Review



# Phagocytosis Checkpoints in Glioblastoma: CD47 and Beyond

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# Phagocytosis Checkpoints in Glioblastoma: CD47 and Beyond

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**Abstract:** Glioblastoma multiforme (GBM) is one of the deadliest human cancers with very limited treatment options available. The malignant behavior of GBM is manifest in a tumor which is highly invasive, resistant to standard cytotoxic chemotherapy and is strongly immunosuppressive. Immune checkpoint inhibitors have recently been introduced in the clinic and have yielded promising results in certain cancers. GBM however is largely refractory to these treatments. The immune checkpoint CD47 has recently gained attention as potential target for intervention as it conveys a "don't eat me" signal to tumor associated macrophages via the inhibitory SIRP alpha protein. In preclinical models, administration of anti-CD47 monoclonal antibodies have shown impressive results with GBM and other tumor models. Several well characterized oncogenic pathways have recently been shown to regulate CD47 expression in GBM cells and Glioma Stem cells (GSCs) include EGFR, beta catenin and LRIG2. Other macrophage pathways involved in regulating phagocytosis including TREM2 and glycan binding proteins are discussed as well. Finally, Chimeric Antigen Receptor Macrophages (CAR)-M could be leveraged for greatly enhancing phagocytosis of GBM and repolarization of the microenvironment in general. Here we comprehensively review the mechanisms that regulate about macrophage phagocytosis of GBM cells.

**Keywords:** Glioblastoma; glioma; macrophage; phagocytosis; CD47; CSF-1R; Siglec

## 1. Introduction

### *1.1. Glioblastoma and Immunotherapy*

Glioblastoma multiforme (GBM) is an almost invariably fatal brain cancer with a median survival from time of prognosis of approximately 14 months [1] . Standard of care involves surgical resection and irradiation with temozolomide therapy. This treatment regimen however is largely ineffective and has considerable side effects. New therapies are urgently needed. Immunotherapy involves harnessing the immune system to mediate destruction of cancer cells. Immune Checkpoint Inhibitors (ICIs), such as monoclonal antibodies against cytotoxic T-lymphocyte antigen-4 (CTLA-4; Ipilumumab) and programmed cell death protein 1 /programmed cell death-ligand 1 (PD-1/PD-L1; nivolumab, Pembrolizumab) have been developed and are currently used in the clinic to treat a variety of cancers. These ICIs have demonstrated remarkable efficacy in a subset of patients however in others such as GBM there has been no measurable response. [2,3]. This is likely due to the fact that patients with immunologically "cold" tumors (such as GBM) are unlikely to mount a sufficient response to tumor antigens [2–4]. Recently, Immune checkpoints involved in tumor-macrophage interactions have gained attention. Macrophages are likely one of the first immune cell types that cancer cells encounter and these early interactions are likely to dictate the progression of the tumor.

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*1.2. Tumor Associated Macrophages*

It is now widely appreciated that the microenvironment plays a central role in dictating the immune response to the tumor and ther overall progression to malignancy [5,6]. The microenvironment consists of many immune cell types including lymphocytes and macrophages (TAMs), the latter being especially prevalent in GBM. Approximately 30-50% of the GBM tumor mass is comprised of macrophages, microglia and other cells of the myeloid lineage [7–11]. Labelling expeiments have shown that most are derived from peripheral blood, although it is likely brain resident microglia also play an important and non-redundant role in tumor progression [12,13]. Extensive paracrine signaling occurs between glioma cells and TAMs [14–19]. In general, glioma cells secrete factors which recruit and induce TAMs to acquire a pro-tumoral phenotype (often referred to as M2). These reprogrammed TAMs in turn promote glioma cell invasion, chemoresistance and immunosuppression of the microenvironment. It is recognized that the standard "M1 vs M2" model of macrophage polarization is an oversimplification and macrophages display phenotypes along a continuum [20,21]. It is generally agreed however that M1 macrophages express cytokines such as IL-12 and cell surface markers CD80 and CD86, whereas M2 polarized macrophages express immunosuppressive cytokines such as IL-10 and TGF-b and cell surface markers such as CD163 and CD206. An attractive strategy is to use therapy to "reprogram" TAMs away from an M2 toward an M1 anti-tumoral phenotype. This is likely to yield substantial benefits as the pro-tumorigenic effects mediated by M2 macrophages including glioma invasion and chemoresistance, and immunosuppression will be reversed and M1 polarized TAMs will have the ability to rectuit and activate potent effector cells. These include cells such as NK cells and cytotoxic CD8+ T lymphocytes to attack tumor antigen expressing glioma cells and the release of additional pro-M1 factors which would propagate a positive feedback loop.

