Glioblastoma (GBM) is the most common and highly lethal form of primary brain tumor in adults. The median survival of GBM patients is approximately 14–16 months despite multimodal therapies. Emerging evidence has substantiated the critical role of symbiotic interactions between GBM cells and noncancerous immune cells (e.g., myeloid cells and T cells) in regulating tumor progression and therapy resistance. Approaches to target the tumor-immune symbiosis have emerged as a promising therapeutic strategy for GBM. Here, we review the recent developments for pharmacological targeting of the GBM-immune symbiosis and highlight the role of such strategies to improve the effectiveness of immunotherapies in GBM.

**Targeting the GBM-immune symbiosis**

GBM is the most common and fastest growing primary brain tumor in adults [1,2]. Despite the aggressive standard-of-care (SOC) treatment that includes maximal surgical resection followed by radiation and/or chemotherapy with temozolomide (TMZ), the median survival for GBM is only approximately 14–16 months [2]. The low therapeutic efficiency of the SOC treatment relates to the challenges that complete resection of GBM tumors is impossible and the blood–brain barrier (BBB) can hinder the systemic therapy [3–5]. Genetic profiling of patient tumors has led to identification of several core signaling pathways in GBM cells, thus motivating clinical trials for testing potential targeted therapies. However, all these efforts have failed to improve GBM patient outcomes, probably due to GBM cell genetic instability and heterogeneity [6]. Conversely, noncancerous cells in the GBM tumor microenvironment (TME) are genetically stable. Increasing evidence demonstrates that the TME is critical for supporting GBM progression, and strategies targeting the GBM TME have emerged as a promising therapeutic approach [3,4]. In recent years, studies using advanced technologies, such as single-cell RNA sequencing (scRNA-seq), whole-exome sequencing, and mass cytometry (CyTOF) followed by functional studies, have revealed a dynamic and diverse immune landscape in the GBM TME with respect to different tumor stages and genetic backgrounds [7–9]. These findings highlight a context-dependent tumor–immune symbiotic interaction, which is critical for promoting tumor progression and therapy resistance (e.g., resistance to the SOC treatment and immunotherapies) in GBM [6].

Immunotherapies, including immune checkpoint inhibitor (ICI) therapies, have been shown to improve patient outcomes in multiple cancer types [10,11]. Unfortunately, such ICI therapies only produce modest clinical benefits in GBM, probably due to lack of intratumoral T cell infiltration [2,7,12]. Apart from that, infiltrating myeloid cells, such as glioma-associated macrophages and microglia (GAMs) and myeloid-derived suppressor cells (MDSCs), induce a robust immunosuppressive TME that inhibits the activity and proliferation of cytotoxic T cells, resulting in an even worse immunotherapy response [2,7,13,14]. T cell-based immunotherapy can reshape the composition and status of myeloid cells in the GBM TME. For example, immunotherapy...
Knowledge of the crosstalk among GBM cells, myeloid cells, and T cells has motivated great efforts to target these symbiotic interactions, with pharmacological tools as the primary focus for translational studies. Here, we review recent advances in pharmacological targeting of the GBM-immune symbiosis and discuss the role and application of such pharmacological tools for improving the effectiveness of immunotherapies in GBM.

**Pharmacological targeting of the GBM–GAM crosstalk**

GAMs are the most abundant cell population in the GBM TME (accounting for up to 50% of total live cells) and composed of bone marrow-derived macrophages (hereafter referred as macrophages) and brain-resident microglia. GAMs contribute to tumor progression through various mechanisms, including secretion of distinct cytokines, ligands, and other factors (Box 1) [13]. Given the profound role of GAMs in GBM, targeting the GBM–GAM symbiosis appears to be a promising therapeutic strategy [16]. Based on the types of targets and molecular mechanisms underlying this crosstalk, we discuss pharmacological approaches to: (i) target receptors on GAMs and GBM cells; (ii) target GAM and GBM cell-secreted chemokines and factors; and (iii) trap extracellular signaling in the TME (Figure 1 and Table 1).

**Targeting receptors on GBM cells and GAMs**

Blockade of GBM cell receptors is a straightforward approach for diminishing the protumor effect of GAM-derived factors (Figure 1 and Table 1). For example, AXL receptor tyrosine kinase (hereafter referred to as AXL) on glioma stem cells (GSCs) can be activated by GAM-derived protein S (PROS1), which, in turn, phosphorylates p65 (a subunit of the NF-κB complex) and promotes tumor growth. Pharmacological inhibition of AXL with its highly selective inhibitor BGB324 abrogates PROS1-induced p65 phosphorylation in GSCs, thus breaking the GSC–GAM crosstalk and inhibiting tumor growth in GBM-bearing mice [17]. A clinical trial is underway evaluating the antitumor effect of BGB324 in recurrent GBM (NCT03965494).

Similarly, inhibition of the protein tyrosine phosphatase receptor type Z1 (PTPRZ1) using (e.g., the combination of ICI therapy and immunovirotherapy) in GBM mouse models results in a significant increase of immunostimulatory macrophages [15]. Together, these findings support a symbiotic interaction between myeloid cells and T cells and imply that this symbiosis may affect the effectiveness of immunotherapy in GBM.

**Box 1. GAMs and their role in GBM progression**

Lineage tracing study has revealed that GBM GAMs originate from both bone marrow-derived macrophages and brain-resident microglia [13,85,95]. Distinguishing macrophages and microglia in the GBM TME is complicated [9]. However, recent studies using advanced technologies have made a decent progress. For instance, studies using cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq) and scRNA-seq have demonstrated that macrophages are the predominant GAM population in recurrent GBM, whereas microglia are accumulated in newly diagnosed tumors [9]. Additionally, the composition of macrophages and microglia in tumor tissues may vary in GBM patients with different genetic backgrounds [7,26,27,85]. Specifically, microglia are highly enriched in IDH-mutated glioma, whereas macrophages are enriched in IDH-WT and PTEN-deficient glioma [7,26]. Within the same TME, microglia and macrophages could compete for space for their activation and function [9].

