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Glioma associated microglia/macrophages, a potential pharmacological target to promote antitumor inflammatory immune response in the treatment of glioblastoma

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ABSTRACT

Glioma associated microglia/macrophages (GAMs) constitute the largest proportion of glioma infiltrating cells, particularly in high grade tumors (i.e., glioblastoma). Once inside the tumors, GAMs usually acquire a specific phenotype of activation that favors tumor growth, angiogenesis and promotes the invasion of normal brain parenchyma. Therefore, treatments that limit or prevent GAMs' recruitment at the tumor site or modulate their immune activation promoting antitumor activities are expected to exert beneficial effects in glioblastoma. In the present paper, we aim at the revision of pharmacological strategies that interfere with GAMs' function and are currently proposed as an alternative/additional option to current approved cytotoxic regimens.

Keywords: Glioblastoma, macrophages, microglia, metalloproteases, pro-inflammatory activation, pro-tumor functions, glioma associated microglia/macrophages targeted therapies, pharmacotherapy

INTRODUCTION

Glioma associated microglia/macrophages (GAMs) constitute the largest proportion of tumor infiltrating cells. They are less abundant in low grade gliomas, but constitute up to 30% of the entire tumor mass in

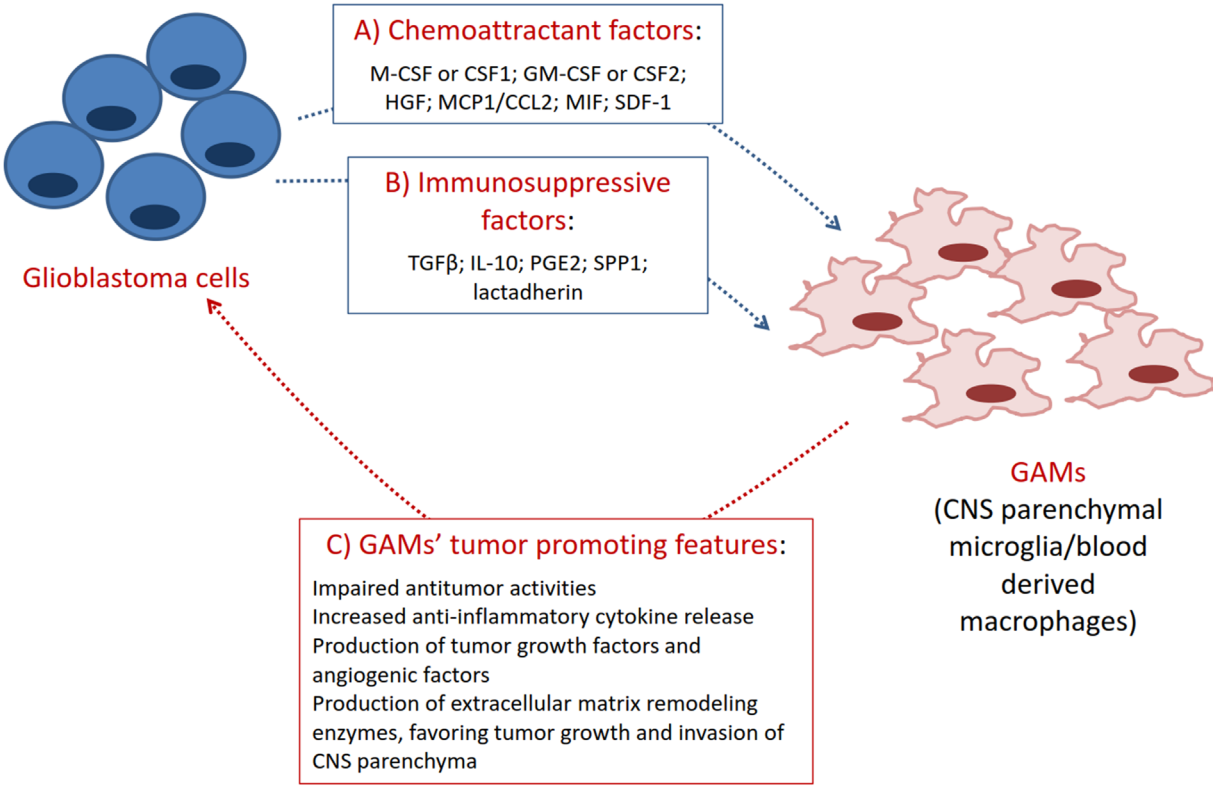

Figure 1. Cross-talk between glioblastoma cells and GAMs. A: Glioblastoma cells produce several chemoattractant factors which promote the recruitment of microglia and macrophages at the tumor site, GAMs; B: once inside the tumor, GAMs are exposed to immunosuppressive/antinflammatory factors and are reprogrammed towards phenotypes that sustain tumor growth, progression and invasion; C: the most relevant tumor promoting features of GAMs are presented. M-CSF: macrophage colony stimulating factor; CSF1: macrophage colony stimulating factor; GM-CSF: granulocyte/macrophage colony stimulating factor; CSF2: granulocyte/macrophage colony stimulating factor; HGF: hepatocyte growth factor; MCP-1/CCL2: monocyte chemotactic protein 1/chemokine (C-C motif) ligand 2; MIF: macrophage inhibitory factor; SDF-1: stromal-derived factor-1; TGF β : transforming growth factor β; IL: interleukin; PGE2: prostaglandin E2; SPP1: osteopontin; GAMs: glioma associated microglia/macrophages; CNS: central nervous system

glioblastoma (IV grade glioma)[1]. On the basis of largescale genomic analyses, glioblastoma can be classified into at least four distinct molecular subtypes[2], among which the mesenchymal subtype tends to have the most relevant immune component[3]. Microglial cells, scattered in normal brain parenchyma, are recruited at the tumor site by glioblastoma-secreted chemoattractant factors[4,5] [Figure 1], while peripheral blood-derived macrophages, normally found in the perivascular space, meninx and choroid plexus, accumulate in glioblastoma trough breakdown of the blood-brain-barrier (BBB)[1] particularly in high grade glioma[6]. Iba1$^+$ cells were consistently detected in a group of 41 glioblastoma specimens, showing preferentially an amoeboid phenotype toward the tumor center and a ramified morphology in the periphery of the tumors[7]. In addition, markers suggesting both pro- and anti-tumoral properties of GAMs were detected. A significant proportion of cells expressing the cluster of differentiation (CD) 163 and the inducible nitric oxide synthase (iNOS) was found in the tumor parenchyma together with a wider distribution of arginase 1 positive cells[7]. GAMs are frequently detected in the perivascular niche of tumor blood vessels, and their number increases with tumor progression[8]. As shown in Figure 1, invading microglia/macrophages play a critical role in the regulation of glioma biology, including tumor growth, progression and invasion[8]. Consistently, depletion of microglia/macrophages *in vivo* experimental models significantly reduced tumor growth[8-12], holding the potential to ameliorate the outcome of current available therapies.

