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Co-evolution of glioma and immune microenvironment

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ABSTRACT

Glioma evolution is governed by a multitude of dynamic interactions between tumor cells and heterogenous neighboring, non-cancerous cells. This complex ecosystem, termed the tumor microenvironment (TME), includes diverse immune cell types that have gained increasing attention for their critical and paradoxical roles in tumor control and tumorigenesis. Recent work has revealed that the cellular composition and functional state of immune cells in the TME can evolve extensively depending on the tumor stage and intrinsic features of surrounding glioma cells. Concurrently, adaptations to the glioma cellular phenotype, including activation of various cellular states, occur in the context of these immune cell alterations. In this review, we summarize important features of the immune TME that play key roles during each stage of glioma progression, from initiation to immune escape, invasion and recurrence. Understanding the complex interplay between tumor and immune cells is critical for the development of effective immunotherapies for glioma treatment.

INTRODUCTION

Gliomas are the most common malignant primary brain tumors in adults.[1] Recent advances in genomic and molecular subtyping of gliomas have led to an update in the WHO classification of these tumors, introduced in 2021.[2] This new classification subdivides adult-type diffuse gliomas into three subtypes: isocitrate dehydrogenase (IDH) 1 or 2-mutant astrocytomas (grade 2, 3 or 4), 1p19q-codeleted oligodendrogliomas (grade 2 or 3), and IDH-wildtype glioblastomas (grade 4) (GBM). Standard of care for gliomas consists of maximal safe resection and in high-grade gliomas (grade 3 or 4) adjuvant radiotherapy and chemotherapy.[3 4] Postoperative management in grade 2 tumors is not as well defined but often based on risk stratification with low-risk patients (eg, aged ≤40 years with absent or modest asymptomatic residual tumor not needing immediate adjuvant treatments) being eligible for a "watch and wait" period where they can be observed without any treatments for further progression.[4] The prognosis of gliomas varies based on their molecular features.[5] For example, IDH-mutant gliomas are less aggressive than IDH-wildtype gliomas, even when an IDH-mutant astrocytoma has necrosis and/or microvascular proliferation, which are traditional histological hallmarks of GBM.[6] Additional studies have highlighted other genomic alterations, such as homozygous deletion of cyclin-dependent kinase inhibitor 2A (CDKN2A) and/or cyclin-dependent kinase inhibitor 2B (CDKN2B) genes, that affect prognosis and response to therapies.[7] Such studies highlight the importance of understanding the molecular features of gliomas and how they may change as gliomas evolve and affect responses to emerging therapeutics.

The complex nature of gliomas is also depicted by their dynamic tumor microenvironment (TME), containing cancer cells intertwined with diverse non-malignant cells, including various types of immune cells, fibroblasts, and endothelial cells.[8] While these host cells were initially viewed as bystanders of tumorigenesis, an increasing number of studies have shown that these non-tumor cells interact with glioma cells to influence the evolution of glioma tumors.[8–10] Reciprocally, glioma cell-intrinsic features, including altered genetic and epigenetic programs, metabolic reprogramming, and dysregulated signaling pathways can reshape the microenvironment to further promote tumorigenesis.[10–12] Importantly, this rewiring of the TME has been proposed as a means for tumor to develop resistance to therapies,[13 14] and accordingly, there has been increasing interest in identifying therapeutic strategies to target non-tumor cells in the TME.

Immunotherapy in recent years has revolutionized treatment for multiple types of cancers.[15] However, the utility of immunotherapy for gliomas has remained limited. For example, a phase III randomized study in newly diagnosed GBM of immune checkpoint inhibitor nivolumab, in combination with standard-of-care radiotherapy and temozolomide, did not improve progression-free or overall survival compared with placebo.[16] In another phase III clinical trial, nivolumab did not improve overall survival compared

with bevacizumab in patients with recurrent GBM.[17] The limited efficacy of immune checkpoint inhibitors may be partially due to the paucity of neoantigens, the plasticity of glioma cells to escape immune clearance, and the immunosuppressive TME.[14] A plethora of recent studies have focused on characterizing the immune components of the TME, demonstrating that tumor-infiltrating immune cells including myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), neutrophils, dendritic cells, and T-cell lymphocytes are critical determinants of tumor progression and outcomes.[18] These cells can be co-opted by glioma cells to promote an immunosuppressive environment and tumor growth via various mechanisms, including secretion of chemokines and cytokines that alter the balance of protumor and antitumor immune responses.[19]

The advent of high-throughput sequencing methods and spatiotemporal microscopy have allowed an in-depth examination of glioma composition and structure at unprecedented resolution and scale. Such studies have surprisingly revealed that the cellular composition and functional state of the TME can differ depending on intrinsic features of glioma cells that evolve over time.[20–23] Notably, single-cell transcriptomic sequencing experiments in GBM tumors have identified distinct clusters of glioma cell transcriptional activation states associated not only with genomic alterations but also with immune cell composition of the surrounding TME.[5 20–24] These clusters, referred to as classical (CL), mesenchymal (MES), neural (NE), and proneural (PN), are spatially distributed in different regions of the tumor with unique TME features. Interestingly, analysis of clinical differences between these subtypes has suggested that MES-predominant tumors correlate with a worse prognosis and often predominate in patients with recurrent GBM following standard-of-care treatment.[24] In addition, reciprocal interactions between cancer and immune cells in the TME can lead to dynamic switching of these different GBM subtypes over time and in response to treatments that alter the immune TME.[14] This highly heterogenous nature and plasticity of GBM is proposed to strongly undermine the efficacy of immunotherapies and highlights the importance of understanding the co-evolution of glioma cells and the immune TME and the associated complex cellular and molecular changes that underly tumor progression and resistance to therapy.

Here, we review the current understanding of fundamental glioma-immune cell interactions that occur as gliomas evolve, from tumor initiation and immune escape to invasion and recurrence. Understanding the dynamic and reciprocal adaptive changes that co-occur in glioma cells and immune TME may lead to new molecular targets or rational combination for the treatment of these currently immunotherapy-resistant tumors.

Proinflammatory immune response during glioma initiation

Early studies in immunodeficient mice[25] and immunocompromised patients[26] showed increased susceptibility to tumor development, underscoring the critical antitumor role the immune system plays in surveilling against tumor initiation. However, this cancer immunosurveillance function can also promote tumor growth through cancer immunoediting, whereby immune attack prunes away immunogenic cancer cells while less immunogenic cells adapt to the selective pressure exerted by the immune system, developing into more aggressive tumor cells that can eventually escape immune surveillance.[27] Studies in immunocompetent and immunodeficient glioma mouse models[28] and in human patients with glioma[29] have shown this immunoediting process shapes the clonal architecture, immune microenvironment, and immunosuppressive gene signature in gliomas. We highlight below important immune-glioma cell interactions that have been shown to occur during early glioma development.

