.

Additionally, increased expression of IL-8 by GB may be involved in the regulation of ECM rearrangements. Blocking of IL-8 binding to its receptor with neutralizing antibodies was shown to reduce glioma stem cell motility and migratory behavior through nuclear factor kappa B (NF- κ B) downregulation [35]. Moreover, IL-8-NF- κ B signaling was found to promote F-actin polymerization and mediate epithelial-mesenchymal transition (EMT) being implicated in regulation of tumor cell migration and invasion [36–38].

Angiogenic factors

Cyclooxygenase-2 (COX-2), TGF- β , IL-8, and IL-6 were demonstrated to contribute to tumor's neoangiogenesis. COX-2, being an enzyme that participates in the production of eicosanoids, plays a major role in GB angiogenesis, by inducing the synthesis of vascular endothelial growth factor (VEGF) [39]. VEGF levels have been correlated with GB patient poor prognosis and recurrence [40].

In addition, glioma cells have been demonstrated to stimulate increased microglial production of TGF- β [41] which further induces proliferation of high-grade gliomas through activation of Ras protein and the mitogen-activated protein kinase (MAPK) pathway, leading to upregulation of VEGF and its receptor [42, 43]. At the same time, TGF- β isoforms cooperate with hypoxia-inducing factor (HIF) to highly stimulate the formation of tumoral vasculature.

Furthermore, IL-8 acts as a powerful activator for angiogenesis by regulating the migration of endothelial cells and their survival and proliferation [42]. In concert to IL-8 function, IL-6 activates the STAT3 signaling cascade which leads to increased VEGF expression levels in GB, contributing to tumor vascularization [44].

Immunosuppressive factors

The neoplastic cells as well as TME resident cells produce several immunosuppressive factors, which direct the immune system to anti-inflammatory responses.

The pro-inflammatory cytokine tumor necrosis factor- α (TNF- α) is normally produced by activated immune cells and suppresses tumor proliferation [45]. Studies have shown that patients with GB secrete lower levels of TNF- α , or express potential inhibitors of its receptor, which allow tumor progression. Another family member, TNF-related apoptosis-inducing ligand (TRAIL), plays a significant role in protecting normal cells and inducing apoptosis of cancer cells [46]. Glioma cells suppress the extrinsic TRAIL-mediated apoptotic pathway by interrupting the extrinsic caspase signaling cascade to further overcome apoptosis [46].

Additionally, increased levels of the immunosuppressive cytokine IL-10 have been observed in GB patients [1]. IL-10 is commonly secreted by the tumor cells in response to M2 macrophage stimulation [47] and activates the transcription factor STAT3 that induces expression of anti-inflammatory molecules [48]. At the same time, IL-10 inhibits the expression of pro-inflammatory molecules, activates Tregs, suppresses the potency of CD8⁺-mediated cytotoxicity, as well as phagocytosis and antigen presentation [49], thus favoring overall tumor progression.

Finally, GB cells also produce increased levels of B7-homolog 1 (B7-H1), an immunosuppressive protein not

expressed by normal brain cells [50]. B7-H1 acts as a ligand to PD-1 and therefore inhibits the T helper cell immune response. Moreover, B7-H1 may also act as a receptor which transmits anti-apoptotic signals, thus inhibiting cancer cell cytotoxicity by cytotoxic CD8⁺ T lymphocytes [51].

Therapeutic approaches targeting TME interactions

Taken all together, the sophisticated crosstalk of neoplastic cells with TME renders GB a complex and heterogeneous tumor with remarkable resistance to therapy. Until now, standard therapy for a newly diagnosed GB includes surgical resection and complementary treatment with the alkylating agent temozolamide (TMZ), in combination with radiation and six cycles of TMZ for maintenance. However, surgical removal of the entire tumor is often impossible, since the initial neoplasm usually invades other healthy brain areas leading to recurrence [52].

Novel compounds that target the cellular components of TME along with neoplastic cells or block their interaction constitute promising new therapeutic approaches for GB (Table 1).

Monoclonal antibodies targeting immune checkpoints

The use of monoclonal antibodies that block upregulated molecules which contribute to an immunosuppressive tumor environment is under extensive investigation in GB preclinical and clinical studies. Cytotoxic T lymphocyte antigen-4 (CTLA-4) and PD-1 are two immunosuppressive molecules whose production is increased in favor of tumor progression [53]. They are both expressed on the surface of T cells and bind to the B71 or B72 (CD80 and CD86) molecules of the APCs. This blocks the co-stimulatory signal from the APC, which is necessary for T cell activation and leads to downregulation of T cells [53]. PD-1 and CTLA-4 blocking antibodies, ipilimumab and tremelimumab, originally investigated in melanoma, have entered clinical phase II/III with promising results in GB cases [54].

The combination of nivolumab (anti-PD-1 antibody) and AXL inhibitor (BGB324) was shown to reduce microglia infiltration and increase survival in mice [55].