In this regard, standard treatment for glioblastoma includes maximal surgical resection (whenever feasible), followed by radiotherapy and concurrent treatment with temozolomide plus additional 6 cycles of adjuvant temozolomide[13]. Despite such multimodal approach, the average survival of patients diagnosed with glioblastoma remains low (14-16 months), with better outcomes observed when tumors display O^6-methylguanine

DNA-methyltransferase promoter methylation[13]. In fact, most glioblastoma tumors tend to recur after being surgically removed. This is partly due to the highly infiltrative nature of these cancer cells, so that radical surgery is difficult to achieve. On the other hand, tumors can regenerate from glioblastoma cancer stem cells (GSCs) that are usually resistant to radio- and chemotherapy. Treatment guidelines for recurrent disease are less defined and may include a second surgery, re-irradiation, or re-exposure to temozolomide at standard dose. Other options comprise systemic chemotherapy with one nitrosourea drug, i.e., carmustine, lomustine, or fotemustine, and in the United States, the monoclonal antibody against vascular endothelial growth factor-A (VEGF-A) bevacizumab[13]. Finally, several targeted therapies have been tested in clinical trials with limited beneficial effects. Interestingly, we observed using primary cultures of rat microglial cells that temozolomide did not reduce microglial cell viability after 24 h treatments in the μmol/L (clinically relevant) dose range, albeit it significantly increased intracellular protein content[14]. Notably, resistance to anti-angiogenic therapy, i.e., bevacizumab, appears to be mediated by changes in the glioblastoma's microenvironment, including the extent of myeloid cell infiltration as well as their biological activities[15-17]. In preclinical models of glioblastoma, it has been shown that ionizing radiations increase the recruitment of myeloid cells with a pro-tumorigenic phenotype at the tumor site, contributing to disease recurrence[18]. Taken together, the evidence suggest a possible involvement of GAMs in the response to standard treatments. Therefore, glioma associated myeloid cells can be envisioned as an alternative or an ancillary pharmacological target to improve the clinical outcome of current available therapies. Noteworthy, the glioblastoma microenvironment includes also other immune cells, namely regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), that concur to the establishment of an immunosuppressive environment, impairing the effector function of infiltrating T cells and natural killer cells and facilitating tumor growth[19]. Therefore, a comprehensive understanding of these different components of the patients' immune system endowed in the tumor microenvironment is necessary to develop therapeutic strategies that increase anti-tumor immunity and clinical benefits. In the present paper, we aim at the revision of pharmacological strategies that interfere with GAMs' function, i.e., cell recruitment at the tumor site, cell inflammatory activation and immune function, and the extracellular matrix remodeling promoted by GAM-secreted factors. For recent advances on the biology of MDSCs and Tregs in the glioblastoma microenvironment and their potential role as therapeutic targets, we refer the readers to other review articles[20,21].