The initiation and early stages of gliomagenesis are marked by a proinflammatory innate immune microenvironment. Single-cell transcriptomic studies performed by Yeo *et al* using a mouse GBM model in which tumors are initiated using conditional (Cre/Lox) overexpression of human epidermal growth factor receptor (EGFR), loss of CDKN2A and phosphatase and tensin homolog (PTEN), all alterations seen in human GBMs,[23] demonstrated that compared with late stage tumors, early tumors has a TME dominated by microglia that expressed proinflammatory markers (ie, chemokine (C-X-C motif) ligand 2 (Cxcl2), Cxcl3, Cxcl10, interleukin (IL)-1β, tumor necrosis factor-alpha (TNF-α), chemokine ligand 3 (Ccl3)), neutrophil chemotaxic proteins (ie, Cxcl10, IL-1β, Ccl4, Ccl3, Cxcl3, Cxcl2, S100a9) and positive regulators of macrophage phagocytosis (IL-1β, Tnf-α, Gata2) . This suggests that an acute inflammatory response may play a specific role in early glioma development. Additionally, a breakthrough study by Gangoso *et al* uncovered a self-reinforcing feedback loop whereby initial immune response drives GBM tumor cells to recruit myeloid cells, which in turn exposes tumors to increased interferon (IFN)-γ.[30] Using serial transplantation of glioma stem cells into immunocompetent mice, the authors were able to enrich for glioma stem cells (GSCs) that acquire features of immune evasion. Genetic analysis of these immune evasive GSCs surprisingly revealed that these cells did not acquire any additional significant genetic alterations compared to parental GSCs from initial tumors. Instead, these GSCs were epigenetically reprogrammed under the influence of continuous IFN-γ signaling which led to the upregulation of several tumorigenic chemokines, particularly Ccl9, which previously has been shown in mouse lung adenocarcinoma models to promote an immunosuppressive environment.[31] Of note, myeloid cells are not typically considered to be a main source of IFN-γ[32]; however, the authors demonstrated that in vitro co-culture of immune naive GSCs and isolated infiltrating macrophage population was able to induce similar transcriptional changes in tumor cells, suggesting that these macrophages can be a source of IFN-γ during early tumor development. How

TAMs acquire this ability to secrete significant amounts of IFN-γ in gliomas and the temporal kinetics of this IFN-γ response has yet to be elucidated. While IFN-γ signaling plays a well-recognized role in orchestrating antitumor responses,[33] a growing body of literature suggests that chronic IFN activation can have a paradoxical effect of inducing immune suppression and contribute to resistance of immune checkpoint blockade.[34–37] This new finding that TAM-mediated IFN signaling can lead to epigenetic immunoediting raises important questions of what other glioma-immune associated interactions alter the epigenome and how the balance of proinflammatory and anti-inflammatory response lead to IFN-γ mediated immune evasion in GSCs.

Glioma stem cell modulation by immune cell interactions

Cancer stem cells are characterized by their ability to undergo self-renewal and have been proposed to play important roles in tumor initiation and progression.[38] The nomenclature for these cells has been controversial as not all cancer stem cells are tumor-initiating cells[39]; nonetheless, studies have identified populations of human brain tumor cells with NE stem cell phenotypes that when isolated and injected into immunocompromised mice can form tumors with similar characteristics as the original tumor,[40 41] suggesting that glioma formation is driven by cells with NE stem cell characteristics. Common pathways activated in cancer stem cells, including signal transducer and activator of transcription 3 (STAT3)[42] and nuclear factor-kappa B (NF-κB),[43] may be induced through immune TME factors (figure 1). For example, IL-6 released by microglia has been shown to promote GSC renewal by activating STAT3 signaling and inhibiting IL-6 can suppress GSC survival and tumor growth.[44] IL-1β expression and secretion by microglia has also been shown to promote a stem cell phenotype in human PN GBM cells.[45] Shi *et al* demonstrated that TAM-mediated secretion of pleotrophin, a cytokine that regulates diverse cellular functions including growth and differentiation and has been shown to predict poor prognosis in several malignant tumors,[46] is critical for GSC maintenance and GBM growth.[47] Pleotrophin binds to its receptor PTPRZ1 expressed preferentially on GSCs and induces phosphoinositide 3-kinase-AKT pathway activation for GSC maintenance. The transforming growth factor beta (TGF-β) signaling pathway can also regulate stem cell-related genes (eg, SOX2 and SOX4) to inhibit differentiation of GSCs and promote GSC maintenance and proliferation.[48] In addition to TAMs, neutrophil infiltration was found to promote glioma stem cell proliferation in vitro by inducing S100A4 expression.[49] Interestingly, tumor associated neutrophils (TANs) were shown to suppress GBM growth in mouse models secondary to increased recruitment of T cells. However, in T-cell deficient mice, TANs promoted stemness and enriched GSCs through secretion of osteopontin, suggesting that TANs exhibit both an inflammatory antitumor and stem-cell promoting protumor response depending on the presence of T-cells in the TME.[50 51] MDSCs have also been shown to affect GSCs through induction of oxidative stress and production of nitric oxide (NO) which can upregulate Notch and IL-6 signaling in GSCs leading to STAT3 activation and promotion of cancer cell stemness.[52] Regulatory T cells (Tregs) can also induce the expression of stemness-related genes (eg, CD133, SOX2, NESTIN) by secreting TGF-β that promote NF-kB or STAT3 signaling pathway.[53]

There is increasing evidence that cancer stem cells can also reciprocally modulate the immune microenvironment to promote tumor growth[54] (figure 1). A recent study demonstrated that naïve microglia can reduce the sphere-forming ability of human GSCs to suppress glioma growth, while microglia or monocytes cultured from glioma patients lost this antitumorigenic potential,[55] suggesting that GSC derived factors in the TME can reprogram microglia to a GSC-supporting phenotype. Indeed, GSCs have been shown to secrete factors including IL-10, macrophage-inhibitory cytokine-1 (MIC-1), and TGF-β to suppress the antitumor function of TAMs and reprogram them to an immunosuppressive, protumor "M2-like" phenotype.[56] Periostin secreted by GSCs has also been shown to modulate TAM recruitment and promote an immunosuppressive state. Inhibition of periostin in GSCs markedly reduced TAM density and inhibited tumor growth in mice implanted with GSC-derived xenografts.[57] Additionally, a gain-of-function screen of epigenetic regulators of GSC renewal identified the circadian regulator circadian locomotor output cycles kaput (CLOCK) as an important candidate for stem cell maintenance and modulation of the immune microenvironment.[58] CLOCK heterodimerizes with basic helix-loop-helix ARNT-like protein 1 (BMAL1) to upregulate metabolic pathways critical for GSC renewal and enhance secretion of olfactomedin-like 3 (OLFML3). OLFML3 in turn promotes GSC secretion of legumain that upregulates CD162 on microglia to promote an immunosuppressive phenotype.[59]

IDH mutations reshape glioma epigenetics and immune microenvironment

Point mutations in the codon 132 of IDH1 or codon 172 of IDH2 lead to the generation of mutant IDH. These mutations lead to de novo production of the oncometabolite R-2-hydroxyglutarate (2-HG).[60] Consequently, 2-HG levels are 10 to 100-fold higher in IDH-mutant gliomas than non-neoplastic or IDH-wildtype GBM. Classically, 2-HG acts as a competitive inhibitor of alpha-ketoglutarate-dependent enzymes which include important DNA and histone demethylases, such as a-ketoglutarate-dependent ten eleven translocation enzymes and Jumonji C domain-containing dioxygenases. This results in increased DNA and histone methylation and silencing of some tumor suppressor genes, such as CDKN2A/B and retinoblastoma-associated protein.[61 62] While the exact epigenetic remodeling mechanisms of 2-HG-mediated gliomagenesis continue to be elucidated, seminal studies using single-cell sequencing have begun