The infiltration of macrophages and microglia into the TME is triggered by multiple GBM cell-secreted chemokines, such as CSF-1, CSF-2, CCL2, OPN, LOX, and monocyte chemotactrant protein 3 (MCP3) [13]. Once infiltrating into the GBM TME, they are educated by GBM cells, and skewed toward an immunosuppressive phenotype to support tumor progression and induce immunosuppression [13,96,97]. Mechanistically, immunosuppressive GAMs release different cytokines and growth factors, such as IL-6 [37], IL-11 [38], IL-1β [27,85], IL-10 [86], and TGF-β1 [86], to promote GBM progression via activation of the protumor signaling in GBM cells [8,19]. Alternatively, GAMs could affect GBM cell survival via suppressing T cell function [83]. Since GAMs are the primary immune cells in the GBM TME, pharmacological targeting of the GBM–GAM symbiosis is a promising approach for GBM treatment [16].

**Glossary**

- **Aptamer:** short single-stranded DNA or RNA molecules that can selectively bind to distinct targets (e.g., peptides, proteins, carbohydrates, toxins, and small molecules).
- **Blood–brain barrier (BBB):** a system of brain microvascular endothelial cells that can protect the brain from toxic substances in the blood, supply brain tissues with nutrients, and filter harmful compounds from brain back into the blood stream.
- **Circadian locomotor output cycles kaput (CLOCK)-BMAL1 complex:** a heterodimeric transcriptional activator that coordinates rhythmic gene expression and controls biological functions of the circadian clock.
- **Glioma-associated macrophages (GAMs):** infiltrating bone marrow-derived macrophages and brain-resident microglia in the GBM TME that originate from the bone marrow and progenitors seeding the embryonic yolk, respectively.
- **Glioma stem cells (GSCs):** a small population of cells within GBM tumors that have self-renewal and tumorigenic ability and can induce tumor recurrence and treatment resistance.
- **Immune checkpoint inhibitor (ICI):** a class of agents that trigger antitumor immune response by targeting immune checkpoint molecules.
- **Mass cytometry (CyTOF):** a variation of flow cytometry that allows the quantification of multiple labeled targets (up to 50) simultaneously on the surface and interior of single cells.
- **Myeloid cells:** a group of immune cells (e.g., macrophages, MDSCs, neutrophils, monocytes, dendritic cells, and mast cells) that originate from hematopoietic stem cells in the bone marrow.
- **Myeloid-derived suppressor cells (MDSCs):** a population of immature myeloid cells (including polymorphonuclear and monocyte MDSCs) that can suppress antitumor immunity and promote tumor progression.
- **Organoids:** 3D multicellular in vitro tissue constructs that can mimic the in vivo TME.
- **Patient-derived xenografts (PDX):** cancer patient-derived xenograft mouse models that can reflect the properties of original patient tumors.
- **Single-cell RNA sequencing (scRNA-seq):** an optimized next-

---

2 Trends in Pharmacological Sciences, Month 2022, Vol. xx, No. xx
neutralizing antibodies blocks the GSC–GAM crosstalk by interrupting the binding of GAM-derived pleiotrophin to its receptor PTPRZ1 on GSCs and induces a robust antitumor effect in GBM mouse models [18].
### Targeting GBM-GAM crosstalk

<table>
<thead>
<tr>
<th>Target</th>
<th>Therapeutic agent</th>
<th>Tumor model</th>
<th>Therapeutic mechanism</th>
<th>Combined with other therapies</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD47</td>
<td>Anti-CD47</td>
<td>GL261 and CT2A models, mouse</td>
<td>Escape of GBM cells from GAM-mediated phagocytosis</td>
<td>TMZ and anti-PD1</td>
<td>[83]</td>
</tr>
<tr>
<td>AXL</td>
<td>BGB324</td>
<td>GSC267 and GSC374, PDX; M57080, mouse</td>
<td>Inhibition of GAM PROS1-induced GSC stemness</td>
<td>Anti-PD1</td>
<td>[17]</td>
</tr>
<tr>
<td>PTPRZ1</td>
<td>Anti-PTPRZ1 antibody</td>
<td>T912 GSC, PDX</td>
<td>Inhibition of GAM pleiotrophin-induced GSC stemness</td>
<td>N/A</td>
<td>[18]</td>
</tr>
<tr>
<td>CSF-1R</td>
<td>BGB324, GSC267 and GSC374, PDX; M57080, mouse</td>
<td>Depletion of GAMs</td>
<td>N/A</td>
<td>[22]</td>
<td></td>
</tr>
<tr>
<td>Anti-CSF-1R antibody</td>
<td>GL261, mouse</td>
<td>Depletion of GAMs</td>
<td>N/A</td>
<td>[6]</td>
<td></td>
</tr>
<tr>
<td>CSF-1R</td>
<td>Anti-CSF-1R antibody</td>
<td>GL261, mouse</td>
<td>Depletion of GAMs</td>
<td>N/A</td>
<td>[23]</td>
</tr>
<tr>
<td>TβRI</td>
<td>SB431542</td>
<td>SETD2-mutated GBM, mouse</td>
<td>Inhibition of GBM cell TGF-β1-induced microglia activation</td>
<td>N/A</td>
<td>[27]</td>
</tr>
<tr>
<td>LOX</td>
<td>β-aminopropionitrile</td>
<td>U87, human; 005 GSCs and QPP7, mouse; and GSC23, PDX</td>
<td>Inhibition of GBM cell LOX-induced macrophage recruitment</td>
<td>N/A</td>
<td>[26]</td>
</tr>
<tr>
<td>CLOCK</td>
<td>SR9009</td>
<td>CT2A, mouse</td>
<td>Inhibition of GSC self-renewal and GSC-induced microglial infiltration</td>
<td>N/A</td>
<td>[29]</td>
</tr>
<tr>
<td>MAGL</td>
<td>JZL184</td>
<td>GL261, mouse</td>
<td>Blockade of prostaglandin E2 production in GSCs, and its role in GAM immunosuppressive polarization</td>
<td>Anti-PD1</td>
<td>[30]</td>
</tr>
<tr>
<td>P-selectin</td>
<td>KF 38789</td>
<td>IAGR53 and GL261, mouse; PD-GB4, PDX</td>
<td>Inhibition of GBM cell P-selectin-induced GAM immunosuppressive polarization</td>
<td>N/A</td>
<td>[33]</td>
</tr>
<tr>
<td>Wnt/β-catenin-WISP1</td>
<td>Carnosic acid</td>
<td>T4121, PDX</td>
<td>Inhibition of GSC W1SP1-induced GAM survival</td>
<td>N/A</td>
<td>[34]</td>
</tr>
<tr>
<td>P3Kγ</td>
<td>KG100-115</td>
<td>GL261, mouse</td>
<td>Inhibition of GAM IL-11-induced GBM stemness and tumorigenicty</td>
<td>TMZ</td>
<td>[36]</td>
</tr>
<tr>
<td>BACE1</td>
<td>MK-8931</td>
<td>GSCs, PDX</td>
<td>Inhibition of IL-6R cleavage in GAMS, suppressing GAM-mediated immunosuppression</td>
<td>N/A</td>
<td>[36]</td>
</tr>
<tr>
<td>IL-6</td>
<td>Anti-IL-6</td>
<td>GL261, mouse</td>
<td>Inhibition of GBM cell IL-6-induced GAM immunosuppressive polarization</td>
<td>Anti-PD1 and anti-CTLA4; CD40 agonist</td>
<td>[41]</td>
</tr>
<tr>
<td>IL-6</td>
<td>GL261, mouse</td>
<td>Inhibition of GBM cell IL-6-induced GAM immunosuppressive polarization</td>
<td>Anti-PD1</td>
<td>[41]</td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>GL261, mouse</td>
<td>Inhibition of GBM cell IL-6-induced GAM immunosuppressive polarization</td>
<td>Anti-PD1 and anti-CTLA4; CD40 agonist</td>
<td>[41]</td>
<td></td>
</tr>
<tr>
<td>SLIT2</td>
<td>SLIT2 ligand trap protein</td>
<td>CT2A, mouse</td>
<td>Inhibition of GBM cell SLIT2-induced GAM chemotaxis and immunosuppressive polarization</td>
<td>Anti-PD1 and anti-4-1BB</td>
<td>[43]</td>
</tr>
<tr>
<td>OPN</td>
<td>4-1BB-OPN aptamer</td>
<td>GL261, mouse</td>
<td>Inhibition of GBM cell OPN-induced GAM migration and immunosuppressive maintenance</td>
<td>N/A</td>
<td>[45]</td>
</tr>
<tr>
<td>CHI3L1</td>
<td>Galectin mimic peptide</td>
<td>GL261, mouse</td>
<td>Inhibition of GBM cell CHI3L1-induced GAM migration and immunosuppressive polarization</td>
<td>N/A</td>
<td>[46]</td>
</tr>
</tbody>
</table>