DRUGS TARGETING GAMS' FUNCTION FOR THE TREATMENT OF GLIOBLASTOMA

Drugs that interfere with GAMs' recruitment at the tumor site

Microglial cells are recruited at the tumor site by several chemoattractant factors which are produced and released by tumoral cells[4,5]. One of the first identified GAMs' chemoattractant factor is the hepatocyte growth factor[22], which binds to and activates the tyrosine kinase receptor, c-Met. The latter plays a role both on microglial motility and cell proliferation[22]. Other glioma-released chemoattractant factors are the myeloid colony stimulating factors (CSFs), i.e., the macrophage colony stimulating factor (M-CSF or CSF1)[23], the granulocyte/macrophage colony stimulating factor (GM-CSF or CSF2)[24]. These factors signal through activation of two different receptors[25]. The M-CSF receptor (CSF1R) is a homodimeric type III receptor, encoded by the *FMS* proto-oncogene, with intrinsic tyrosine kinase activity, whereas the GM-CSF receptor (CSF2R) is a heterodimer composed of a specific ligand-binding subunit (the α-chain) and a common β-chain. The latter is the signal transduction subunit and is shared with the receptors for interleukin (IL)-3 and IL-5. Activation of the CSF2R is known to stimulate at least three pathways: the Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway, the mitogen-activated protein kinase (MAPK) pathway and the phosphoinositide 3-kinase pathway[25]. In addition, the monocyte chemotactic proteins (MCPs), particularly MCP-1/chemokine (C-C motif) ligand 2 (CCL2)[26,27], and the stromal-derived factor-1 (SDF-1)[28], have shown to play a role in the recruitment of microglial cells to the tumor site [Figure 1]. In addition a substantial number of peripherally derived macrophages can be consistently detected in glioma GL261 implanted tumors, since the early phases of disease[29]. Once inside the tumors, GAMs usually acquire a specific phenotype of activation[30] that favors tumor growth, angiogenesis and promotes the invasion of normal brain pa-

renchyma[5]. Therefore, pharmacological treatments that prevent or reduce GAMs' recruitment at the tumor site are expected to exert beneficial effects in glioblastoma. In this regard, it has been shown that the immunosuppressant agent cyclosporine A (CsA), a drug normally used in clinical practice, significantly reduces the number of infiltrating microglia/macrophages in implanted glioma tumors. This effect, together with a modulation of GAM's inflammatory activation, results in a significant reduction of tumor growth[9]. However, chronic immunosuppression associated with systemic use of CsA increases the risk of developing tumors, and may probably limit the use of this drug for the treatment of glioblastoma. In addition, several tumor favoring mechanisms are associated with CsA, including increased production of transforming growth factor β (TGFβ) and VEGF together with an inhibitory action on the DNA repairing ability of the cells[31]. In the human U-87 glioma cell line, CsA significantly reduced the expression level of the human microRNA (miRNA, miR-)195, together with the modulation of several other miRNAs[32]. Interestingly, miR-195 seems to play a tumor suppressor function in both glioma cell lines and human gliomas[33,34]. On the other hand, the nuclear factor of activated T cells (NFAT1), i.e., the main intracellular target of CsA, appears to be a crucial regulator of glioma invasion-related genes. Thus, a direct inhibition of NFAT1 activity in glioma cells can limit their ability to infiltrate normal brain parenchyma, and may be considered as a potential adjuvant therapy for glioblastoma[35].