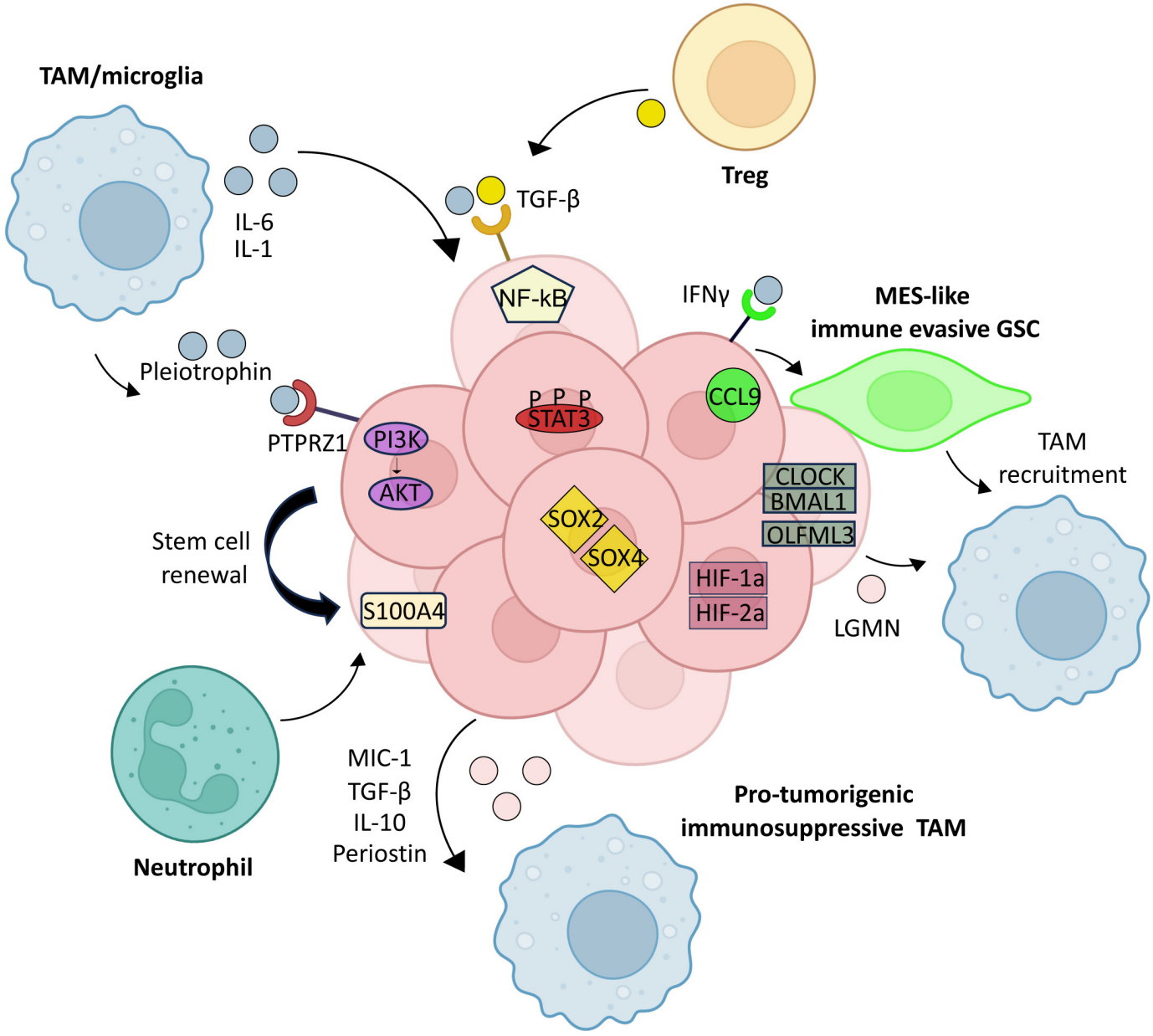

Figure 1 Glioma stem cell (GSC) maintenance and renewal are modulated by immune cell factors. Various factors in the immune TME promote GSC features, including TAM, neutrophil and Treg-secreted factors and hypoxia. These molecules have been shown to modulate signaling pathways in GSCs to promote the expression of stem-cell-related genes (eg, SOX2 and SOX4). Concurrently, GSCs can reorganize the immune TME by upregulating the expression and release of factors that recruit TAMs and reprogram them into an immunosuppressive state. TAM recruitment can also lead to GSCs expressing mesenchymal-like transcriptional programs which enables them to evade the immune system. BMAL1, basic helix-loop-helix ARNT-like protein 1; CCL, chemokine ligand; CLOCK, circadian locomotor output cycles kaput**;** IFN, interferon; IL, interleukin; LGMN, legumain; MES, mesenchymal; MIC-1, macrophage-inhibitory cytokine-1; NF-κB, nuclear factor-kappa B; OLFML3, olfactomedin-like 3; PI3K, phosphoinositide 3-kinase; STAT3, ignal transducer and activator of transcription 3; TAM, tumor-associated macrophages; TGF-β**,** transforming growth factor beta TME, tumor microenvironment; Treg, regulatory T cell.

to uncover essential differences in the glioma immune microenvironment architecture dependent on IDH-mutational status which may be attributable to upregulation of 2-HG. Studies have shown that 2-HG can be released into the TME where it can suppress antitumor T-cell activity and promote immunosuppression.[63–66] Accordingly, IDH-mutant tumors exhibit fewer tumor-infiltrating lymphocytes than IDH-wildtype gliomas.[67] Analysis of the immune cell composition using The Cancer Genome Atlas (TCGA) RNA-sequencing data, as well as experiments using syngeneic glioma models, demonstrated that IDH-mutant gliomas more strikingly downregulate several immune signaling pathways compared to IDH-wildtype gliomas, including chemotaxis factors such as CCL-2/3, CXCL-1/2/4/16, granulocyte-macrophage colony-stimulating factor (GM-CSF), and

IL-2/6/16.[68 69] Histological examination confirmed low expression levels of T-cell markers in IDH-mutant glioma, and revealed significant enrichment of naive CD4+T cells and reduction of memory T cells.[69] In addition, lower numbers of dendritic cells and Tregs (Foxp3+) were also seen, particularly in oligodendrogliomas[70]. 2-HG has also been shown to inhibit dendritic cell maturation and suppress major histocompatibility complex (MHC) class I and II-mediated antigen presentation leading to reduced activation of T cells.[71] 2-HG can be taken up by myeloid cells to induce tryptophan-2,3-dioxygnease-2-dependent conversion of L-tryptophan to L-kynurenine which binds to and activates the aryl-hydrocarbon receptor (AHR).[72] Activated AHR leads to an increase in anti-inflammatory cytokines IL-10 and TGF-β, further augmenting the immunosuppressive macrophage phenotype in IDH-mutant tumors. Inhibition of AHR has been shown to reverse suppression of antitumor immunity in IDH1-mutant macrophages. In addition to 2-HG modulation of TME immune cells, recent studies have also demonstrated a direct, cell-autonomous role for IDH1 mutations in modulating innate immunity through epigenetic silencing of the cGAS-STING signaling and attenuation of IFN-γ production.[73 74] Importantly, these IDH-mutant effects can be reversed with IDH-mutant inhibitors. It will be interesting to investigate whether such inhibitors can act synergistically with immune checkpoint inhibitors to improve their efficacy.

In addition to IDH mutations, studies have examined other prominent mutations in IDH-mutant gliomas that play pivotal roles in glioma progression and immune evasion. Combination of Alpha-thalassemia/mental retardation, X-linked (ATRX) and p53 loss was found to promote tumorigenesis in gliomas through epigenetic remodeling towards an astrocytic lineage .[75] Moreover, ATRX loss induces immunosuppressive ligand (eg, programmed death-ligand 1 (PD-L1)) and cytokine (eg, IL-6, CXCL3/8/9) expressions which results in T-cell apoptosis and promotion of protumorigenic, anti-inflammatory macrophages. Further studies are needed to decipher how unique genetic alterations associated with IDH-mutant versus wildtype gliomas differentially impact the immune TME.

