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Pharmacological targeting of the tumor–immune symbiosis in glioblastoma

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Abstract

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)** (see Glossary) 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

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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 (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.

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 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].

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 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)-β1 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 locomotor 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 E_2 (PGE_2). Consequently, GSC-derived PGE_2 promotes GAM immunosuppressive polarization [30-32]. Suppressing PGE_2 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 [63], 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 $CD73^{high}$ 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 actives 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.

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 G BM-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.

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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 and microglia (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 monocytic 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-generation sequencing (NGS) strategy that provides the gene expression profiles at a single-cell level

Symbiotic interaction
a type of interaction between cells (e.g., GBM cells and myeloid cells) in which at least one cell type benefits

T cell exhaustion
a state of T cell dysfunction in cancer that is characterized by poor effector function and enhanced expression of inhibitory immune checkpoint molecules/receptors

T cell tolerance

a state of unresponsiveness of T cells toward specific self or non-self-antigens, which negatively affects an individual's immune system

Th1-like CD4 T cells
a subset of CD4 T cells that can secrete inflammation-related cytokines (e.g., IFNγ and TNFα) and exhibit an antitumor immune response

Treg cells
a specialized population of helper T cells that suppress antitumor immunity

Tumor microenvironment (TME)
stromal components (e.g., blood vessels, immune cells, fibroblasts, signaling molecules, and extracellular matrix) of a tumor that support tumor progression

Tumor-on-a-chip
a microfluidic device that can maintain the structural and functional units of tumor cells *in vitro*

Whole-exome sequencing
a technology that can sequence all protein-coding regions of the genome

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Highlights

Myeloid cells, such as glioma-associated macrophages and microglia (GAMs) and myeloid-derived suppressor cells (MDSCs), are highly infiltrated into the glioblastoma (GBM) tumor microenvironment (TME) and exhibit symbiotic interactions with GBM cells and other components (e.g., T cells) of the TME. Such symbiosis not only promotes tumor growth but also induces an immunosuppressive TME in GBM.

Pharmacological blockade of the tumor–immune symbiosis (e.g., the GBM-GAM, GBM–MDSC, and GBM-T cell symbiosis) inhibits tumor progression by modulating the biology of both GBM cells and immune cells.

Immunotherapies, such as immune checkpoint inhibitor (ICI) therapies, offer limited clinical benefits in GBM patients. Pharmacological targeting of the tumor-myeloid cell symbiosis increases the infiltration and activation of T cells and synergizes with ICI therapies in GBM.

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?

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 [6]. 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 [8]. 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 [8].

The infiltration of macrophages and microglia into the TME is triggered by multipleGBM cell-secreted chemokines, such as CSF-1, CSF-2, CCL2, OPN, LOX, and monocyte chemoattractant 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 [36], IL-1β [27,85], IL-10 [98], and TGF-β1 [99], to promote GBM progression via activation of the protumor signaling in GBM cells [6,16]. 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].