### Targeting GBM-MDSC crosstalk

<table>
<thead>
<tr>
<th>Target</th>
<th>Therapeutic agent</th>
<th>Tumor model</th>
<th>Therapeutic mechanism</th>
<th>Combined with other therapies</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR2</td>
<td>CCX872</td>
<td>KR158 and 005 GSC, mouse</td>
<td>Inhibition of GBM cell CCL2-induced M-MDSC migration</td>
<td>Anti-PD1</td>
<td>[54]</td>
</tr>
</tbody>
</table>

*Table 1. Recent in vivo studies using pharmacological strategies to block the tumor-immune symbiosis in GBM"*
In addition to receptors on GSCs and/or GBM cells, receptors on GAMs are also promising targets for blockade of the GBM–GAM crosstalk (Figure 1 and Table 1). Colony-stimulating factor 1 receptor (CSF-1R) is crucial for regulating macrophage development [19], and it is plausible that inhibition of CSF-1R should affect the GAM biology. BLZ945 is a selective brain penetrant CSF-1R inhibitor [20,21] that can reprogram GAMs from an immunosuppressive phenotype to an immunostimulatory phenotype in the GBM TME. As a result, treatment with BLZ945 significantly decreases tumor growth and prolongs survival in GBM mouse models [21,22]. Similarly, preclinical studies with another CSF-1R inhibitor PLX3397 effectively depletes GAMs and inhibits tumor growth in GBM-bearing mice [8]. Detailed characterization in GBM tumors has revealed that PLX3397 treatment reduces the percentage of macrophages, but does not affect monocytes, suggesting a potential role of this inhibitor in blocking macrophage differentiation [8]. Therefore, we conclude that inhibition of CSF-1R with its inhibitors BLZ945 and PLX3397 reduces GBM tumor growth via blocking macrophage differentiation and immunosuppressive

---

**Table 1. (continued)**

<table>
<thead>
<tr>
<th>Target</th>
<th>Therapeutic agent</th>
<th>Tumor model</th>
<th>Therapeutic mechanism</th>
<th>Combined with other therapies</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR4</td>
<td>C021</td>
<td>GL261, mouse</td>
<td>Inhibition of GBM CCL2-induced M-MDSC migration</td>
<td>N/A</td>
<td>[51]</td>
</tr>
<tr>
<td>MIF</td>
<td>Ibudilast</td>
<td>GL261, mouse</td>
<td>Inhibition of GBM cell MIF-induced M-MDSC activation</td>
<td>N/A</td>
<td>[48]</td>
</tr>
<tr>
<td>Exosomal miR-1246/ HIF-1α</td>
<td>2-Methoxyestradiol</td>
<td>U87, human</td>
<td>Inhibition of GBM cell exosomal miR-1246-induced M-MDSC differentiation and activation</td>
<td>N/A</td>
<td>[50]</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Rilonacept; anti-IL1β</td>
<td>GL261 and SB28, mouse</td>
<td>Inhibition of systemic PMN-MDSCs</td>
<td>N/A</td>
<td>[50]</td>
</tr>
<tr>
<td>G-CSF</td>
<td>Recombinant G-CSF</td>
<td>IDH1 mutated and WT glioma models, mouse</td>
<td>Inhibition of GSC G-CSF-induced PMN-MDSC expansion</td>
<td>TK/Fit3L/immune stimulatory gene therapy</td>
<td>[53]</td>
</tr>
</tbody>
</table>