The "escape" phase of glioma progression is linked to an immunosuppressive TME

Tumor progression is driven by adaptations of tumor cells in the context of changes to their surrounding microenvironment. These evolving tumor cells can reciprocally reprogram the immune TME to a protumorigenic profile. Longitudinal profiling of glioma composition over time has shown that the progression of glioma development from an initial quasi-stagnant state to explosive growth expansion is preceded by a dramatic change in the immune TME to an immunosuppressive phenotype and an accumulation of myeloid cells.[23]

TAMs represent the most abundant population of tumor infiltrating immune cells. They release of a wide array of molecular factors that play critical tumor-supportive roles. Glioma cells can promote TAM recruitment through the secretion of various chemokines including monocyte chemoattractant protein-1 (MCP-1) (also known as CCL2), CCL7, CXCL12, colony-stimulating factor 1 (CSF-1), lysyl oxidase and glial cell-derived neurotrophic factor.[76] Genomic alterations in glioma cells such as NF1 deficiency,[14] PTEN deficiency,[77] and EGFR overexpression and its truncation mutant variant (EGFRvIII)[78] can induce expression of these chemokines to promote TAM infiltration into the TME. Some chemokines such as CSF-1 and MCP-1 have been shown to act not only as a chemoattractant for TAMs but also reprogram them into an immunosuppressive, protumor phenotype.[79] Single-cell sequencing and pseudotime progression analyses have also highlighted an evolution in TAM phenotype from early to late glioma. TAMs lose their proinflammatory polarization over time during glioma progression, as highlighted by increased expression of negative regulators of inflammatory response including Cxcl13, a chemokine associated with anti-inflammatory macrophage polarization,[80] as well as immediate early genes (Fos, Jun, Egr1, Zpf36, Nfkbia, Dusp1)[23] that are induced by the immunosuppressive reprogramming factor CSF-1.[81] Moreover, upon infiltration to the TME, TAMs also interact with glioma cells to enhance their proliferation and progression. TAMs release cytokines such as IL-12, IL-1B, CCL8, and IL-6 as well as other factors such as stress-inducible protein 1 (STI1), epidermal growth factor (EGF), TGF-β, and matrix metalloproteinase (MMP)-2 that have been shown to enhance glioma cell proliferation.[76] Chemokines such as CCL2, 5, 20, and 22 released by TAMs also help recruit Tregs which subsequently suppress the antitumor activity of cytotoxic T cells (CTLs), natural killer (NK) cells, and antigen-presenting cells (APCs).[82 83] Fas-ligand (FasL) secreted by TAMs can also bind Fas receptors on invading T cells to induce their apoptosis.[84]

MDSCs are known to mediate antitumor immune responses and their intratumoral density increases with glioma progression.[85] Gliomas cell promote expansion of MDSCs in the bone marrow and recruit them to the TME through various molecular factors including IL-6, IL-8, IL-10, CSF-1, CCL2, CXCL2, prostaglandin E2 (PGE2), and TGF-β.[18 76] Subsequently, MDSCs in the TME suppress the antitumor activity of CTLs, NK cells, macrophages and dendritic cells (DCs) through the production of NO and secretion of proinflammatory cytokines such as IFN-γ and TNF-α.[86] MDSCs can also release TGF-β which induces Treg differentiation, NK cell anergy and immunosuppressive polarization of TAMs.[87 88] Longitudinal analysis of single-cell sequencing data showed that neutrophil and MDSCs have differential genes expression during their evolution through glioma progression.[23] These include downregulation of inflammatory response genes and upregulation of NFAT signaling and hypoxia response genes. While NFAT signaling is recognized as a proinflammatory pathway in normal immune cell function,[89] how this signaling pathway and other aforementioned

pathways may be altered in immunosuppressive neutrophil subsets remains unknown.

Gliomas can also reprogram T cells into a dysfunctional state to evade lymphocyte-induced immune attack through multiple mechanisms. GBM exhibits a noteworthy capacity of eliciting a full array of T-cell dysfunction, including anergy, tolerance, and exhaustion. Tolerance, the programmed induction of T-cell unresponsiveness, in GBM can occur by elimination of CTL via FasL-mediated apoptosis and Treg-induced immunosuppression. Indeed, both CD4+ and CD8+ T cells demonstrate increased susceptibility to apoptosis in patients with GBM. One study found that 22.6% of GBM tumor-infiltrating lymphocytes are in early stages of apoptosis and less than 50% are viable.[90] Gliomas also promote the recruitment of Treg, which has been shown to correlate with glioma progression. Patients with higher-grade gliomas have increased Tregs and reduced CD4+T cells.[91] Various factors produced by gliomas can induce Treg infiltration including CCL22 and indoleamine 2,3-dioxygenase 1 (IDO1) mediated secreted factors.[76] Tregs production of immunosuppressive cytokines (IL-10, IL-35, and TGF-β), conversion of ATP to adenosine, and consumption of IL-2 contribute to the inhibition of antitumor cytotoxic T cells.[76] Tregs can also express cytotoxic T-lymphocyte associated protein 4 (CTLA-4) that suppresses the function of APCs[92] and secrete granzyme or perforin molecules to destroy effector cells.[93] Downregulation of MHC-I expression through modulation of the cyclic GMP-AMP synthase (cGAS)-stimulator of Interferon Genes (STING)-type I IFN pathway has also been shown to limit T-cell activation in glioma TME.[94–96] T-cell anergy, whereby lymphocytes become perpetually unresponsive following antigen exposure, in GBM has been attributed to diminished IL-2 cytokine production required for T-cell clonal expansion.[97] T-cell exhaustion is marked by a hyporesponsive state of T cells with increased inhibitory receptors, decreased effector cytokines and impaired cytotoxicity. Immune checkpoints such as programmed cell death protein 1 (PD-1) and CTLA-4 contribute to this process and are upregulated by glioma cells through various mechanisms ultimately resulting in inhibition of clonal T-cell proliferation. For example, hypoxic stress promotes the expression of hypoxia-inducible factor 1α (HIF1α) that impairs metabolic function and promotes expression of exhaustion-related genes including upregulation of inhibitory immune receptors.[97] In IDH-mutant gliomas, 2-HG can enhance this metabolic and epigenetic dysfunction to further promote exhaustion.[66] Additionally, Tregs and TAMs can bind to tumor-infiltrating lymphocytes or secrete factors such as IL-10 and TGF-β that promote signaling pathways that enhance T-cell exhaustion.[97] In addition to the classic immune checkpoints, PD-1 and CTLA-4, additional immune checkpoints have been characterized including T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), which mediates immune suppression by binding to galectin 9 and carcinoembryonic antigen (CEACAM).[98] TIM-3 expression correlates with higher grade gliomas and lower Karnofsky Performance Status scores.[98] Infiltrating lymphocytes in GBM co-expressing PD-1, TIM-3 and other checkpoint molecules have been shown to be non-functional.[99] Several clinical trials are now underway investigating inhibitors of these immune checkpoints in conjunction with PD-1 inhibitors.[100] Interestingly, recent studies using single-cell RNA and epitope sequencing analyses have shown that the majority of T cells in GBM tumors do not exhibit canonical exhaustion markers but rather are in a transitional state with enrichment of GZMK+T cells.[101] Further investigation is needed to understand how these T cells may differ from canonically exhausted T cells and the effects of GZMK expression on T-cell function and immune therapy response.