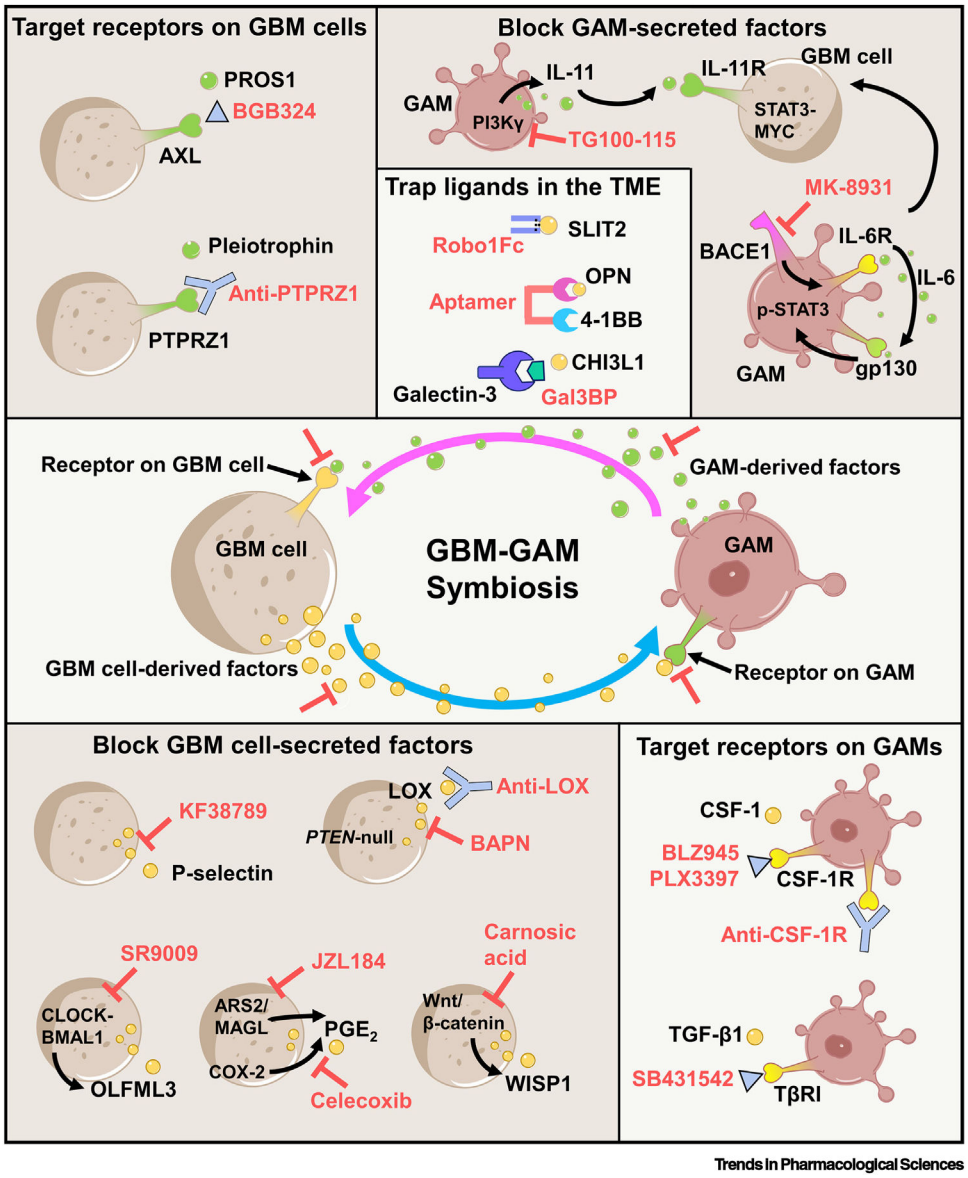

Figure 1. Pharmacological approaches to target the GBM–GAM crosstalk.

Depending on targeted cell types and molecular mechanisms underlying the GBM–GAM symbiosis, pharmacological strategies of targeting the symbiosis include: (i) targeting receptors on GBM cells; (ii) targeting receptors on GAMs; (iii) targeting GBM cell-secreted chemokines; (iv) targeting GAM-secreted factors; and (v) trapping extracellular signaling in the TME. The key targets and associated drug candidates are indicated. Abbreviations: 4-1BB, tumor necrosis factor receptor superfamily member 9; ARS2, arsenite-resistance protein 2; AXL, AXL receptor tyrosine kinase; BACE1, β-site amyloid precursor protein-cleaving enzyme 1; CHI3L1, chitinase-3-like 1; CLOCK, circadian locomotor output cycles kaput; COX-2, cyclooxygenase-2; GAMs, glioma-associated macrophages and microglia; CSF-1R, colony-stimulating factor-1 receptor; GBM, glioblastoma; Gal3BP, galectin 3-binding protein; LOX, lysyl oxidase; MAGL, monoacylglycerol lipase; OPN, osteopontin; PGE_2, prostaglandin E_2; PI3Kγ, phosphoinositide-3-kinase gamma; PROS1, Protein S;

PTPRZ1, tyrosine phosphatase receptor type Z1; SLIT2, slit guidance ligand 2; TME, tumor microenvironment; TβRI, transforming growth factor beta receptor I; WISP1, Wnt-induced signaling protein 1.