Interestingly, novel compounds, interfering with known chemoattractant factors, are in different stages of development for the treatment of glioblastoma. For example, it has been shown that the CSF-1R inhibitor BLZ945, which blocks the signaling pathways activated by M-CSF, significantly increased survival in different preclinical models of glioblastoma[36]. This pharmacological treatment induced the regression of established tumors in engineered mice and abated tumor growth in human xenografts. The drug is a small molecular weight CSF-1R inhibitor, with optimal BBB penetration properties. However, despite the chemoattractant properties of M-CSF and its established role in promoting macrophage survival, BLZ945 did not reduce the number of tumor infiltrating microglia/macrophages in these models. GAMs appeared indeed protected from BLZ-induced cell death by glioma-secreted cytokines such as GM-CSF, interferon γ (IFNγ) and the C-X-C motif chemokine 10 (CXCL10)[36]. On the other hand, BLZ945 modulated the inflammatory activation of GAMs, favoring their antitumor activities which explains the beneficial effects observed with the treatment (see next section). In a different preclinical model of glioblastoma, consisting of tumors derived by implantation of GSCs lacking tumor suppressor phosphatase and tensin homolog, p53 and neurofibromin 1 (NF1), BLZ945 efficiently blocked GAMs' recruitment at tumor site together with reducing tumor growth[37]. Interestingly, a first-in-human study employing BLZ945 (NCT02829723) is currently ongoing. It is a phase I/II with BLZ945 given as a single agent or in combination with PDR001 [a novel monoclonal antibody against the immune checkpoint programmed death-1 (PD-1) receptor, by Novartis Oncology], which aims at the characterization of the safety, tolerability, pharmacokinetics, pharmacodynamics, and antitumor activity of BLZ945 in adult patients with advanced solid tumors. Moreover, another selective CSF-1R inhibitor (PLX3397) has been recently tested in a phase II clinical trial in patients affected by recurrent glioblastoma (NCT01349036). The drug was well tolerated, showed good BBB penetration, and reduced the amount of $Iba1^+$ cells within the tumors. However, no significant improvement in the progression free survival compared with historical controls was observed in PLX3397 treated patients[38]. Moreover, it has been recently shown that genetic reduction of MCP-1/CCL2 significantly reduces macrophage infiltration within the tumors extending the survival time of tumor bearing animals[39]. However, previous attempts to block MCP-1/CCL2 with monoclonal antibodies demonstrated modest clinical efficacy. The drugs were instead effective in combination with temozolomide, significantly increasing mice survival[27]. Interestingly, it has been shown that the production of MCP-1 by glioma cells can be efficiently reduced by non-cytotoxic drugs, including the antibiotic minocycline, the angiotensin II receptor inhibitor telmisartan and the bisphosphonate zolendronic acid[40]. These drugs have a good BBB penetration and will be tested in combination as an ancillary therapy to improve the outcome of currently approved cytotoxic regimens.

Recently, a small molecular weight inhibitor of the AXL receptor tyrosine kinase has been shown to exert

relevant antiproliferative effects on different preclinical models of glioblastoma. The drug, namely BGB324 (also known as R428) significantly increased neurological free survival particularly in the group of mice bearing high-AXL expressing tumors[37]. In addition, BGB324 treatment reduced the amount of infiltrating $CD45^+$ leukocytes and $CD11b^+$ GAMs. Interestingly, the anti PD-1 inhibitor nivolumab increased the protective effects of BG324, and effectively prolonged the survival of tumor bearing mice[37]. Nivolumab *per se* displayed no survival benefits in these animals, while increasing both AXL kinase activity and GAMs' tumor infiltration. In line with these observations, a phase III clinical trial (NCT02017717) set to compare the efficacy and safety of nivolumab administered alone versus bevacizumab in patients diagnosed with recurrent glioblastoma failed to demonstrate its efficacy[19]. Immune PD-1 check point inhibitors, including nivolumab, have proven efficacy in various malignancies and the number of clinical approved indications is constantly increasing[41]. The use of these drugs is associated with specific toxicities, often termed immune-related adverse events. The most common side effects involve the skin, colon, endocrine organs and liver. Rarely, neurological complications have been described[41], including recent case reports on nivolumab-induced autoimmune encephalitis[42] and progressive multifocal leukoencephalopathy[43].

Finally, microglial/macrophages' infiltration of GSC-derived tumors was efficiently blocked by the integrin inhibitor arginine-glycine-aspartic acid (RGD) peptides albeit interfering with GSC-secreted periostin[44]. Consistently, genetic ablation of periostin reduced GAMs' recruitment at tumor site and modulated their immune functions, thus inhibiting tumor growth and increasing survival of glioma bearing animals. Similar beneficial effects were expected by pharmacological inhibition of integrin signaling pathways in human glioblastoma. However, despite promising phase I/II results, a recent phase III clinical trial failed to demonstrate clinical efficacy of cilengitide, a cyclic RGD pentapeptide that selectively inhibits the avβ3 and avβ5 integrins when added to standard temozolomide treatment in glioblastoma patients[45]. A possible explanation for these negative findings can be retrieved in part in the unfavorable pharmacokinetic profile of cilengitide[46]. In fact, the relevance of the signaling pathways downstream the integrin receptors, αvβ3 and αvβ5, is further supported by a recent proteomic analysis of the glioma secretome. These data suggest the involvement of osteopontin (SPP1) and lactadherin in the reprogramming of GAMs' immune responses towards pro-tumoral functions via integrin signaling[47].