Another upregulated immune checkpoint molecule, the carcinoembryonic antigen-related cell adhesion molecule I (CEACAMI), is involved in hemophilic interactions between CEACAMI⁺ tumor cells and tumor-infiltrating lymphocytes. This interaction inhibits T cell-mediated cytotoxicity and shields the tumor from immune attacks [56]. CEACAMI monoclonal antibody is currently under extensive investigation in four ongoing clinical trials with promising results.

Table 1 Therapeutic approaches in GB targeting TME

Type of therapy	Target	Function	Study phase	References
Ipilimumab	PD-1 inhibitor	Prevents T lymphocyte suppression	Phase II/III	[53, 54]
Tremelimumab	CTLA-4 inhibitor	Prevents T lymphocyte suppression	Phase II/III	[53, 54]
Nivolumab and BGB324	PD-1 and AXL inhibitor	Reduce microglia infiltration	Preclinical	[55]
CEACAMI antibody	CEACAMI inhibitor	Prevents CD8 ⁺ lymphocyte suppression	Phase I	[56]
Pexidartinib (PLX3397)	CSF-1R inhibitor	Reduces GAM infiltration	Phase II	[57]
Emactuzumab (RG7155)	CSF-1R inhibitor	Alters microglia polarization	Preclinical, Phase I	[58]
Maraviroc (MRV)	CCR5 inhibitor	Alters microglia polarization	Preclinical	[59]
Plerixafor (AMD3100)	CXCR4 antagonist	Promotes GAM M1-like polarization. Inhibits the invasion and angiogenic potential of CXCR4/CXCR7-expressing glioblastoma stem-like cells	Preclinical	[60, 61]
WP1066	STAT3 inhibitor	Promotes GAM M1-like polarization. Enhances the radiosensitivity of GSCs under coculture with astrocytes and when grown as orthotopic xenografts	Phase I	[62, 63]
GM-CSF-producing vaccine	GM-CSF	Increases tumor antigen presentation and T lymphocyte coordination	Preclinical	[64]
IL-2 vaccine	IL-2	CD8 ⁺ lymphocyte and NK cell activation	Preclinical	[65]
IL-4 vaccine	IL-4	Increases tumor infiltration by CD8 ⁺ lymphocytes	Preclinical	[66]
IL-12 vaccine	IL-12	Prevents tumor growth	Preclinical	[65]
STDENVANT (DC-based vaccine)	Upregulates PD-1 and PD-L1 on effector T cells	Increases tumor antigen presentation. Decreases Tregs when co-administered with an anti-PD-L1 antibody	Preclinical	[67]
ICT-07 vaccine	GSC tumor antigens (gp100, IL13R α 2, AIM-2, HER-2, TRP-2, MAGE1)	Decreases GSC number, improves progression-free survival	Phase I	[68]
SOX2 vaccine	GSC SOX2 transcription factor	Enhances systemic and local immune response, prolongs overall survival	Preclinical	[69]

The anti-CSF-1R antibody, pexidartinib (PLX3397), which blocks the recruitment and polarization of GAMs was shown to exhibit significant reduction of GAMs infiltration in tumor-bearing mice, decrease tumor volume, and improve their survival. A phase II clinical trial in GB patients showed that pexidartinib was well-tolerated but with reduced efficacy [57]. Another anti-CSF-1R antibody emactuzumab (RG7155) was found to alter GAM polarization and block glioma progression. However, glioma cell-derived IL-4 was reported to rescue GAM viability and counteracts CSF-1 treatment, suggesting that CSF-1R-targeting agents will not benefit patients with elevated IL-4 expression [58]. However, RG7155 has recently entered a phase I study in combination with immune checkpoint inhibitor against the ligand of PD-1, PD-L1.

Monoclonal antibodies targeting the T cell co-stimulatory molecular pathways to induce immune response in the GB tumor microenvironment have also been investigated. The CD226/TIGIT co-stimulatory axis has been found to regulate T cell function since both CD226 and TIGIT bind to PVR in

order to exert their opposite actions, being immune-inducing and immunosuppressing, respectively [70]. Lozano et al. demonstrated that blocking of TIGIT resulted in elevated IFN- γ and T-box transcription factor TBX21 (T-bet) expression and increased CD226 binding to PVR, leading to CD4⁺ T cell activation [71]. The opposite effect was observed upon CD226 blockade that leads to enhanced TIGIT activity.

CD28 is another co-stimulator of TCR that may be targeted by monoclonal antibodies. CD28 being a co-receptor that binds CD80 and CD86 leads to activation of NF- κ B, a transcription factor necessary for the induction of inflammatory responses [72]. Until recently, CD28 was considered to be activated in conjunction with the TCR, in order to induce NF- κ B activity. However, it is now clear that both CD28 and TCR lead to increased NF- κ B activity individually, while simultaneous activation of both receptors leads to a higher induction of NF- κ B activity in a cooperative manner [72]. CD28 downregulation is the hallmark of senescent CD8⁺ cells [73], whereas T cell energy is the result of deficient CD28 co-stimulation [74]. Therefore, targeting of CD226 or CD28 by

monoclonal antibodies may prove particularly effective in stimulating patient's immune response against the tumor's immunosuppressive mechanisms.