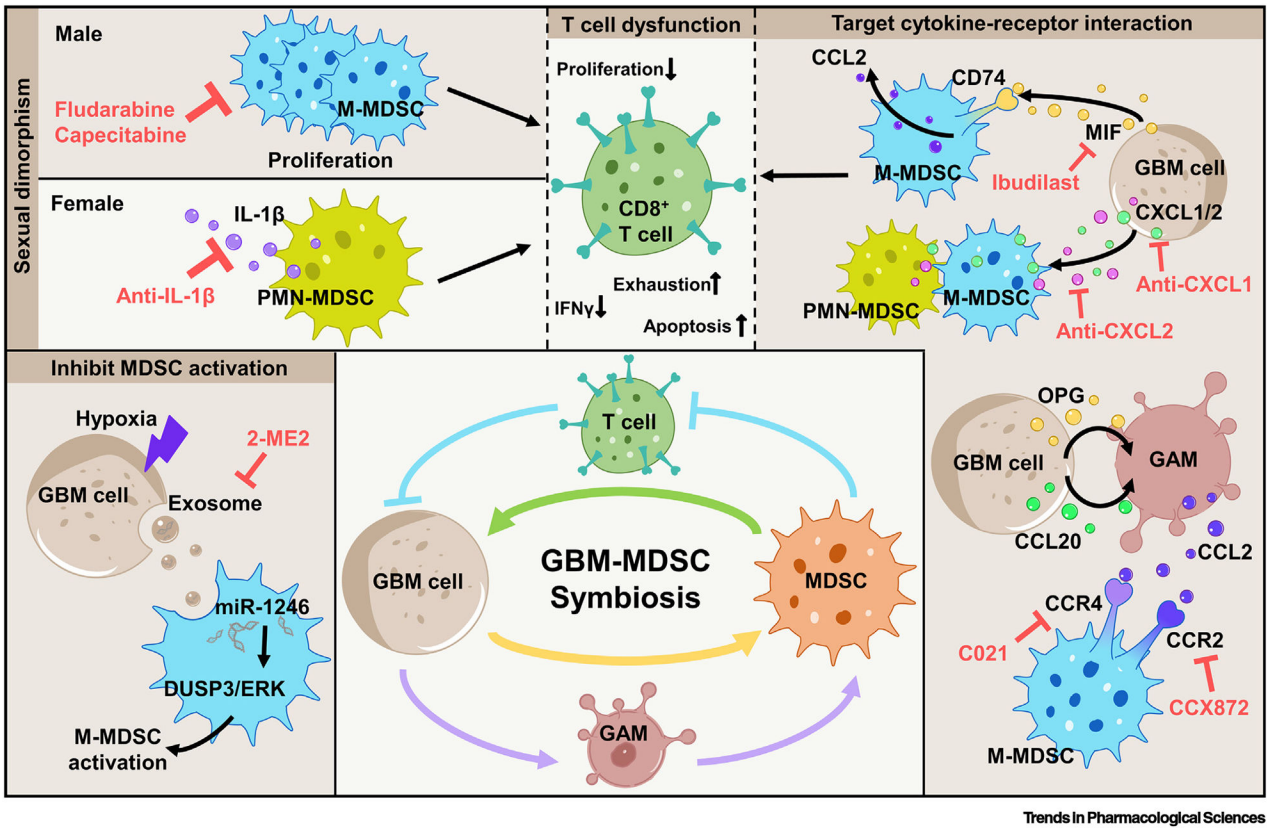

Figure 2. Pharmacological tools to target the GBM–MDSC symbiosisw.