Immunologic induction of glioma phenotypic switching and invasion

A hallmark of high-grade gliomas is their ability to invade into surrounding parenchymal tissue.[102] This is exemplified by GBM cells which show aggressive invasion potential as dramatically illustrated in Walter Dandy's 1928 series of patients who died from recurrent GBM following hemispherectomy.[103] Transcriptomic and spatiotemporal analysis of GBM tissues have identified four distinct clusters of GBM cells, referred to as CL, MES, NE, and PN, which are associated with distinct genomic abnormalities[5 24] and immune microenvironment features.[20–23 104] PN subtype is associated with younger age, PDGFRA abnormalities, and IDH1 mutations with or without TP53 mutations, which have previously been observed in "secondary GBM".[5 24] CL subtype harbors Chr 7 amplifications, Chr 10 deletions, EGFR amplification and Ink4a/ARF deletions, while lacking abnormalities in TP53, NF1, PDGFRA or IDH1. MES subtype is characterized by high expression of CHI3L1 and MET as well as a high frequency of NF1 mutation or deletion. MES tumors show an enrichment in TAMs compared with other subtypes and are associated with worse prognosis.[20–23 104] NE tumors demonstrate differentiated gene expression signatures similar to neurons, astrocytes, and oligodendrocytes.[5 24] Understanding these molecular subtypes is important for prognosis and treatment, as different subtypes may respond differently to specific therapies.

Interestingly, recent studies have suggested that glioma progression and invasion are modulated by crosstalk interactions with the TME and associated with switch from the PN, NE stem-cell-like phenotype to cancer cells with MES features.[20–23] Immune cells, as highlighted below, are important regulators of this transition and glioma invasion, in addition to promoting angiogenesis, which is another important feature of tumor growth and invasion.

Epithelial-to-mesenchymal transition (EMT) of cancer cells is associated with enhanced cell migration, local invasion, and distant metastasis. Expression of EMT markers correspondingly correlates with poor prognosis.[105] Early sequencing studies identified subtypes of GBMs that expressed MES-like features that are associated with

worse patient survival.[5 106] These cells express stem cell markers and can adapt to interventions such as chemotherapy and radiotherapy. Experiments in patient-derived glioma cultures and xenograft models have shown that the MES signature is lost on explantation from the TME, suggesting that TME factors are necessary for the acquisition and maintenance of an MES phenotypic state.[107] TAMs, which are increasingly recruited to the TME as the tumor progresses, have been shown to strongly associate with the development of MES state.[20–23 108] These TAMs express proinflammatory (eg, TNF-α), anti-inflammatory (eg, TGF-β), and pro-angiogenic (eg, vascular endothelial growth factor (VEGF)) factors, which have been shown to contribute to the MES transition through activation of the NF-kB pathway, leading to the upregulation of master transcription factors, including STAT3, CCAAT/enhancer binding protein beta (C/EBPB), and transcriptional coactivator with PDZ-binding motif (TAZ) that are implicated in mesenchymal reprogramming.[107] Oncostatin-M secreted by TAMs has also been shown to induce the transition to an MES-like state in glioma cells.[108] Oncostatin interacts with receptors on GBM cells that subsequently activates STAT3, a key transcription factor that initiates the MES program. TAM-secreted factors such as TGF-β and STI1 can induce MMP-2 and MMP-9 expression leading to degradation of the extracellular matrix (ECM) and glioma cell invasion.[109] Versican, a proteoglycan released from glioma cells has been shown to induce toll-like receptor-2 signaling in TAMs that leads to increased expression of MT1-MMP, a key regulator of cell migration.[110]

Recently, there has been an increased focus on how neuronal activity can promote glioma growth.[111 112] Recent studies suggest that a reciprocal neuron-glioma interactions can lead to remodeling of NE circuits in the human brain that further promote tumor progression.[113] Venkataramani *et al* found a distinct subpopulation of GBM cells with neuronal and NE-progenitor-like states that drive brain invasion through neurogliomal synaptic activity.[114] Using patient-derived GBM cells implanted in mice, they showed that tumor cells that are classified as "unconnected" (ie, GBM cells that do not have any gap-junction connectivity with astrocytes or other GBM cells) have features of neuronal NPC-like states and are enriched in the tumor margin. In contrast, MES-like non-neuronal cell states are found in the tumor core and enriched in "connected" GBM cells. Time-lapse microscopy revealed that "unconnected" GBM cells showed greater migration and invasiveness than their "connected" counterparts, which remained primarily stationary. While this work challenges the role of EMT in GBM invasion, indirect glioma-immune cell interactions (eg, via cytokine secretion) may still play a role in the promotion of these "unconnected" phenotypes. Additionally, whether immune-mediated stem cell maintenance and renewal, as discussed above, is associated with these "unconnected" features of invasive GBM cells remains an important question for further studies. Exciting new findings have found differential immune programs in highly connected regions compared with lowly connected ones. Highly connected regions are found to have downregulation of proinflammatory pathways such as IFN-γ and TNF-α[115] resulting in increased infiltration of alternatively activated, immunosuppressive TAMs. These findings pave the way for further studies to examine the effect of neuronal activity on the immune microenvironment and open new avenues for combination therapies targeting neuronal activity and glioma-immune-neuronal crosstalk.

Changes in TME associated with tumor recurrence and treatment

The standard of care for GBM is maximal safe surgical resection followed by radiation therapy (RT) and temozolomide (TMZ) chemotherapy.[116] GBM tumors invariably recur, and recent efforts have focused on elucidating the temporal evolution of these tumors before and after therapy in hopes of identifying treatment resistance-related factors. GBM has long been hypothesized to progress along a PN-to-MES axis,[5] and work from TCGA Network suggest that non-MES subtypes typically acquire MES features at recurrence.[24] Single-cell transcriptomic analysis of paired primary and recurrent GBM tumors after treatment with TMZ and RT show that non-MES primary tumors most commonly switched to an MES signature on recurrence.[21] Recurrence in all subtypes is associated with increase in TAM density, with the MES subtype unsurprisingly having the highest TAM infiltration.

RT and TMZ have been proposed to induce a proinflammatory microenvironment. RT induces proinflammatory cytokines such as TNF-α, IFN-γ, CXCL9/10/16 which can promote antitumor T-cell recruitment.[117] RT-TMZ combination has been shown to increase PD-L1 expression in preclinical GBM models.[118] The delicate balance between stimulation and suppression of the immune response following TMZ and RT is still being investigated. Potentially, there may be a switch from an initial proinflammatory response to an immunosuppressive TME as tumor recurs, and elucidating the timing of these changes would have significant importance for determining when additional immunotherapies should be given. Interestingly, comparing GBM patients with short-term (<6 months of progression-free survival) versus late-term (>12 months) relapses following RT-TMZ treatment found short-term relapse patients have higher infiltration of alternatively-activated, pro-tumor "M2-like" macrophages.[20] M2-like macrophages have been implicated in mediating RT resistance,[119] which suggests that macrophage inhibitory therapy[79] can be beneficial for short-term relapse patients. Further work is needed to fully elucidate the functional importance of these immune cell types in tumor recurrence following standard therapy.