*Abbreviations: 4-1BB, tumor necrosis factor receptor superfamily member 9; CCR2, C-C motif chemokine receptor 2; CH3L1, chitinase-3-like 1; CSF-1R, colony-stimulating factor 1 receptor; CTLA4, cytotoxic T-lymphocyte associated protein 4; Gal3BP, galectin 3-binding protein; GAMs, glioma-associated macrophages and microglia; G-CSF, granulocyte colony-stimulating factor; GITR, glucocorticoid-induced TNFR-related receptor; GSC, glioma stem cell; HIF-1α, hypoxia-inducible factor 1-alpha; IDH, isocitrate dehydrogenase; IDO1, indoleamine 2,3-dioxygenase 1; IL-6R, IL-6 receptor; MAGL, monoacylglycerol lipase; MIF, migration inhibitory factor; M-M-MDSCs, monocytic myeloid-derived suppressor cells; OPN, osteopontin; PD1, programmed cell death protein 1; PDX, patient-derived xenograft; PI3Kγ, phosphoinositide-3-kinase gamma; PMN-MDSC, polymorphonuclear MDSC; PROS1, protein S; PTPRZ1, protein tyrosine phosphatase receptor type Z1; SETD2, SET domain containing 2; SLIT2, slit guidance ligand 2; STAT3, signal transducer and activator of transcription 3; TβRI, transforming growth factor beta receptor I; TCR, T cell receptor; TK/Flt3L, thymidine kinase/FMS-like tyrosine kinase 3 ligand; WISP1, Wnt-induced signaling protein 1.
polarization. Recent efforts of developing anti-CSF-1R neutralizing antibodies also prove this conclusion, where anti-CSF-1R antibodies show a robust antitumor effect in GBM mouse models [23]. These preclinical studies have motived clinical trials (e.g., NCT02829723 and NCT01349036) testing the antitumor effect of CSF-1R inhibitors in GBM patients. Unfortunately, a Phase II clinical trial (NCT01349036) with PLX3397 has revealed a minimal antitumor effect in recurrent GBM patients [24]. However, it should be noted that the progression-free survival in two of 37 GBM patients is significantly extended following PLX3397 treatment. Genetic profiling studies demonstrated that these two patients are mesenchymal subtype [24], in which PTEN deletion/mutation is common [25]. Together with recent studies showing that macrophages are highly enriched in PTEN-deficient GBM [26], these findings encourage further clinical trials with CSF-1R inhibitors in PTEN-deficient and/or mesenchymal GBM patients. Further evidence demonstrates that SETD2 mutation in GBM cells produces transforming growth factor (TGF)-β to activate microglia via the TGF-β receptor I (TβRI) [27]. Inhibition of microglial TβRI using its inhibitor SB431542 impairs tumor growth in GBM-bearing mice [27]. Together, these findings suggest that pharmacological targeting of the receptors (e.g., CSF-1R and TβRI) on GAMs shed light on inhibiting tumor progression by breaking the context-dependent GBM–GAM symbiosis.

**Targeting GBM-secreted chemokines**

GAMs infiltration is usually triggered by GBM cell-secreted chemokines. Pharmacological inhibition of such factors is an effective strategy to block the GBM–GAM symbiosis (Figure 1 and Table 1). Lysyl oxidase (LOX) has been identified as a potent and novel macrophage chemoattractant in PTEN-deficient GBM. Mechanistically, GBM cell-secreted LOX interacts with β1 integrin on macrophages, which, in turn, promotes macrophage migration through activation of the proline-rich tyrosine kinase 2 (PYK2) signaling [26]. The LOX-β1 integrin-PYK2 axis-mediated interaction between GBM cells and macrophages may explain the early observation that macrophages are highly infiltrated in PTEN-mutated GBM tumors [28]. Given the critical role of LOX in macrophage recruitment, the antitumor effect of β-aminopropionitrile (BAPN, a LOX inhibitor) and LOX neutralizing antibodies has been observed in PTEN-deficient GBM mouse models [26]. In addition to directly targeting GBM cell-derived chemoattractants, pharmacological blockade of key factors that regulate the expression of chemokines is also promising. For example, the circadian loco-motor output cycles kaput (CLOCK)-BMAL1 complex can upregulate olfactomedin-like 3 (OLFML3) transcription in GSCs, which, in turn, induces microglial infiltration into the GBM TME [29]. Inhibition of the CLOCK-BMAL1 complex using SR9009 (an agonist of nuclear receptors REV-ERBs, which show a negative feedback loop to repress the CLOCK-BMAL1 complex) reduces tumor growth and progression in vivo via impairing microglial infiltration [29]. Together, these findings highlight a significant symbiotic interaction between GAMs and GBM cells/GSCs harboring specific genetic alterations (e.g., PTEN deficiency and CLOCK amplification) and suggest that pharmacological targeting of this context-dependent symbiosis should be embedded in developing personalized medicine for GBM patients.