GBM cell-derived ligands, exosomes, and cytokines recruit and activate MDSCs, which, in turn, inhibit T cell proliferation and function and promote GBM tumor growth. Pharmacological approaches targeting MDSC infiltration and activation during the GBM–MDSC symbiosis are proposed. The key targets and associated drug candidates are indicated. Sexual dimorphism of MDSCs also appears to be a target for GBM therapy: low dose of chemotherapy (fludarabine and capecitabine) inhibits M-MDSC proliferation in male GBM. By contrast, anti-IL-1β treatment inhibits PMN-MDSC function and enhances $CD8^+$ T cell-mediated antitumor immunity in female GBM. Abbreviations: 2-ME2, 2-Methoxyestradiol; CCL2, C-C motif chemokine ligand 2; CCR2/4, C-C motif chemokine receptor 2/4; CLXCL1/2, C-X-C motif ligand 1/2; DUSP3, dual specificity phosphatase 3; ERK, extracellular signal-regulated kinase, GAMs, glioma-associated macrophages and microglia; GBM, glioblastoma; IL-1β/R, interleukin-1β/receptor; INFγ, interferon gamma; MIF, macrophage migration inhibitory factor; M-MDSCs, monocytic myeloid-derived suppressor cells; OPG, osteoprotegerin, PMN-MDSC, polymorphonuclear myeloid-derived suppressor cells.