Drugs that interfere with GAMs' inflammatory activation and immune function

Under the influence of glioma cells, the antitumor functions of GAMs appear mostly suppressed. As shown in Figure 1, tumor cells indeed produce several immunosuppressive molecules, such as TGFβ, IL-10, and various prostaglandins (i.e., prostaglandin E2, PGE2), thus favoring the acquisition of a pro-tumorigenic phenotype of activation by GAMs[30,48,49]. Pharmacological strategies that promote antitumor activities of GAMs, i.e., production of cytotoxic molecules and increased phagocytosis, or that reduce the release of pro-tumorigenic (i.e., growth factors) may exert beneficial effects in glioblastoma. In this regard, amphotericin B (AmpB), an antifungal compound clinically used to treat life-threatening fungal infections[50], has been shown to promote macrophage activation via toll like receptor activation and increase pro-inflammatory cytokine release[51]. In view of these properties, AmpB was recently tested in preclinical models of gliomas. In an experimental model consisting of human-derived GSC tumors implanted in nonobese diabetic/severe combined immunodeficiency (NOD-SCID) mice, systemic administration of AmpB significantly reduced tumor growth and increased animal survival[52]. The drug did not exert direct anti-tumor activity on GSCs *in vitro* and its pharmacological benefits *in vivo* were abated by depletion of myeloid cells. This suggests that the beneficial effects of AmpB were mediated by modulation of GAMs' functions. Increased tumor infiltration of $Iba1^+$ microglial cells and macrophages was detected in AmpB treated animals. This effects has been recently confirmed using ultrasmall iron oxide nanoparticles as contrast agents for magnetic resonance imaging, in order to detect monocyte infiltration into brain tumors[53]. In addition, tumor infiltrating $Iba1^+$ cells in response to AmpB showed a significant up-regulation of iNOS, that most likely results in increased production of cytotoxic nitric oxide (NO)[52] Beneficial effects of AmpB were also observed in immunocompe-

tent C57BL/6 mice, against highly aggressive tumors derived from enriched stem-like $CD133^+$ GL261 glioma cells. Notably, the antitumor effects of AmpB *in vivo* are achieved with lower doses than those maximally tolerated in humans.

Another promising class of therapeutics for the treatment of glioblastoma are the inhibitors of the mechanistic target of rapamycin (mTOR) kinase and/or other related kinases. The mTOR kinase is a central regulator of several intracellular processes related to cellular growth, metabolism, and proliferation[54]. Robust evidence have highlighted the crucial role of this pathway in glioblastoma biology, together with the demonstration of significant antiproliferative effects obtained by its pharmacological inhibition[55]. Several drugs targeting this activity are currently in clinical development for the treatment of different types of cancer[56], including those with an optimal pharmacokinetic profile for the treatment of glioblastoma[56,57]. Notably, we have shown that inhibition of mTOR activity in rat microglial cells can promote their antitumor properties while restricting pro-tumorigenic features[58]. Therefore, mTOR inhibitors have the potential to target both glioblastoma and GAMs' functions. Similarly, the chemokine receptor C-C chemokine receptor type 5 (CCR5) inhibitor maraviroc, in the same *in vitro* model, showed both direct antiproliferative activities on rat glioma C6 cells together with immune modulatory actions on glioma stimulated rat microglial cultures[59].