There are subtleties in how the tumor interacts with the myeloid compartment of the microenvironment. For example, so called glioma stem cells (GSCs) are a subset of tumor cells which express a different profile of chemokines, growth factors and interleukins and other factors from bulk tumor population [22–26] As a consequence of this differential cytokine expression GSCs are thought to interact in a more specialized way with TAMs. GSCs also express specific cell surface proteins such as CD44, CD133 and nestin which could mediate special interactions with TAMs within the microenvironment [27]. In addition to heterogeneity among the glioma cells, as mentioned previously there are various subpopulations of myeloid cells within the microenvironment including resident brain microglia, macrophages derived from monocytes in the blood, CD11c+ dendritic cells, immature Gr1+ monocytoid cells and myeloid derived suppressor cells (MDSCs). Each of these cells in turn can have overlapping and distinct effects on tumor progression [9,11,28–33]. Further complicating the investigation of the relative contribution of these macrophage/myeloid subpopulations on GBM progression is that there are substantial differences in the cell specific markers between mice and humans employed to distinguish between macrophage subsets [34].

*1.2. Macrophage Phagocytosis of Glioma Cells*

Most of myeloid cell types associated with GBM tumors, including macrophages, microglia and dendritic cells, retain the capacity to carry out phagocytosis under certain conditions [35–43]. The current paradigm is that tumor cells experiencing oncogenic cellular stress express and presentation of "Eat Me" signals such as surface exposed calreticulin , High mobility group box 1 protein (HMGB1), phosphatidyl serine (PS) among others [44,45]**.** When these signals are detected by tissue macrophages, the result rapid and efficient phagocytosis of the tumor cell. However, tumor cells eventually upregulate surface "don't eat me" receptors, most well characterized of which is CD47. CD47 is a transmembrane protein containing an extracellular immunoglobulin (Ig) domain which engages SIRP receptors on macrophages that strongly inhibits phagocytic signaling pathways [46]. There are three SIRP receptors in humans, the most studied being SIRP alpha [47]. The cytoplasmic domain of SIRPa contains an immunoreceptor tyrosine-based inhibition motif (ITIM) which recruit SHP1/2 phosphatases involved in downregulating immune signaling particularly pathways that are involved in phagocytosis [48]. The Weissman lab has pioneered the use of therapeutic anti-CD47

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antibodies to treat solid tumors in a variety of mouse models [49–52]. Preclinical studies with anti-CD47 blocking antibodies have been promising in many cancer models and a humanized anti-CD47 monoclonal antibodies such as Hu5F9-G4 (Magrolimab) has been developed [53]. Unfortunately as is the case with most current immune checkpoint therapies, anti CD47 seems to be significantly less effective against solid tumors in clinical trials conducted thus far [54]. In addition, CD47 is expressed on high levels in red blood cells and other cell types which could make it less than ideal as a targeted monotherapy. Anti-SIRP approaches are currently in development to perhaps escape these limitations [55]. Other "Don't Eat Me" surface immune checkpoint molecules include certain sialic acid containing glycoproteins and glycolipids which engage the Sialic acid-binding immunoglobulin type lectins (Siglec) proteins which inhibit the immune response.