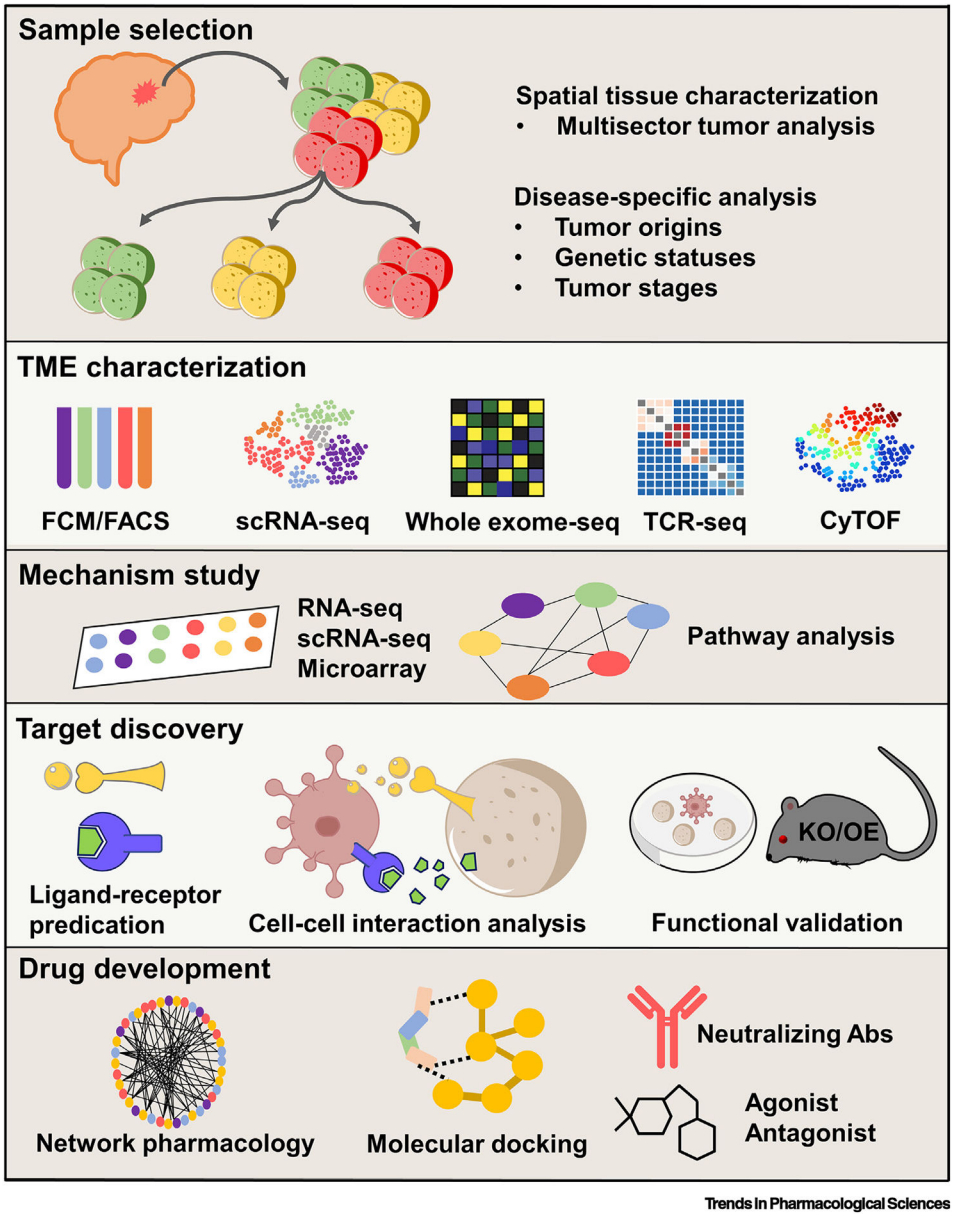

Figure 3. Workflow of developing pharmacological tools for targeting the glioblastoma (GBM)-immune symbiosis.

Spatial tissue characterization and disease-specific analyses are critical for establishing the immune landscape of GBM patient tumors with different tumor origins, genetic statues, 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 statues 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.

Table 1.

Recent *in vivo* studies using pharmacological strategies to block the tumor–immune symbiosis in GBMA[a]

Targeting GBM–GAM crosstalk					
Target	**Therapeutic agent**	**Tumor model**	**Therapeutic mechanism**	**Combined with other therapies**	**Refs**
CD47	Anti-CD47	GL261 and CT2A models, mouse	Escape of GBM cells from GAM-mediated phagocytosis	TMZ and anti-PD1	[83]
AXL	BGB324	GSC267 and GSC374, PDX; MS7080, mouse	Inhibition of GAM PROS1-induced GSC stemness	Anti-PD1	[17]
PTPRZ1	Anti-PTPRZ1 antibody	T0912 GSC, PDX	Inhibition of GAM pleiotrophin-induced GSC stemness	N/A	[18]
CSF-1R	BLZ945	TS573 and U251, human	Depletion of GAMs	N/A	[22]
	PLX3397	Patients with recurrent GBM	Depletion of GAMs	N/A	[24]
		GL261 model, mouse	Depletion of GAMs	N/A	[8]
	Anti-CSF-1R antibody	GSCs, mouse	Depletion of GAMs	N/A	[23]
	Anti-CSF-1R antibody	GL261, mouse	Depletion of GAMs	Anti-PD1 and anti-CTLA4	[75]
TβRI	SB431542	SETD2-mutated GBM, mouse	Inhibition of GBM cell TGF-β1-induced microglia activation	N/A	[27]
LOX	β-aminopropionitrile	U87, human; 005 GSCs and QPP7, mouse; and GSC23, PDX	Inhibition of GBM cell LOX-induced macrophage recruitment	N/A	[26]
CLOCK	SR9009	CT2A, mouse	Inhibition of GSC self-renewal and GSC-induced microglial infiltration	N/A	[29]
MAGL	JZL184	GL261, mouse	Blockade of prostaglandin E_2 production in GSCs, and its role in GAM immunosuppressive polarization	Anti-PD1	[30]
P-selectin	KF 38789	iAGR53 and GL261, mouse; PD-GB4, PDX	Inhibition of GBM cell P-selectin-induced GAM immunosuppressive polarization	N/A	[33]
Wnt/β-catenin-WISP1	Carnosic acid	T4121, PDX	Inhibition of GSC W1SP1-induced GAM survival	N/A	[34]
PI3Kγ	TG100-115	GL261, mouse	Inhibition of GAM IL-11-induced GBM stemness and tumorigenicity	TMZ	[36]
BACE1	MK-8931	GSCs, PDX	Inhibition of IL-6R cleavage in GAMs, suppressing GAM-mediated immunosuppression	N/A	[38]
IL-6	Anti-IL-6	GL261, mouse	Inhibition of GBM cell IL-6-induced GAM immunosuppressive polarization	Anti-PD1 and anti-CTLA4; CD40 agonist	[41]
		GL261, mouse	Inhibition of GBM cell IL-6-induced PD-L1 expression in myeloid cells	Anti-PD1	[79]
SLIT2	SLIT2 ligand trap protein	CT2A, mouse	Inhibition of GBM cell SLIT2-induced GAM chemotaxis and immunosuppressive polarization	Anti-PD1 and anti-4-1BB	[43]
OPN	4-1BB-OPN aptamer	GL261, mouse	Inhibition of GBM cell OPN-induced GAM migration and immunosuppressive maintenance	N/A	[45]