Changes in the TME following tumor recurrence can also impact the efficacy of immunotherapies. PD-1 blockade counteract tumor-induced T-cell dysfunction by interfering with PD-1/PD-L1 signaling, thereby activating antitumor immune response. Unfortunately, PD-1

blocking therapy has not shown much promise to date in patients with GBM, potentially due to low CD8+T cells infiltration in GBM.[120] Interestingly, neoadjuvant PD-1 blockade has been shown to enhance local and systemic antitumor immune responses compared to adjuvant PD-1 inhibition. Patients who recevied neoadjuvant treatment had more robust tumor-specific T-cell clonal expansion, downregulation of PD-1 expression on peripheral blood T cells, and decreased monocytic population in the TME.[121] Such results suggest that the timing of immunotherapies is an important consideration to be addressed in future clinical trials.

Studies have suggested a critical role of lymphatic vasculature in modulating T-cell trafficking to the tumor and PD-1 blockade response.[122] Vascular endothelial growth factor C (VEGF-C), a lymphangiogenesis-promoting factor, has been shown to be downregulated in GBM tumors. Higher expression of VEGF-C correlates with increased infiltration of T cells and treatment efficacy with anti-PD-1.[121] In addition, limited tumor mutational burden in GBM may be another factor limiting PD-1 therapy. While it has been suggested that RT and TMZ-induced somatic mutations can generate neoantigens to promote anitumor immunity following PD-1 therapy, recent evidence suggest that TMZ-induced hypermutation does not enhance PD-1 blockade responses, possibly due to the low quality or subclonal nature of neoantigens.[123] In addition, in patients with recurrent GBM who received recombinant poliovirus or immune checkpoint blockade, low tumor mutational burden was found to correlate with longer survival and upregulation of inflammatory markers, suggesting an inverse relationship between immune response and mutational burden.[124] Further studies are needed to examine the effect of tumor mutational burden in GBM on immunotherapies. Interestingly, an alternative effect of PD-1 therapy on the GBM TME has been proposed. In mouse models of GBM, the effect of anti-PD-1 is attributed to blockade of PD-L1 signaling in TAMs, resuling in induction of apoptosis in microglia via antibody-dependent cellular cytotoxicity and polarization of TAMs toward a proinflammatory state.[125] Whether this is a relevant mechanism in human GBM patients remains unclear.

Chimeric antigen receptors T cells (CAR-T cells) which redirect T cells to recognize unique tumor cell surface markers have gained increasing attention in glioma immunotherapy research over the past decade. While early preclinical experiments using CAR-Ts showed encouraging results in mice,[126] subsequent studies in GBM patients demonstrated an initial antitumor response followed by disease recurrence, target-antigen loss and immune escape.[127] CAR-T function can be suppressed by factors in the TME including Tregs, MDSCs, TAM-secreted cytokines, and amino-acid-depleting enzymes such as arginase 1 or IDO.[76] Further understanding of how RT-TMZ treatment alter TME in ways that affect CAR-T efficacy is needed to fully realize their theraputic potential.

Other forms of immunotherapies that modulate the immune microenvironment of GBM are under active investigations. Cytokine therapies that aim to enhance antitumor immunity have limited efficacy thus far. For example, inhibition of colony stimulating factor 1 receptor (CSF1R), using PLX3397, to block TAM recruitment showed no improvement in progression-free or overall survival.[128] Similarly, TGF-β receptor type I inhibition in combination with lomustine did not improve overall survival compared to lomustine alone in newly diagnosed patients with GBM.[129] Recent investigations into gene therapy for immune activation using adeno-virus or retro-virus vectors have demonstrated exciting preliminary results. Adenoviral vectors expressing IL-12 have shown promise in increasing T-cell infiltration into tumors and are currently being investigated in conjunction with PD-1 blockade.[130] In addition, a novel combined cytotoxic and immune-stimulating approach using two adenoviral vectors, one expressing FMS-like tyrosine kinase 3 ligand to recruit DCs and another expressing herpes simplex virus type 1 (HSV1) thymidine kinase which converts the prodrug valacyclovir into a nucleotide analog that kills dividing glioma cells, is recently shown to be safe and feasible in patients with GBM in a phase I clinical trial.[131] The recent phase I trial of Toca 511, which is a non-lytic retroviral that selectively delivers cytosine deaminase gene product to tumor cells to convert the prodrug Toca-5-fluorocytosine into cytotoxic metabolite 5-fluorouracil, in patients with GBM showed excellent tolerability and may prove to be useful in augmenting immunotherapy responses.[132]

Oncolytic viruses which can target and lyse tumor cells and enhance antitumor immune responses in the TME are also a new avenue of immunotherapy for gliomas.[133] DNX-2401, a tumor-selective engineered adenovirus, in combination with pembrolizumab has shown promising results in a phase I/II clinical trial in recurrent malignant gliomas with a total of 56.2% of patients demonstrating a clinical benefit defined as stable disease or better.[134] DNX-2401 has also been studied in 12 patients with pediatric diffuse intrinsic pontine glioma and was found to elicit immune-mediated anti-glioma response.[135] Tumor-targeting HSV-1 CAN-3110 has also recently been shown to improve survival in patients withrecurrent GBM with HSV-1 seropositivity and to dramatically increase tumor-infiltrating lymphocytes.[136] Vaccine-based therapies can also elicit antitumor immune responses by enhancing T-cell activity through increased antigen presentation.[137] Rindopepimut, an EGFRvIII-targeting peptide vaccine, has recently been studied in a phase III clinical trial for patients with newly diagnosed GBM who had undergone maximal resection and RT with TMZ.[138] The trial showed that Rindopepimut alone did not improve overall survival but suggested that combination therapy with other immunotherapies may be beneficial. These and other selected ongoing trials targeting the immune TME are highlighted in table 1.

Table 1 Selected clinical trials for immunotherapies in gliomas

Type of immunotherapy	Study name (trial ID)	Treatment	Results	PMID
Cytokine	Colony stimulating factor 1 receptor inhibitor PLX3397 in recurrent glioblastoma: an Ivy Foundation Early Phase Clinical Trials Consortium phase II study (NCT01349036)[128]	CSF1R inhibitor	No improvement in progression-free or overall survival in patients with recurrent GBM	26449250
	Safety and efficacy of L19TNF in patients with isocitrate dehydrogenase (IDH) wildtype WHO grade 3/4 glioma at first relapse (GLIOMOON) (NCT03779230)[142]	L19 antibody fused with TNF in IDH-WT gliomas	L19-TNF fusion protein was safe and increased tumor necrosis and infiltration of CD4+ and CD8+ T cells	33028706
	Study of Ad-RTS-hIL-12+veledimex in combination with cemiplimab in subjects with recurrent or progressive glioblastoma (NCT04006119)[130]	IL-12 adenovirus	Phase I trial showing safety and good brain penetration. Preliminary overall survival analysis demonstrated improvement in VDX with nivolumab	34850166
	Study of IDO inhibitor and temozolomide for adult patients with primary malignant brain tumors	IDO1 inhibitor (indoximod)+TMZ	Ongoing in pre-published results, indoximod was well tolerated and 1 out of 12 patients with TMZ-refractory GBM had a reduction in tumor size	
Immune checkpoint inhibitors	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) (NCT02017717)[17]	Nivolumab vs bevacizumab	Median overall survival comparable between nivolumab and bevacizumab in recurrent GBM	32437507
	An investigational immuno-therapy study of nivolumab compared with temozolomide, each given with radiation therapy, for newly-diagnosed patients with glioblastoma (CheckMate 498 phase III trial) (NCT02617589)[143]	Nivolumab+RT vs TMZ+RT	TMZ+RT demonstrated a longer median overall survival than nivolumab+RT	35419607
	Randomized phase II and biomarker study of pembrolizumab plus bevacizumab vs pembrolizumab alone for patients with recurrent glioblastoma (NCT02337491)[144]	Pembrolizumab+bevacizumab or Pembrolizumab alone vs bevacizumab alone	No improvement in progression-free survival or overall survival with pembrolizumab treatment compared with bevacizumab alone	33199490
	Nivolumab alone or in combination with ipilimumab before surgery for the treatment of surgically accessible relapsed IDH-wildtype glioblastoma (NCT04606316)	Neoadjuvant nivolumab, or Nivolumab+ipilimumab, phase I trial	Ongoing	
	A study to evaluate safety and efficacy of ACT001 and anti-PD-1 in patients with surgically accessible recurrent glioblastoma multiforme (NCT05053880)	ACT001, an inhibitor of plasminogen activator inhibitor-1, in combination with anti-PD-1 treatment in recurrent GBM	Ongoing	
CAR T-Cell therapy	CAR T-cell receptor immunotherapy Targeting EGFRvIII for patients with malignant gliomas expressing EGFRvIII (NCT01454596)[127 145]	EGFRvIII-targeting CAR T cells	Results pending, previous studies have shown initial antitumor response observed, however, later disease recurrence and tumor escape observed	28724573 28029927