Macrophages are also responsible for clearing apoptotic GBM cells [56–58]. Paradoxically, the pathways that regulate phagocytosis of apoptotic cells has been shown in many contexts to induce an anti-inflammatory response [59]. For example, bone marrow derived macrophage (BMDM) phagocytosis of GBM cells was shown in-vitro to result in lower secretion of IL-1b and TNFa and an increase in anti inflammatory IL-10 cytokine production [60]. Phagocytosis also resulted in an increase in macrophage expression of PD-L1 and PD-L2. These mechanisms almost certainly evolved to prevent an inappropriate inflammatory response during the clearance of apoptotic cells which occurs during normal physiological processes. It is unclear if inducing apoptosis of tumor cells will augment or inhibit *in-vivo* antigen presentation of tumor antigens. There has been some work showing alternative forms of cell death can be induced in cancer cells to overcome this immunosuppressive effect. For example downregulation of HSP70 resulted in a caspase-independent "apoptosis-like" effect in breast and colorectal tumor cells whereby phagocytic clearance was not accompanied by immunosuppresive cytokines [61]. Nonetheless, understanding the full spectrum of molecular pathways which govern TAM phagocytosis of GBM cells will likely result in great strides toward additional checkpoint inhibitor therapy. Here we review these pathways in a comprehensive fashion.

## 2. Pathways Regulating TAM Phagocytosis of GBM Cells

### *2.1. CD47/SIRP Pathway*

As mentioned above, the "don't eat me" signal protein CD47 has emerged as an authentic immune checkpoint target and humanized anti-CD47 monoclonal antibodies are currently in clinical trials [54]. Preliminary data from preclinical mouse models have shown anti-CD47 therapy to be very effective in treating breast, ovarian and GBM [49,53,62]. Anti-CD47 treatment of mice harboring orthotopically injected luciferase-expressing GBM xenografts resulted in almost complete inhibition of tumor growth as measured by bioluminescence photon flux [49]. An added benefit of anti-CD47 is that it only promotes phagocytosis but can promote M1 macrophage polarization which should promote a more immunologically "hot" tumor [63].

Preclinical studies have also focused on the ability of anti-CD47 to work in combination with other therapy. Anti-CD47 can synergize with IR or TMZ to prolong survival of human GBM xenograft implanted mice [64]. A recent study showed that TMZ was important for anti-CD47 induced phagocytosis of GBM and this was dependent on Stimulator of Interferon Genes (STING) [65]. Another study simultaneously blocked VEGF and CD47 using a novel bispecific fusion protein VEGFR1D2-SIRP$\alpha$D1 [66]. This was effective at both enhancing phagocytosis as well as lowering blood vessel density and angiogenesis. This study also noted that preventing autophagy using chloroquine was able to further enhance these effects. Delivery of therapeutics has always been a challenge for GBM due in part to the blood brain barrier (BBB). Another laboratory employed an oncolytic virus which encodes an anti-CD47 antibody to improve survival in orthotopic human GBM models [67]. Several groups have been using implantation of hydrogel formulations into the cavity of freshly resected GBM in rodent brains [68,69]. Song et al injected hydrogels infused with TMZ and vectors encoding shRNA targeting CD47 [68]. In another study, use of temperature-sensitive hydrogel system hydroxypropyl chitin (HPCH) copolymer which encapsulates both anti-CD47 and

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TMZ was able to provide a full curative effect in approximately 50% of the animals harboring GL261 murine glioma tumors [69].

As mentioned above, interfering with CD47 might have side effects as this protein is expressed on other cell types, most notably red blood cells [70]. Alternative approaches to interfere with CD47 function involve the use of a SIRPa-Fc fusion protein to bind to and prevent tumor CD47 from engaging SIRP receptors on TAMs [71–73]. Using SIRPa-Fc in an immunocompetent mouse model of GBM was effective in reducing tumor size, particularly when used in combination with autophagy inhibitors [74]. Interestingly, the SIRPa protein has also been detected in normal astrocytes and lower grade glioma however its expression decreases with increasing tumor grade [75,76]. Aggregation of the U373MG cell line resulted in an increase in tyrosine phosphorylation of SIRP which was attenuated with an anti-CD47 blocking antibody. It is unclear what role tumor-expressed SIRP proteins play in carcinogenesis or immune-evasion.

As alluded to above, therapeutic approaches which convert the GBM from an immunologically "cold" to a "hot" with the aim of rendering the tumor more amenable to immune checkpoint therapies [77,78]. Using TLR3 and TLR9 agonists, Huang et al were able to stimulate microglia phagocytosis and clearance of GBM cells in tissue culture, brain slices and in the GL261 mouse model [79]. Another study used "trojan horse" nanoparticles to deliver anti-CD47 along with a STING agonist into the brains of GBM bearing mice. It was observed in this study that polarization of TAMs within the tumor was strongly towards M1 phenotype [80].