Interestingly, both glioma and infiltrating GAMs express the Ca^{2+}-activated K^+ channels (KCa3.1), whose inhibition using 1-(2-chlorophenyl) diphenylmethyl-1H-pyrazole (TRAM-34) induced a switch of GAMs toward a pro-inflammatory, antitumor phenotype[60]. In addition, *in vivo* treatments with TRAM-34 significantly decreased the extent of tumor growth in glioma-bearing mice[60]. Moreover, stimulation of microglia with pro-inflammatory IL-12 is associated with increased phagocytic activity[61]. Consistently, intracranial injection of a recombinant adeno-associated viral vector (rAAV2) encoding for IL-12 augmented the brain levels of IL-12 and IFNγ in tumor-bearing animals, favoring microglial infiltration into the tumor and restoring their antitumor functions. Increased immune activation of GAMs significantly reduced tumor growth and prolonged animal survival time[62]. Similarly, systemic administration of miR-142-6p, whose expression level is consistently downregulated in GAMs, extended animal survival in different glioma models. These beneficial effects relied on reduced GAMs' infiltration at the tumor site and increased antitumor activities[63]. Inhibition of the C-X-C chemokine receptor type 4 (CXCR4) by a newly synthetized receptor antagonist, peptide R, reduced tumor growth, glioma cell invasiveness, and intratumor vessel formation while directing GAMs' immune activation toward a pro-inflammatory/antitumor phenotype[64]. Notably, SDF-1 suppression in a murine glioma resulted in delayed tumor growth and invasiveness, lower microvascular density, and higher density of microglia/macrophages in non-hypoxic compared to hypoxic regions. These findings suggest that tumor-secreted SDF-1 stimulates glioma invasiveness and recruitment of GAMs towards hypoxic areas[65]. In addition, it has been recently shown that the antitumor activity of vosaroxin, a first in class cytotoxic agent that intercalates DNA and inhibits topoisomerase II, are also linked to increased recruitment of myeloid cells at the tumor site together with an augmented pro-inflammatory activation[66]. Likewise, the antitumor effects of chlorogenic acid (5-caffeoylquinic acid) (CHA) found in pre-clinical models of glioblastoma were associated with increased antitumor immune activations of GAMs. CHA is phenolic compound found in the human diet, in coffee, apples, pears and in green tea[67]. Finally, a recent paper describes the beneficial effects of a single chain antibody (X7Ab) directed against the chemokine receptor ACKR3/CXCR7. Reduction of tumor growth and improved survival were observed *in vivo* in different preclinical models of glioblastoma, particularly when X7Ab was used in combination with standard doses of temozolomide. Interestingly, increased mean fluorescence intensity of classical activated (major histocompatibility complex class II, $MHCII^+$) tumor infiltrating macrophages was detected, suggesting augmented pro-inflammatory (i.e., antitumor) activation of these cells within the tumor microenvironment[68].

Drugs that interfere with matrix remodeling promoted by GAM-secreted factors

Besides their immune functions which may either restrict or favor astrocyte malignant transformation,

GAMs are directly involved in the degradation of the extracellular matrix. Thus, these cells are key regulators of a central process involved in the expansion of tumors as well as in the invasion of normal brain parenchyma[69]. In fact, microglial cells significantly increase the invasive phenotype of GL261 glioma cells *in vivo*[70]. Consistently, the invasiveness of glioma cells is diminished in microglial-depleted organotypic brain slices inoculated with GL261 glioma cells[71]. Matrix metalloproteases (MMPs), i.e., the enzymes involved in the remodeling of the extracellular environment[72], are largely produced by tumor cells, infiltrating microglia/macrophages, or other infiltrating leukocytes, particularly at the invasive tumor edge facilitating tumor growth and invasion[71,73,74]. As detailed in our recent review[4], a complex crosstalk exists between glioma cells and infiltrating GAMs which increases the activity of MMP enzymes, including MMP-2 and MMP-9. Notably, the latter is over-expressed in GAM cells sorted from human glioblastoma tissues[75].

Consistently, several pharmacological treatments displayed beneficial effects in glioblastoma by limiting the release of MMPs. For example, minocycline, a highly lipophilic tetracycline antibiotic with a good BBB penetration property, reduced the expression of MT1-MPP in invading microglia/macrophages by suppressing p38 MAPK activation[76]. The drug also reduced secretion of MMP-9[75] and other pro-inflammatory cytokines from microglia and tumor cells resulting in an overall decrease of glioblastoma cell migration[76]. Notably, minocycline is also able to reduce MCP-1 secretion by glioblastoma cells, thus potentially limiting GAMs' recruitment at tumor site (as discussed above). The same inhibitory effects on MT1-MMP were displayed *in vitro* by the lipid lowering agent, atorvastatin[77]. In addition, propentofylline, an atypical methylxanthine with central nervous system (CNS) glial modulating and antinflammatory actions, significantly reduced tumor growth by targeting microglial production of MMP-9. The drug restricted also the migratory capacity of both glioma CNS-1 cells and microglia *in vitro*[78]. Invasion and infiltration of the normal brain parenchyma interfere with radical surgical resections of glioblastoma, that often recur after the first aggressive treatment. Pharmacological reduction of glioma cell motility and invasiveness thus hold the potential to improve the outcome of current therapeutic approaches, by limiting the infiltration extent of normal brain parenchyma[69].

Other features of GAMs

In vitro, microglia co-cultured in the presence of glioma cells appear to be morphologically activated although phagocytosis is largely impaired[10]. Nevertheless, another promising therapeutic approach for the treatment of glioblastoma consists in the use of nanoparticles which are internalized by GAMs increasing their antitumor immune activation[79,80]. Moreover, GAMs produce a vast array of growth and angiogenic factors which further sustain proliferation of tumor cells[8,48,52] as well as tumor vessel formation[81]. Interestingly, genetic and pharmacological ablation in GAMs of neuropilin 1, a co-receptor that amplifies signaling through the VEGF-A and TGFβ pathways, is associated with reduced glioma growth and blood vessel formation and increased survival time of glioma bearing mice[82].