**Targeting GBM-secreted factors skewing GAM polarization**

In addition to preventing GAM infiltration, pharmacological inhibition of their immunosuppressive polarization by suppressing GBM cell-derived factors sheds light on GBM therapy (Figure 1 and Table 1). One example is JZL184, a specific inhibitor of monoacylglycerol lipase (MAGL), which has been shown to impair GAM immunosuppressive polarization via downregulating arsenite-resistance protein 2 (ARS2) in GSCs [30]. Mechanistically, ARS2 promotes GSC proliferation by directly activating its transcriptional target MAGL, which further increases the production of prostaglandin E2 (PGE2). Consequently, GSC-derived PGE2 promotes GAM immunosuppressive polarization [30–32]. Suppressing PGE2 production in GSCs using MAGL inhibitor JZL184 and cyclooxygenase-2 inhibitor celecoxib impairs tumor growth and prevents GAM accumulation in
the GBM TME [30]. Another example is KF 38789, a specific inhibitor of P-selectin that is essential for the GBM-microglia symbiosis [33]. In detail, GBM cell-secreted P-selectin binds to P-selectin glycoprotein ligand-1 (PSGL-1, also known as CD162) on microglia and promotes microglial immunosuppressive polarization. As a result, KF 38789 treatment exhibits a significant antitumor effect in GBM mouse and patient-derived xenograft (PDX) models [33]. From another angle, pharmacologically inhibiting the survival of immunosuppressive GAMs can effectively suppress GBM tumor growth. For example, GSC-secreted Wnt-induced signaling protein 1 (WISP1, a downstream target of Wnt/β-catenin signaling) can improve the survival of immunosuppressive GAMs via activation of the integrin α6β1-AKT signaling pathway. Inhibition of the Wnt/β-catenin-WISP1 axis by β-catenin inhibitor carnosic acid (a natural benzenediol abietane diterpene) leads to GAM apoptosis and inhibits GBM tumor growth [34]. Additionally, blocking exosomal secretion from GBM cells using dimethyl amiloride suppresses GAM immunosuppressive polarization [35]. Together, these findings suggest that pharmacological targeting GAM immunosuppressive polarization via inhibiting GBM cell-secreted factors and exosomes is an actionable therapeutic strategy.

Blocking GAM-secreted cytokines

Once infiltrating into the TME, macrophages and microglia are educated by GBM cells and skewed toward an immunosuppressive phenotype, which, in turn, promote tumor growth via distinct mechanisms, including secretion of different cytokines and growth factors (Box 1). Interleukin 11 (IL-11) has been identified as one of such cytokines that is highly secreted by GBM-associated microglia and promotes GBM tumor growth and chemotherapy resistance via activation of the STAT3-MYC signaling in GBM cells [36]. The expression of IL-11 in GBM-associated microglia is regulated by phosphoinositide-3-kinase gamma (PI3Kγ), and inhibition of PI3Kγ using its inhibitor TG100-115 does not inhibit GBM cell growth in vitro, but extends the survival of GBM-bearing mice via specifically inhibiting microglial IL-11 (Figure 1) [36]. Along a similar line, IL-6 is a cytokine expressed by immunosuppressive macrophages in the GBM TME, where it promotes tumorigenesis by stimulating GBM cell aerobic glycolysis [37]. Neutralization of macrophage-derived IL-6 inhibits macrophage-induced GBM cell glycolysis, proliferation, and tumorigenesis in vivo [37]. In addition to acting on GBM cells, IL-6 may induce macrophage immunosuppressive polarization via the STAT3 signaling through an autocrine manner. A very recent study revealed that β-site amyloid precursor protein-cleaving enzyme 1 (BACE1) is expressed specifically in GAMs and contributes to GAM immunosuppressive polarization. Pharmacological inhibition of BACE1 with its inhibitor MK-8931 downregulates the IL-6-sIL-6R-STAT3 axis in GAMs, promotes GAM-mediated phagocytosis of GBM cells, and impairs tumor progression in vivo (Figure 1 and Table 1) [38]. Since BBB-penetrating BACE1 inhibitors (e.g., E2609, AZD3293, and CNP520) have been widely used for treating Alzheimer’s disease [39], it will be very promising to test their antitumor effect in GBM mouse models and patients. Together, these findings highlight a therapeutic potential of pharmacological inhibiting GAM-derived cytokines (e.g., IL-11 and IL-6) in GBM. However, it is worth noting that these cytokines may not be secreted preferentially by GAMs. GBM-associated endothelial cells (ECs) also express and secrete IL-6, which can activate GAMs to promote tumor growth, and inhibition of EC-derived IL-6 genetically and pharmacologically impairs GBM tumor growth in vivo [40,41].

Trapping extracellular signaling in the TME

Although directly targeting the receptors and factors expressed on or secreted by GBM cells and GAMs are the most common approach to block the GBM–GAM crosstalk, recent studies have also made a decent effort to develop therapeutic molecules trapping the signaling transduction between GAMs and GBM cells (Figure 1 and Table 1) [42]. For example, GBM cells secrete polypeptide SLIT2 into the GBM TME, which promotes GAM infiltration and immunosuppressive
polarization through transmembrane Roundabout (ROBO) receptors [43]. In line with genetic studies, SLIT2 ligand trap protein (Robo1Fc) has been developed to systemically inhibit SLIT2, and treatment with Robo1Fc shows a robust antitumor effect in GBM-bearing mice [43]. A similar, but not identical, strategy for trapping GBM-derived molecules is to use aptamer [44]. Osteopontin (OPN) is a potent chemokine that not only recruits macrophages into the GBM TME but also maintains these macrophages in an immunosuppressive phenotype to suppress T cell function [45]. The 4-1BB-OPN bispecific aptamer has been developed to inhibit OPN and activate antitumor immunity simultaneously, and it exhibits a significant antitumor effect in GBM-bearing mice [45]. The third strategy is to develop mimetic peptides. For example, GBM cells can secrete chitinase-3-like 1 (CHI3L1) to promote GAM infiltration and immunosuppressive polarization via binding to galectin-3 on macrophages [46]. Molecular docking studies demonstrated that galectin-3 binding protein (Gal-3BP) and galectin-3 could compete for the same binding pocket in CHI3L1. As a result, treatment with Gal-3BP mimetic peptide inhibits tumor growth and extends survival in GBM-bearing mice via impairing the accumulation of immunosuppressive GAMs [46].

**Pharmacological targeting of the GBM–MDSC crosstalk**

MDSCs have emerged as an important type of myeloid cells contributing to GBM immunosuppression [47]. Depending on their phenotypic and morphological features, MDSCs can be subdivided into polymorphonuclear (PMN) and monocytic (M) MDSCs, which may play different roles in cancer progression and drug treatment response [48,49]. Among them, M-MDSCs are enriched in the tumor tissues of male GBM patients, whereas PMN-MDSCs are widely distributed in the circulating system of female GBM patients [50]. The sexual dimorphism spurs researchers to develop gender-specific MDSC-targeting therapeutic strategies in GBM. Preclinical studies demonstrated that the function of M-MDSCs and PMN-MDSCs in male and female GBM patients could be targeted by antiproliferative agents (e.g., fludarabine) and IL-1β blockade (e.g., anti-IL-1β antibodies), respectively [50] (Figure 2). However, further studies are still needed to elucidate molecular mechanisms underlying the sex-specific manner of MDSCs in GBM.