Epigenetic regulation of the phagocytic immune checkpoint has gained attention recently. Tacedinaline (CI-994), a class I Histone Deacetylase (HDAC) inhibitor was identified in a screen as a apoptosis inducer in MYC-driven cancer cell lines including GBM lines [81]. CI-994 treatment significantly enhanced the expression of "eat me" signals calreticulin and HMGB1 on the surface of the medulloblastoma cell lines tested. Administration of CI-994 combined with anti-CD47 was effective in treating medulloblastoma in orthotopically injected mice. In macrophages, HDAC activity appears to inhibit phagocytosis [82]. Expression of miRNA22 in TAMs represses HDAC6 expression which in turn results in a higher level of phagocytosis of GBM cell lines. These studies suggest that HDAC inhibition could be an effective with checkpoint inhibitor therapy as it activates pro-phagocytosis pathways in both GBM and TAMs.

In addition to its ability to engage and activate SIRP receptors on TAMs, CD47 plays cell autonomous roles in GBM [83,84]. The matricellular protein Thrombospondin-1 (TSP-1) can also activate CD47 [85]. Activation of CD47 using a TSP-1 derived peptide agonist increased proliferation of U87 and U373 cells but not normal human astrocytes [83]. CD47 mediates invasion via the Phosphoinositol-3-Kinase (PI3K) pathway in U87 and T98G GBM lines [84]. It was also observed that ablation of CD47 in mouse and human GBM cells resulted in an increase in notch pathway signaling and concomitant upregulation of the extracellular matrix protein Tenascin-C [86]. This increase in Tenascin-C expression resulted in a higher level of TAM infiltration and phagocytosis. Work with glioma stem cells (GSC) has elucidated other mechanisms of CD47 regulation in this population of cells. It is known that irradiation of GBM is rarely effective as GSCs are radioresistant and have the ability to recreate the tumor within a short period of time [87]. It has been demonstrated that GSCs express higher levels of CD47 [88]. This increased CD47 expression seems to contribute to GSC proliferation and migration as treatment with anti-CD47 slows each of these processes. In a recent paper, it was shown that IR induces CD47 expression on GSCs via the 5' AMP-activated protein kinase (AMPK) pathway [89]. GSCs also typically display altered metabolic pathways relative to the bulk tumor. For example, GSCs utilize fatty acid oxidation (FAO) to a much greater extent than bulk GBM tumor cells [90]. In a study by Jiang et. al, the FAO pathway resulted in activation of NF-kB which in turn upregulates CD47 expression specifically in GSCs [91]. Blockade of FAO using etoximir, an inhibitor of a key enzymes in the FAO pathway, synergizes with anti-CD47 to strongly increase GBM phagocytosis and decrease tumor volume.

Some insight has been gained in understanding the molecular pathways which regulate CD47 expression and stability on GBM cells. The Leucine-Rich Repeats and Ig-Like Domain (LRIG) family of transmembrane receptors have been associated with neurological tumors [92,93]. There are three

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LRIG genes in humans and they primarily regulate growth factor receptors by targeting them for ubiquitination. In glioma cells, LRIG1 and 3 act as tumor suppressors while LRIG2 has seemingly oncogenic function [92,94,95] A recent study by Hu et al showed that LRIG2 can strongly prevent phagocytosis of GBM cells by upregulating components of the CD47 pathway [96]. In this report, soluble LRIG2 generated by A disintegrin and metalloprotease 17 (ADAM17)-mediated cleavage from GBM cells was shown to recruit TAMs and induce SIRPa expression on their surface. In addition to this, LRIG2 was shown to enhance the expression of CD47 on GBM cells via transcriptional activation of the CD47 gene. This study strongly implicates LRIG2 as a key regulator of the CD47 immune checkpoint via upregulation of both CD47 and SIRPa. The well-characterized beta-catenin oncogenic pathway can also stimulate CD47 expression in GBM cells [97]. Another study demonstrates how the EGFR pathway cooperates with CD47 to promote tumor progression. EGFR is commonly altered in GBM although single agent therapy with EGFR inhibitors have displayed modest efficacy at best [98]. In a recent study, EGF treatment or expression of the constitutively active truncation mutant EGFRvIII was able to increase the expression of CD47 in multiple established human GBM cell lines [99]. This effect was found to be mediated by SRC phosphorylation of CD47 at Y288 which prevents binding and polyubiquitination by Tripartite motif-containing protein 21 (TRIM21), an E3 ubiquitin ligase. GBM cells expressing a CD47 Y288F mutant are phagocytosed in-vivo at much higher levels and animals orthotopically injected with these cells survive longer than wild type injected counterparts.