CONCLUSION

GAMs represent the most relevant population of tumor infiltrating cells that significantly contribute to the pathogenesis of glioblastoma by favoring tumor growth and invasion of the normal brain parenchyma. Preclinical evidence supports the notion that GAMs are a viable pharmacological target whose function can be modulated in order to prevent their pathological activation. Current available data, summarized in Table 1, suggest that the immune activation of GAMs can be genetically or pharmacologically modulated so that these cells can be efficiently instructed to perform anti-tumor activities. In addition, it is possible to control their recruitment at the tumor site, and the production of extracellular matrix remodeling enzymes, thus limiting tumor growth and the ability to infiltrate normal brain parenchyma. One of the main limitations to systemic chemotherapy for glioblastoma is represented by the inability of most drugs to effectively penetrate the BBB and achieve cytotoxic concentrations in the cerebrospinal fluid and brain parenchyma. In fact, sev-

Table 1. Drugs targeting GAMs' functions within the glioblastoma microenvironment

Drug name and approval status	Drug properties	Molecular target	Pharmacological actions on GAMs	Other effects	Clinical outcome	Ref.
Preclinical evidence						
Amphotericin B Approved for clinical use by FDA and in EU member states	Small MW compound	Toll-like receptors	↑ GAM's tumor infiltration ↑ GAMs' antitumor immune activation ↑ iNOS expression and NO production	No direct antiproliferative effects on GSCs *in vitro*	↓ Tumor growth ↑ Survival	[52, 53]
Cyclosporine A Approved for clinical use by FDA and in EU member states	Small MW compound	Calcineurin/ NFAT1	↓ GAMs' tumor infiltration ↓ IL10, ARG1 and GM-CSF ↓ MMP2	↑ TGFβ and VEGF ↓ DNA repair ↓ miR195 and other miRNAs	↓ Tumor growth Potential tumor promoting activities	[9,31,32]
Minocycline Approved for clinical use by FDA and in EU member states	Small MW compound	p38-MAPK	↓ MT1-MPP, ↓ MMP-9 production by GAMs ↓ Tumor cells' migration	↓ Pro-inflammary cytokines by microglia ↓ MCP-1 by glioma cells	↓ Tumor growth ↑ Survival	[40,75,76]
Nivolumab Approved for clinical use by FDA and EMA	Biologic (mAb)	PD-1	↑ GAMs' tumor infiltration ↑ AXL kinase activity	↑ Protective effects of BG324	No survival benefits *per se*	[37]
mTOR kinase inhibitors Approved/ investigational drugs	Small MW compound	mTOR kinase	↑ Pro-inflammatory activation of microglia *in vitro*	Direct antiproliferative effects	↓ Tumor growth	[55,57,58]
BGB324 (R428) Investigational	Small MW compound	Receptor tyrosine kinase AXL	↓ CD11b+ GAMs' tumor infiltration ↓ CD45+ leukocyte tumor infiltration		↑ Survival	[37]
BLZ945 Investigational	Small MW compound	CSF-1R	↑ survival of GAMs ↑ GAMs' phagocytic activity ↓ GAMs' protumor immune activation	↑/↓ GAMs' tumor infiltration	↓ Tumor growth ↑ Survival	[36,37]
CHA Investigational	Small MW compound	STAT factors	↑ GAMs' antitumor immune activation		↓ Tumor growth	[67]
Propentofylline Investigational	Small MW compound	Phosphordiesterase	↓ MMP-9 by GAMs	↓ Migratory capacity of microglia and glioma	↓ Tumor growth	[78]
TRAM-34 Investigational	Small MW compound	KCa3.1 channels	↑ GAMs' antitumor immune activation		↓ Tumor growth	[60]
Vosaroxin Investigational	Small MW compound	DNA and TOPO-II	↑ GAMs' tumor infiltration ↑ GAMs' antitumor immune activation		↓ Tumor growth	[66]
Peptide R Investigational	Synthetic peptide	CXCR4	↑ GAMs' antitumor immune activation	↓ Glioma invasiveness, ↓ Intratumor vessel formation	↓ Tumor growth	[64]
RGD peptides Investigational	Synthetic peptides	Integrins	↓ GAMs' tumor infiltration ↑ GAMs' antitumor immune activation	↓ GSC-secreted periostin	↓ Tumor growth	[44]
IL-12 or rAAV2-mediated IL-12 Investigational	Biologic (protein or engineered viral vector)	IL-12 receptor	↑ GAMs' tumor infiltration ↑ GAMs' antitumor immune activation ↑ GAMs' phagocytic activity	↑ IFNγ and IL-12 intratumoral levels induced by rAAV2.	↓ Tumor growth ↑ Survival	[61,62]
miR-142-6p Investigational	Biologic (Synthetic oligonucleotide)	mRNA	↑ GAMs' tumor infiltration ↑ GAMs' antitumor immune activation		↓ Tumor growth ↑ Survival	[63]
X7Ab Investigational	Biologic (single-chain antibody)	ACKR3 / CXCR7	↑ GAMs' antitumor immune activation	Increased therapeutic effects of TMZ	↓ Tumor growth ↑ Survival	[68]