MDSCs respond to GBM cell- and/or GAM-secreted chemokines and cytokines, such as C-C motif chemokine ligand 2 (CCL2), macrophage migration inhibitory factor (MIF), C-X-C motif ligand 1/2 (CXCL1/2), and G-CSF, in the TME [48,51–53]. A growing body of evidence demonstrates that targeting the cytokine–receptor interaction during the GBM–MDSC symbiosis appears to be a primary therapeutic approach (Figure 2). For example, inhibition of this symbiosis by blockade of the MIF–CD74 axis using the CD74 inhibitor Ibudilast in GBM-bearing mice decreases M-MDSC recruitment and GBM cell proliferation, and increases CD8+ T cell infiltration [48]. In addition, GBM cell-derived CCL20 and osteoprotegerin (OPG) upregulate CCL2 production in GAMs, which, in turn, increases the infiltration of M-MDSCs through the C-C motif chemokine receptor 2 (CCR2) and CCR4. Pharmacological inhibition of CCR2 and CCR4 (using CCX872 and C021, respectively) significantly extends the survival of GBM-bearing mice by decreasing MDSC infiltration [51,54]. Similarly, inhibition of MDSC infiltration using anti-CXCL1 and anti-CXCL2 neutralizing antibodies exhibits a significant antitumor effect in several different GBM mouse models [52]. Worth noting, in addition to MDSCs, the CCL2–CCR2 axis and CXCL1/2 may also affect the biology of GAMs and other immune cells [55,56]. Therefore, identification of specific MDSC-related chemokines and their potential clinical translation could accelerate personalized drug development and avoid unexpected side effects.

These recruited MDSCs need to be further activated to gain immunosuppressive function in the GBM TME. Multiple cytokines have been reported to activate MDSCs, including M-CSF, GM-CSF, IL-6, IL-10, TGF-β, B7-H1, and INFγ [47,57]. Additionally, exosomal miRNAs have been reported to be essential for MDSC differentiation and activation [58–61]. Functional studies
have revealed that GBM-derived extracellular vesicles containing miR-1246 induce MDSC differentiation from donor monocytes under hypoxic conditions [59]. Inhibition of miR-1246 transcription and exosomal packaging using 2-methoxyestradiol impairs GBM tumor growth and MDSC infiltration [59].

**Pharmacological targeting of GBM–T cell crosstalk**

A growing body of studies using CyTOF and scRNA-seq have revealed a unique landscape of T cell populations and T cell receptors in brain tumors [7,62–64]. Compared with IDH-mut glioma, IDH-WT tumors express higher T cell-specific genes and cytotoxicity signatures [83], suggesting a potential GBM–T cell crosstalk. Here, we discuss the recent progress of pharmacological tools targeting T cell exhaustion and T cell tolerance in GBM (Table 1).

In the GBM TME, exhausted T cells exhibit reduced effector function and increased expression of immune checkpoints (e.g., PD1, CTLA4, TIM3, TIGIT, LAG3, BTLA, 2B4, CD39, and CD160) [14,65,66]. Pharmacological approaches to target these immune checkpoints have been developed to treat GBM patients. For instance, a randomized, multi-institution clinical trial demonstrated that
neoadjuvant anti-PD1 therapy significantly improves the overall survival and progression-free survival of recurrent GBM patients [67]. This result is consistent with another single-arm Phase II clinical trial (NCT02550249) in which researchers observed a significant antitumor effect of neoadjuvant anti-PD1 therapy on newly diagnosed or relapsed GBM [68]. However, a recent clinical trial (NCT02017717) with anti-PD1 therapy failed to increase patient overall survival in recurrent GBM patients [69]. We speculate that these controversial results may relate to tumor genetic status and altered core signaling pathways in GBM cells, and their associated GBM-T cell symbiosis. Indeed, genomic profiling in GBM patient tumors has revealed that PTEN mutations are enriched in anti-PD1 therapy nonresponders, whereas MAPK pathway alterations (e.g., PTPN11 and BRAF) are enriched in responders [28]. A recent study further demonstrated that phospho-ERK1/2 expression in GBM cells is predictive of overall survival following adjuvant anti-PD1 therapy in recurrent GBM patients [70]. Together, these findings suggest that context-dependent GBM-T cell crosstalk is critical for designing effective ICI therapy in GBM. Additional clinical trials are underway for testing immunotherapies, including anti-LAG3 combined with anti-PD1 (NCT02658981), anti-TIGIT combined with anti-PD1 (NCT04656535), and anti-CD39 (NCT04306900), in GBM patients.

T cell tolerance represents the programmed induction of unresponsiveness due to misexpressed self-antigens in GBM [14], a process that is regulated by the expansion of Treg cells [71]. Mechanistically, GSCs express and secrete distinct factors (e.g., TGF-β and CCL2) to control Treg cell infiltration and expansion in GBM [72]. Several pharmacological tools have been developed to target Treg cells in GBM. First, glucocorticoid-induced TNFR-related receptor (GITR) has been shown to be critical for Treg cell differentiation into CD4 effector T cells [71]. Targeting GITR using an agonistic antibody (anti-GITR) improves the survival of GBM-bearing mice via converting Treg cells to Th1-like CD4 T cells [71]. Moreover, the anti-GITR therapy synergizes with anti-PD1 therapy in GBM-bearing mice, and the synergy is further amplified when combined with the SOC treatment [71]. The other approach is to block indoleamine 2,3-dioxygenase 1 (IDO1), given previous studies have shown that GBM cell IDO1 promotes Treg cell expansion [73]. Although IDO1 inhibitor BGB-5777 alone is not enough to inhibit GBM tumor growth, treatment with this inhibitor synergizes with anti-PD1 therapy in GBM mouse models [73].