Targeting GBM–GAM crosstalk

Target	Therapeutic agent	Tumor model	Therapeutic mechanism	Combined with other therapies	Refs
CHI3L1	Gal3BP mimetic peptide	GL261, mouse	Inhibition of GBM cell CHI3L1-induced GAM migration and immunosuppressive polarization	N/A	[46]

Targeting GBM–MDSC crosstalk

Target	Therapeutic agent	Tumor model	Therapeutic mechanism	Combined with other therapies	Refs
CCR2	CCX872	KR158 and 005 GSC, mouse	Inhibition of GBM cell CCL2-induced M-MDSC migration	Anti-PD1	[54]
CCR4	C021	GL261, mouse	Inhibition of GBM cell CCL2-induced M-MDSC migration	N/A	[51]
MIF	Ibudilast	GL261, mouse	Inhibition of GBM cell MIF-induced M-MDSC activation	N/A	[48]
Exosomal miR-1246/ HIF-1α	2-Methoxyestradiol	U87, human	Inhibition of GBM cell exosomal miR-1246-induced M-MDSC differentiation and activation	N/A	[59]
IL-1β	Rilonacept; anti-IL1β	GL261 and SB28, mouse	Inhibition of systemic PMN-MDSCs	N/A	[50]
G-CSF	Recombinant G-CSF	*IDH1* mutated and WT glioma models, mouse	Inhibition of GSC G-CSF-induced PMN-MDSC expansion	TK/Flt3L/immune stimulatory gene therapy	[53]

Targeting GBM–T cell crosstalk

Target	Therapeutic agent	Tumor model	Therapeutic mechanism	Combined with other therapies	Refs
PD-1	Neoadjuvant pembrolizumab	Patients with recurrent GBM	Promotion of $CD8^+$ T cell infiltration, systemic activation, and clonal selection	N/A	[67]
	Neoadjuvant nivolumab	Patients with resectable GBM	Promotion of chemokine expression, immune cell infiltration, and augmented TCR clonal diversity	N/A	[68]
IL-7R	NT-I7	GL261, mouse	Promotion of T cell migration	N/A	[93]
GITR	Anti-GITR	GL261, CT2A and 005 GSC, mouse	Promotion of Treg cell differentiation into CD4 effector T cells	Anti-PD1	[71]
IDO1	BGB-5777	GL261 and CT2A, mouse	Restoration of effector T cells antitumor activity	Anti-PD1 and whole-brain radiotherapy	[73]
STAT3	WP1066	GL261, mouse	Restoration of effector T cells antitumor activity	Whole-brain radiotherapy	[94]

[a]Abbreviations: 4-1BB, tumor necrosis factor receptor superfamily member 9; CCR2, C-C motif chemokine receptor 2; CHI3L1, 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.