Chemokine inhibitors

Glioma cells secrete a variety of cytokines whereas at the same time, microglia express chemokines and their receptors being involved in tumor progression. Therefore, targeting chemokine receptors to modulate microglia and tumor interaction is a promising approach with new inhibitors being currently under investigation.

In a preclinical study, the CCR5 inhibitor Maraviroc (MRV) was shown to reduce the M2-like markers IL-10 and ARG-1 and induce the expression of M1-like markers IL-1 β and NO in microglia [59].

Furthermore, targeting of CXCL12 (SDF-1) that is involved in GAMs recruitment by SDF-1 receptor (CXCR4) antagonist, plerixafor (AMD3100), has been demonstrated to promote GAM polarization to M1 phenotype while it also impaired glioma proliferation in preclinical studies [60]. In addition, plerixafor was shown to inhibit the invasion and angiogenic potential of CXCR4/CXCR7-expressing glioblastoma stem-like cells [61]. In accordance, another CXCR4 antagonist, peptide R, exhibited similar beneficial results in glioma xenografts.

The cytokine downstream signaling transcription factor, STAT3, has also been investigated in gliomas. Its inhibitor WP1066 has been reported to block glioma growth and activate the M1-like phenotype of GAMs *in vivo* [62]. Furthermore, it was found to enhance the radiosensitivity of GSCs under coculture with astrocytes and when grown as orthotopic xenografts [63]. It is currently investigated in phase I clinical trial of recurrent glioma patients [62].

Cytokine-based vaccines

The use of cytokine releasing vaccines is being investigated in the hopes of activating the patient's immune system, as well as suppressing the TME. Experimental studies have demonstrated the positive effects of vaccines with irradiated tumor cells engineered to secrete GM-CSF. GM-CSF was demonstrated to boost tumor antigen presentation by DCs and macrophages, as well as the coordination of CD4⁺ and CD8⁺ T cell functions, leading to an increase in median survival in mice models [64]. Additionally, vaccination with IL-2-producing cells showed enhanced activation of CD8⁺ cytotoxic and NK cells in mice with GB, whereas injection of IL-2 secreting fibroblasts in healthy brain areas prevented brain tumor development [65]. The use of IL-4 secreting vaccines in clinical studies resulted in increased tumor infiltration by CD8⁺ cells and prolonged mice survival [66]. Finally, injections of IL-12-

expressing cells in rats led to reduced tumor growth and improved survival [65].

Dendritic cell-based vaccines

DC vaccines have also been proposed as a potential therapy for GB due to their safety and high tumor-specific toxicity even in the immunologically privileged brain [75]. Several tumor antigens can be coupled to DCs including synthetic or glioma peptides and autologous glioma lysates. Direct fusion of DCs with tumor cells can be achieved or, alternately, transfection with tumor cDNA or RNA to elicit systemic toxicity and intracranial T cell infiltration. Several tumor lysate-loaded DC-based vaccines (DCvax) have been investigated in newly diagnosed [76] and recurrent GB patients with encouraging survival outcome [77].

A new glioma vaccine (STDENVANT) has been assessed in the orthotopic mouse model GL261-C57BL/6 consisting of DCs, GSC lysate, and Toll-like receptor (TLR) 9 agonist CpG motif-containing oligodeoxynucleotides (CpG ODNs) [67]. It was found to upregulate PD-1 and its ligand PD-L1 on T cells, DCs, and gliomas and induce the accumulation of Tregs in the brain. However, co-administration with an anti-PD-L1 antibody decreased Treg population and improved survival, suggesting that blockade of PD-L1 may enhance tumor regression via STDENVANT and increase its efficacy in glioma mouse model [67].

In one clinical trial, ICT-07 vaccine that targets a variety of tumor antigens associated with GSCs tumorigenesis (including gp-100, IL13R α 2, AIM-2, HER-2, MAGE1) was found to improve progression-free survival and reduce GSC number in patients requiring double surgery, indicating its efficacy in targeting this population [68]. Finally, vaccines with SOX2 peptides, the main transcription factor of GSC population, have shown enhancement of immune response in animal models and improved overall survival, indicating a new active immunotherapy approach to treat GSCs [69].

Conclusions

Taken all together, the dynamic interaction of glioma cells with their TME plays a pivotal role in tumor growth and progression while it presents a major challenge for treatment. Among the various cell types, GAMs seem to play the most critical role in glioma growth since they can interact with both malignant tumor cells as well as immune cells to enhance immunosuppression. Therefore, elucidation of immunoregulatory soluble factors that confer to GAM plasticity including both morphological and functional changes presents a major research field for future studies. In accordance, novel therapies that modulate the phenotype of microglial and astrocyte cells

to a pro-inflammatory state show promising results for GB management. Furthermore, immunotherapy-based treatment options employing the use of cytokine or DC vaccines in combination with immune checkpoint inhibitors may prove effective in the battle against GB.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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