**Pharmacological targeting of tumor–immune symbiosis to improve the effectiveness of immunotherapy**

Given the critical role of the tumor-immune symbiosis in regulating innate and adaptive immunity in GBM, blockade of this symbiosis may affect the effectiveness of immunotherapies. This concept is also supported by the emerging evidence showing that high infiltration of myeloid cells correlates with increased immunotherapy resistance in GBM patients [70,74,75]. This section summarizes recent findings highlighting pharmacological targeting of myeloid cells (e.g., GAMs and MDSCs) to improve immunotherapy efficiency in GBM (Table 1).

GAMs are a heterogeneous population of cells exhibiting a potent immunosuppressive function in GBM. A growing body of evidence demonstrates that blockade of GAM immunosuppressive function through different strategies may overcome immunotherapy resistance. First, distinct subsets of GAMs may play different roles in affecting ICI therapy efficiency [41,76,77]. Unbiased CyTOF and scRNA-seq studies in GBM tumors have revealed a unique population of CD73high macrophages that persist following anti-PD1 therapy. Depletion of CD73 in GBM-bearing mice exhibits a robust synergistic antitumor effect with anti-PD1 and anti-CTLA4 [74]. Although anti-CD73 antibody has been developed [78], further studies are needed to validate the antitumor effect of the combination therapy with anti-CD73 and ICIs in GBM. The second approach is to
suppress cytokine/ligand-receptor interactions during the GBM–GAM symbiosis. GBM cell-derived IL-6 is essential and necessary for PD-L1 expression in tumor-associated myeloid cells, including macrophages. Inhibition of IL-6 with neutralizing antibodies synergizes with anti-PD1 therapy in GL261 tumor-bearing mice [79]. However, the antitumor effect of anti-IL-6 antibodies is context dependent. For example, EC-derived IL-6 can induce macrophage immunosuppression in a genetic GBM mouse model, but anti-IL-6 therapy is insufficient to activate antitumor immune response and does not sensitize tumors to ICIs [41]. The failure of synergy between EC IL-6 inhibition and ICIs may relate to the dual effect of IL-6 in GBM. In addition to the protumor effect, IL-6 inhibits tumor growth by stimulating CD40 expression [41], suggesting a therapeutic potential of dual targeting IL-6 and CD40. Indeed, combination of anti-IL-6 therapy and CD40 stimulation induces a robust antitumor immunity and synergizes with ICIs (e.g., anti-PD1 and anti-CTLA4) in GBM mouse models [41]. In a similar way, blockade of the PROS1-AXL axis-mediated GAM–GSC symbiosis using AXL inhibitor BGB324 synergizes with anti-PD1 therapy in GBM-bearing mice [17]. The third approach is to disrupt the GBM–GAM crosstalk via blockade of the CD47-signal regulatory protein alpha (SIRPα) pathway. CD47 is a ‘don’t eat me’ signaling that helps GBM cells to evade GAM-mediated phagocytosis [80]. In vivo pharmacological studies have demonstrated that anti-CD47 blockades significantly extends survival of GBM-bearing mice by regulating GAM-mediated innate immune response [81,82], and that this antitumor effect is further amplified upon TMZ treatment [83]. In addition to regulating the innate immune response, anti-CD47 therapy also activates adaptive immunity. Combining anti-CD47 and TMZ treatment significantly sensitizes GBM tumor to anti-PD1 therapy [83]. The final appealing strategy is to reprogram GAMs from an immunosuppressive to an immunostimulatory phenotype. For example, inhibition of macrophage immunosuppressive polarization by combined rapamycin and hydroxychloroquine treatment not only reduces the expression of the CD47-SIRPα signaling axis in GBM cells and GAMs but also synergizes with anti-PD1 therapy in GBM-bearing mice [84]. Consistently, blockade of GAMs immunosuppressive polarization using additional several pharmacological approaches (e.g., MAGL-specific inhibitor JZL184, Robo1Fc, CSF-1R inhibitor AFS98) shows robust synergy with ICIs (e.g., anti-PD1, anti-CTLA4, or anti-4-1BB) in different GBM mouse models [30,43,75].

Similar to inhibition of the GBM–GAM crosstalk, targeting the GBM–MDSC symbiosis could also enhance the effectiveness of ICI therapies (Table 1). This conclusion is supported by the recent findings showing that treatment with CCR2 inhibitor CCX872 or IDO1 inhibitor BGB-5777 not only impairs MDSC infiltration but also shows robust synergy with anti-PD1 therapy in GBM-bearing mice [54,73]. In addition to ICI, immunostimulatory gene (e.g., TK/Flt3L) therapy is also affected by MDSCs. A very recent study demonstrates that due to the high infiltration of PMN-MDSCs in the TME, IDH1 WT gliomas do not respond to the TK/Flt3L therapy [53]. However, reprogramming immunosuppressive PMN-MDSCs into nonsuppressive granulocytes using recombinant G-CSF significantly enhances TK/Flt3L therapeutic efficacy in GBM-bearing mice [53].

Concluding remarks and future perspectives

With its vital role in regulating tumor progression and the effectiveness of immunotherapies, the tumor–immune symbiosis embodies critical therapeutic targets for GBM. This review has outlined recent pharmacological approaches to target the tumor–immune crosstalk (e.g., GBM–GAM, GBM–MDSC, and GBM–T cell crosstalk) in GBM, which not only directly inhibit tumor progression but also turn the TME from ‘cold’ to ‘hot’, thus improving the effectiveness of immunotherapies. Also, a range of pharmacological tools have been developed to target the GBM-immune symbiosis (Figures 1 and 2, and Table 1), demonstrating tremendous clinical translation potential.

Outstanding questions

How can we pharmacologically target the context-dependent GBM-immune symbiosis effectively?

How do we choose appropriate GBM mouse models for pharmacological testing drug candidates targeting the GBM-immune symbiosis? How can we translate promising preclinical studies into the clinic?

Is there a better way to embed advanced technologies (e.g., scRNA-seq, CyTOF, whole-exome sequencing, nanotechnology, CRISPR KO screening, high throughput screening, brain tumor organoids, tumor-on-a-chip system, and exosome delivery system) to identify novel GBM-immune symbiosis and develop drug candidates targeting the symbiosis?