Continued

Table 1 Continued

Type of immunotherapy	Study name (trial ID)	Treatment	Results	PMID
	Phase 1 study of phase 1 study of autologous anti-EGFRvIII synNotch receptor induced anti-EphA2/ IL-13R alpha2 CAR (E-SYNC) T cells in adult participants with EGFRvIII+glioblastoma (NCT06186401)	synNotch-CAR T targeting EGFR vIII	Ongoing	
	Phase I clinical trial of autologous HER2 CMV bispecific CAR T cells for progressive glioblastoma (NCT01109095)[146]	HER2 CMV bicspecific CAR T cells	Phase I trial showing well tolerated, and clinical benefit in 33% of patients with recurrent GBM	28426845
Oncolytic viruses/ vaccines	A phase II, multicenter, open-label study of a conditionally replicative adenovirus (DNX-2401) with pembrolizumab for recurrent glioblastoma or gliosarcoma (NCT02798406)[134]	Oncolytic DNX-2401 virotherapy+pembrolizumab	Well tolerated, notable survival benefit in some patients with recurrent GBM	37188783
	An international, randomized, double-blind, controlled study of rindopepimut/ GM-CSF with adjuvant temozolomide in patients with newly diagnosed, surgically resected, EGFRvIII-positive glioblastoma (the “ACT IV” study) (NCT01480479)[138]	Vaccine targeting EGFRvIII	In patients with newly diagnosed EGFRvIII-positive GBM who had undergone maximal surgical resection and standard chemoradiation, adjuvant rindopepimut did not increase median overall survival	28844499
	SurVaxM vaccine therapy and temozolomide in treating patients with newly diagnosed glioblastoma (NCT02455557)[147]	A vaccine targeting surviving peptide, an anti-apoptotic protein highly expressed in GBM cells	SurVaxM therapy was associated with higher median overall survival (86.6 weeks)	27576783
Gene therapies	Combined cytotoxic and immune-stimulatory therapy for glioma (NCT01811992)[131]	Combined adenoviral delivery of Fit3L and HSV1-TK along with prodrug valacyclovir	Phase I trial demonstrating well-tolerated treatment	37657463
	The Toca 5 trial: Toca 511 and Toca FC vs standard of care in patients with recurrent high-grade glioma (Toca5) (NCT02414165)[132]	Retroviral delivery of cytosine deaminase in conjunction with prodrug Toca 5-fluorocytosine	Phase I trial demonstrating Good tolerability, improved overall survival compared with external control	27252174

AHR, aryl-hydrocarbon receptor; APCs, antigen-presenting cells; ATRX, Alpha-thalassemia/mental retardation, X-linked; BMAL1, basic helix-loop-helix ARNT-like protein 1; CAR, chimeric antigen receptor; CAR-T cells, Chimeric antigen receptors T cells; CDKN2A, cyclin-dependent kinase inhibitor 2A; CDKN2B, cyclin-dependent kinase inhibitor 2B; CEACAM, galectin 9 and carcinoembryonic antigen; C/EBPB, CCAAT/enhancer binding protein beta; cGAS, cyclic GMP-AMP synthase; CL, classical; CLOCK, circadian locomotor output cycles kaput; CMV, Cytomegalovirus; CSF-1, colony-stimulating factor 1; CSF1R, colony stimulating factor 1 receptor; CTLA-4, cytotoxic T-lymphocyte associated protein 4; CTLs, cytotoxic T cells; CXCL, chemokine (C-X-Cmotif) ligand; DCs, dendritic cells; ECM, extracellular matrix; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; EGFRvIII, epidermal growth factor receptor and its truncation mutant variant ; EGFRvIII, EGFR truncation mutant variant; EMT, Epithelial-to-mesenchymal transition; FasL, Fas-ligand; GBM, glioblastoma; GM-CSF, granulocyte-macrophage colony-stimulating factor; GSC, glioma stem cells; HER2, human epidermal growth factor receptor 2; 2-HG, R-2-hydroxyglutarate; HIF1α, hypoxia-inducible factor 1α; HSV1, herpes simplex virus type 1; HSV1-TK, herpes simplex virus type 1-thymidine kinase; IDH, isocitrate dehydrogenase ; IDO1, indoleamine 2,3-dioxygenase 1 ; IDO1, indoleamine 2,3-dioxygenase 1; IFN, interferon; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; MDSCs, myeloid-derivedsuppressor cells; MHC, major histocompatibility complex; MIC-1, macrophage-inhibitory cytokine-1; MMP, matrix metalloproteinase; NE, neural; NF-κB, nuclear factor-kappa B; NK, natural killer; NO, nitric oxide; OLFML3, olfactomedin-like 3; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PGE2, prostaglandin E2; PMID, PubMed Identifier; PN, proneural; PTEN, phosphatase and tensin homolog; RT, radiation therapy ; STAT3, signal transducer and activator of transcription 3; STI1, stress-inducible protein 1; STING, stimulator of Interferon Genes; TAMs, tumor associated macrophages; TANs, tumor associated neutrophils; TAZ, transcriptional coactivator with PDZ-binding motif; TCGA, The Cancer Genome Atlas; TGF-β, transforming growth factor beta; TIM-3, T-cell immunoglobulin and mucin-domain containing-3; TME, tumor microenvironment; TMZ, temozolomide; TNF, tumor necrosis factor; Tregs, Regulatory T cells; VEGF-C, Vascular endothelial growth factor C; VGEF, vascular endothelial growth factor.