Clinical evidence						
Nivolumab FDA and EMA approved	Biologic (mAb)	PD-1		Phase III	No superior survival *vs.* bevacizumab	[19]
BLZ945/PDR001 Investigational	Small MW compound/ Biologic (mAb)	CSF-1R/PD1		Phase I/II		Clinicaltrials.gov
PLX3397 Investigational	Small MW compound	CSF-1R	↓ Iba1+ cells within the tumors	Phase II (recurrent glioblastoma)	No significant effects on PFS compared with historical controls	[38]
Cilengitide Investigational (in combination with TMZ)	Synthetic cyclic RGD pentapeptide	avβ3 and avβ5 integrins		Phase III	No superior survival *vs.* TMZ alone	[45,46]

In the table we reported the main features of drugs that interferes with biological functions of GAMs (name, characteristics and molecular target) together with the pharmacological actions on GAMs and the clinical outcomes on glioblastoma. Drugs are listed based on the level of evidence, i.e., preclinical (*in vitro* and *in vivo*) or clinical testing, according to the following criteria: (1) approved for clinical use (1st, small molecular weight compounds, 2nd biologics); and (2) investigational drugs (1st, small molecular weight compounds, 2nd biologics). ↑: increased; ↓: reduced. AmpB: amphotericin B; ARG1: arginase 1; BBB: blood-brain-barrier; CD: cluster of differentiation; CCL2: chemokine (C-C motif) ligand 2; CCR5: C-C chemokine receptor type 5; CHA: chlorogenic acid (5-caffeoylquinic acid); CsA: cyclosporine A; CSFs: colony stimulating factors; CSF1: macrophage colony stimulating factor; CSF1R: M-CSF receptor; CSF2R: GM-CSF receptor; CSF2: granulocyte/macrophage colony stimulating factor; GAMs: glioma associated microglia/macrophages; GM-CSF: granulocyte/macrophage colony stimulating factor; GSCs: glioblastoma cancer stem cells; JAK: Janus kinase; HGF: hepatocyte growth factor; Iba1: ionized calcium-binding adapter molecule 1, i.e., a specific myeloid lineage marker; IFN γ : interferon γ ; IL: interleukin; iNOS: inducible nitric oxide synthase; mAb: monoclonal antibody; MAPK: mitogen-activated protein kinase; MCP: monocyte chemotactic protein; M-CSF: macrophage colony stimulating factor; MDSCs: myeloid-derived suppressor cells; mTOR: mechanistic target of rapamycin kinase; MMP: matrix metalloprotease; miRNA, or miR: microRNA; NFAT1: nuclear factor of activated T cells; MW: molecular weight; PFS: progression free survival; PG: prostaglandin; PI3K: phosphoinositide 3-kinase; PD-1: programmed death-1; rAAV2: recombinant adeno-associated viral vector; SDF-1: stromal-derived factor-1; SPP1: osteopontin; STAT: signal transducer and activator of transcription; TGF β : transforming growth factor β ; TMZ: temozolomide; TOPO-II: topoisomerase-II; TRAM-34: 1-(2-chlorophenyl) diphenylmethyl-1H-pyrazole; Tregs: regulatory T cells; VEGF-A: vascular endothelial growth factor-A; X7Ab: single chain antibody

eral strategies attempt to overcome this restriction such as improved drug formulation (i.e., nanoparticles or lipid based formulation), local drug delivery (including gene therapy[61,62]), or transient BBB permeabilization[83,84], to name a few. Among the above mentioned drugs, minocycline and rapamycin for example, have increased BBB penetration properties; and novel mTOR inhibitors with improved pharmacokinetic properties are also under development. It is possible to envision the use of pharmacological compounds, targeting GAMs' functions, as a complement to current available therapeutic approaches.

DECLARATIONS

Authors' contributions

Conceived the paper and wrote the primary draft: Dello Russo C
Contributed to the literature revision and manuscript editing: Cappoli N
Read and approved the final manuscript: Dello Russo C, Cappoli N

Availability of data and materials

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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