Can we design personalized ICIs for GBM patients based on their specific GBM–T cell symbiosis? Can we develop pharmacological tools targeting the context-dependent GBM–myeloid cell crosstalk to overcome resistance of immunotherapies, including ICIs?

In addition to GAMs, MDSCs, and T cells, are there context-dependent symbiosis between GBM cells and other immune cells, such as NK cells, dendritic cells, and B cells? Can we design effective pharmacological approaches to target such GBM-immune symbiotic interactions?
Figure 3. Workflow of developing pharmacological tools for targeting the glioblastoma (GBM)-immune symbiosis. Spatial tissue characterization and disease-specific analyses are critical for establishing the immune landscape of GBM patient tumors with different tumor origins, genetic statuses, disease stages, and immunotherapeutic responses. The immune and genetic landscapes of specific GBM tumors can be determined via flow cytometry/fluorescence-activated cell sorting (FCM/FACS), single-cell RNA sequencing (scRNA-seq), whole-exome seq, T cell receptor (TCR)-seq, and mass cytometry (CyTOF). Integration of these techniques could help identify and determine the relationships between GBM cell genetic statuses and immune landscape, and their association with tumor progression and the effectiveness of immunotherapies. Unbiased profiling (e.g., scRNA-seq, RNA-seq, and microarray) and its associated pathway analysis followed by in vitro and in vivo functional studies are essential for validating which pathways and factors are crucial for the context-dependent GBM-immune crosstalk. Network pharmacological studies and molecular docking could help to identify novel therapeutic drug candidates, such as neutralizing antibodies (Abs) and small-molecule agonists/antagonists, for translational studies.
GBM has a unique immunosuppressive TME with infiltration of various types of immune cells (e.g., macrophages, microglia, MDSCs, neutrophils, Treg cells, and T cells), and each type of these immune cells exhibit phenotypic heterogeneity and multifaceted functions [7,8,62,85,86]. Despite the success of developing many pharmacological approaches in GBM mouse models (Table 1), many challenges remain regarding how to translate these preclinical findings into the clinic, and how to develop novel, effective and specific pharmacological tools targeting the GBM-immune symbiosis (see Outstanding questions). Apart from few examples, this concept has not been translated into the clinic for GBM treatment. One reason would be the choice of GBM mouse models, which may not completely recapitulate the immune landscape and genomic heterogeneity of GBM patients. Studies using humanized mice with genetically engineered GBM system via CRISPR/Cas9 could better evaluate the drug effect on blocking the GBM-immune symbiosis [23,87,88]. Additionally, organoids and tumor-on-a-chip systems may provide more comprehensive platforms for rapid drug screening [21,89]. The second reason would be the limitation of effective therapeutic targets. Further studies using both bottom-up and top-down strategies will help to develop new and effective therapeutic tools aiming to block the GBM–immune crosstalk (Figure 3). The classical treatment design starts with the investigations focusing on the cellular and molecular mechanisms underlying the GBM-immune symbiosis. The alternative research strategy may begin with drug screening by determining which pathways/genes are affected by drug candidates [38,90]. Machine-learning approach could help to zero in on pathways/genes that are essential for the GBM-immune symbiosis. The last challenge could be the major barriers (e.g., BBB, blood–cerebrospinal fluid barrier, and brain-resident lymphatic barrier) that can limit drug delivery into the GBM TME. To overcome this challenge, both invasive and noninvasive approaches have been developed to improve drug delivery into the brain by hijacking the cellular and molecular barriers of the BBB [5]. In addition, other approaches (e.g., therapeutic strategies with therapeutic vaccines, adoptive cell therapy, and oncolytic viruses) have also been tested for targeting the GBM TME, although they are not classical catalog of pharmacological drugs [15,91,92]. However, further studies are still needed to optimize appropriate pharmacological treatments combining the GBM-immune symbiosis-targeted therapy and the strategy of enhancing the BBB-penetrating ability. Although a growing body of preclinical data has largely accelerated drug discovery, clinical trials are still needed to validate the clinical benefit of these drug candidates targeting the GBM-immune symbiosis. We anticipate that success in translating current known pharmacological tools into the clinic, and developing novel therapeutic strategies targeting the GBM-immune symbiosis will ultimately improve GBM patient outcomes.

Acknowledgments
This work was supported by National Institutes of Health (NIH) R00 CA240896, Department of Defense (DoD) Career Development Award W81XWH-21-1-0380, Cancer Research Foundation Young Investigator Award, Lynn Sage Scholar Award, American Cancer Society Institutional Research Grant IRG-21-144-27, philanthropic donation from Mindy Jacobson and the Bill Bass Foundation, Northwestern University start-up funds, and the Robert H. Lurie Comprehensive Cancer Center (all to P.C.).

Declaration of interests
No potential conflicts of interest were disclosed by the authors.

References
14 Trends in Pharmacological Sciences, Month 2022, Vol. xx, No. xx


33. Yelin, E. et al. (2021) P-selectin axis plays a key role in microglia immunophenotype and glioblastoma progression. Nat. Commun. 12, 1912


48. Alban, T.J. et al. (2020) Glioblastoma myeloid-derived suppressor cell subsets express differential macrophage migration inhibitory factor receptor profiles that can be targeted to reduce immune suppression. Front. Immunol. 11, 1191


52. Hu, J. et al. (2021) Regulation of tumor immune suppression and cancer cell survival by CXCL1/12 elevation in glioblastoma multiforme. Sci. Adv. 7, eabc2511

53. Albrecht, M.S. et al. (2021) G-CSF secreted by mutant DH1 glioma stem cells abolishes myeloid cell immunosuppression and enhances the efficacy of immunotherapy. Sci. Adv. 7, eabh3243


57. Otros, B. et al. (2016) Cancer stem cell-secreted macrophage migration inhibitor factor stimulates myeloid derived suppressor
64. Arrieta, V.A. et al. (2021) Neoadjuvant PD-1 blockade induces T cell and cDC1 activation but fails to overcome the immunosuppressive tumor associated macromolecules in recurrent glioblastoma. Nat. Commun. 12, 6938.