Concluding remarks

Tumors cells together with cells of the TME in glioma create a complex milieu that interact in a dynamic manner that ultimately promotes tumor adaptability and disease progression. The advent of single-cell sequencing technologies has allowed us to appreciate how changes in the immune microenvironment influence tumor cell state and how adaptations of glioma cells in turn remodel immune cell composition to their survival advantage. The studies highlighted above illustrate how these glioma-immune interactions dynamically evolve over the course of glioma development and recurrence following chemotherapy and radiotherapy (figure 2). These interactions can occur simultaneously in different

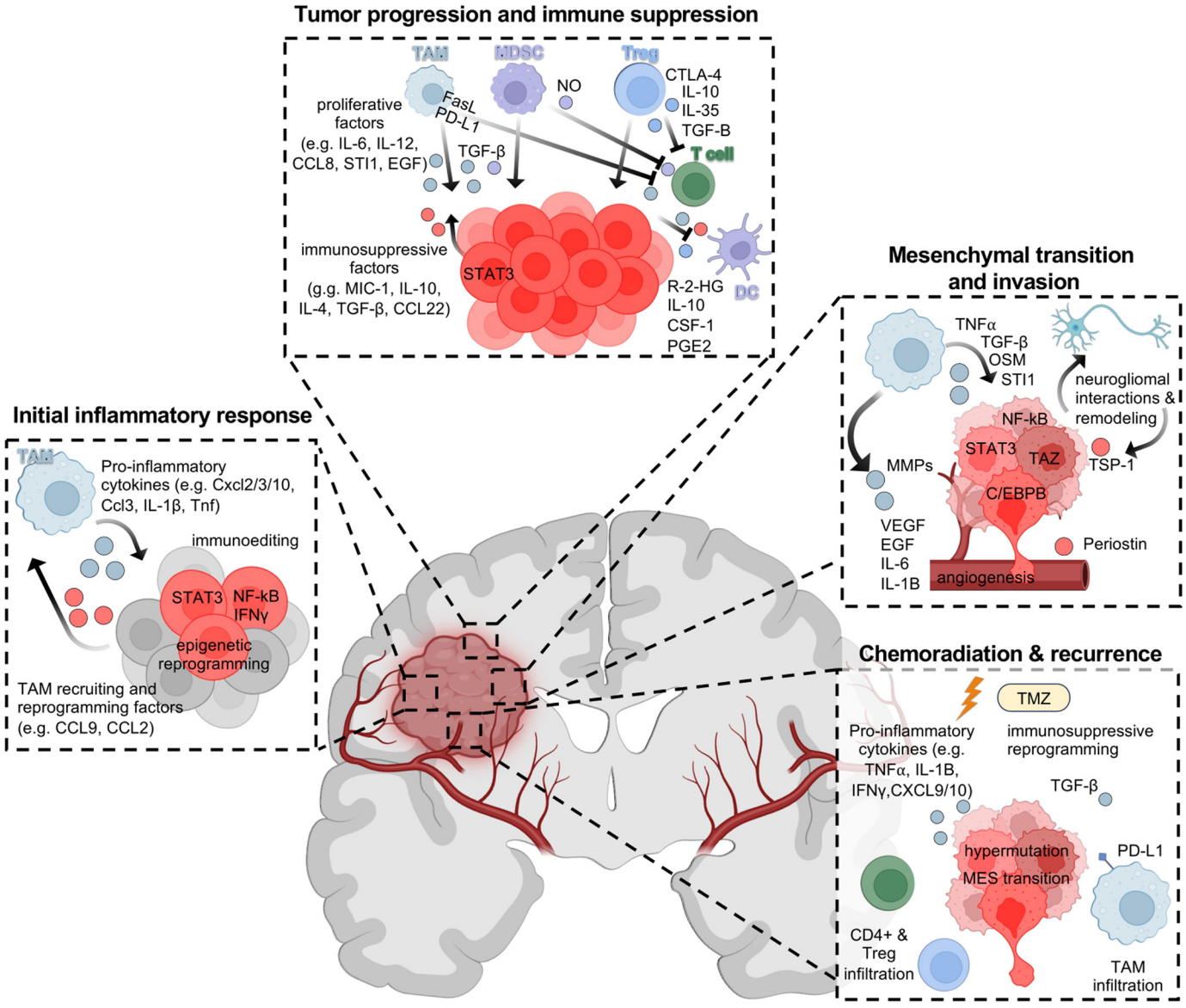

Figure 2 Heterogeneous and dynamic glioma-immune interactions governing different stages of glioma progression. Immune cell interactions with glioma cells regulate various signaling pathways across the life cycle of glioma progression and recurrence. During tumor initiation, a proinflammatory signature leads to epigenetic reprogramming of glioma cells and stem cell renewal. Concurrently, glioma cells secrete TAM recruiting and immunosuppressing factors. Glioma progression is marked by dramatic immune suppression and immune evasion mediated by multiple cell types and secreted molecules. Continued tumor growth results in hypoxia and the release of angiogenic factors. Invasion into surrounding parenchyma has been proposed to be the result of a mesenchymal transition of glioma cells as well as neuroglioma interactions, both of which can be modulated by immune factors. Tumor recurrence is highlighted by a mesenchymal-like state as well as perturbances in proinflammatory and anti-inflammatory responses. CCL, chemokine ligand; CSF-1, colony stimulating factor 1; CXCL, chemokine (C-X-C motif) ligand; EGF, epidermal growth factor; FasL, Fas-ligand; IFN, interferon; IL, interleukin; MES, mesenchymal; MDSC, myeloid-derived suppressor cell; MIC-1, macrophage-inhibitory cytokine-1; MMP, matrix metalloproteinase; NO, nitric oxide; OSM, oncostatin-M; R-2, R-2-HG, hydroxyglutarate; STI1, stress inducible protein 1; TAM, tumor-associated macrophages; TMZ, temozolomide; TNF, tumor necrosis factor; Treg, regulatory T cell; TSP-1, thrombospondin-1; VEGF, vascular endothelial growth factor.

regions within the heterogenous tumor, as depicted by GBM tumors which contain distinct tumor cell states in different regions of the tumor with unique immune TME features. While significant work has been done to characterize the macrophage/microglia population in gliomas, there remains many questions regarding the influence of other immune cell populations on glioma evolution. For example, how other immune populations, despite being relatively sparse compared with TAMs, interact with MES and, less studied, non-MES subtypes will surely be of interest. While many recent studies have focused on genetic and protein-coding transcriptome analysis, other complementary-omics approaches to decipherer changes in epigenetic, non-protein coding, and alternatively

spliced transcript landscape during tumor evolution will be important for future investigations. Notably, the implications of the studies highlighted above showing the dynamic changes occurring in glioma over time and in distinct spatial regions emphasizes the need for multiple resections and biopsies from different regions within the tumor to accurately encapsulate glioma and microenvironmental changes. While multiple surgical procedures for this purpose is limited, the development of new technologies such as implantable microdialysis catheter devices[139] that allow sampling of the tumor over time may provide a partial solution to this problem. The immune-glioma changes that occur at tumor relapse and following treatment with TMZ, RT and other immunotherapies have only begun to be elucidated. Further in-depth experiments using advanced molecular techniques are needed to uncover the full range of dynamic interactions and cellular changes that occur at tumor relapse and following chemoradiation and immunotherapy treatment. Recent technological advances such as Zman-seq, which uses fluorescent-based temporal tracking coupled with single-cell sequencing, identified hyperacute changes in immune cells, including immediate activation of TGF-β signaling within ~24 hours after infiltrating into the tumor.[140] In addition, profiling approaches that go beyond characterizing transcriptomic and genomic changes may uncover new mechanisms of tumor growth and immune modulation, as highlighted by a recently developed mouse glioma cell line to precisely isolate MHC-I peptides and explore antigen presentation in vivo.[141] Such tools may help discover post-translational events that occur during gliomagenesis and identify unique tumor antigens that can be targeted with vaccine therapies. A better understanding of the glioma-immune landscape is essential for developing efficacious treatment for this universally deadly tumor.

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