### *2.2. CSF-1/CSF-1R Pathway*

The macrophage growth factor Colony Stimulating Factor-1 (CSF-1) is strongly pro-tumorigenic in most cancers a s activation of Colony Stimulating Factor-1 Receptor (CSF-1R) promotes M2 polarization of TAMs [100–103] . It was originally observed that breast carcinoma invasion and metastasis is severely diminished in CSF-1 null (op/op) background mice [104]. Since that discovery, CSF-1 was shown to be associated with many malignant cancers and the establishment of a generally immunosuppressive microenvironment [105]. Our laboratory has shown that glioma cells express CSF-1 which results in TAM recruitment and invasion of tumor cells into normal brain parenchyma [14,106,107]. Blockade of CSF-1R signaling with PLX-3397, a CSF-1R pharmacological inhibitor that crosses the blood brain barrier, strongly attenuated invasion and was curative in some preclinical GBM models [14,108]**.** In the context of CSF-1R contribution to the phagocytic checkpoint**,** the balance of evidence points to an important role for CSF-1R signaling to rearrange actin during the various stages of phagocytosis [109]. Recently it was demonstrated that GW2580, another pharmacological inhibitor of CSF-1R, induced MHC-II expression, phagocytosis and T cell mediated killing of GBM tumor in patient derived 3D organoid models [110]. Interestingly, PLX3397 did not display these features. This discrepancy is likely because these inhibitors hit different off-target RTKs [111,112]. Under standard conditions, CSF-1 is released by cancer cells as soluble ligand which act on macrophages via the CSF-1R in a paracrine and possibly endocrine fashion [113]. However it was discovered that there is a splice variant of the CSF-1 gene which contains a transmembrane domain and results in a surface membrane bound form of CSF-1, referred to as mM-CSF [114]. Interestingly, glioma cells expressing mM-CSF are highly susceptible to phagocytosis and cytotoxic killing by TAMs [114–116]. The mechanism is unclear however the membrane bound mM-CSF is functional as it has the ability to stimulate macrophage colony formation [114]. Therefore, a potential therapeutic approach might be to promote alternative splicing of the CSF-1 gene in GBM tumors towards the membrane bound isoform.

### *2.3. BACE-1*

A recent intriguing study identified the β-site amyloid precursor protein cleaving enzyme 1 (BACE1) as playing a role in preventing tumor phagocytosis. BACE1 is responsible for generating β-amyloid peptides which form plaques in the brains of Alzheimer Disease (AD) patients resulting in neuronal dysfunction associated with that disease [117]. Several groups have developed BACE-1 specific inhibitors for potential AD therapy. In a paper by Zhai et al, a screen for phagocytosis of

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fluorescently labeled human patient derived GSCs was conducted using a library of compounds which have good blood brain barrier permeability and low toxicity [118]. The BACE-1 inhibitor MK-8931 was found to enhance phagocytosis. Furthermore, it was demonstrated that BACE-1 is expressed in tumor promoting macrophages and administration of MK-8931 along with IR was able to suppress malignant GBM growth in-vivo. Interestingly, oligomers of beta amyloid protein that are associated with AD can stimulate microglia phagocytotic activity against glioma cells [119]. As with many neurodegenerative disorders, microglia dysfunction seems to be a central feature of both gliomagenesis and AD [120].

### *2.4. TREM2*

Several very recent exciting, yet controversial studies have focused on the potential role of Triggering Receptor Expressed on Myeloid Cells 2 (TREM2) in regulating phagocytosis in GBM [121,122]. TREM2 is expressed primarily on microglial cells and can be activated by a variety of ligands including bacteria, polyanionic molecules, lipoproteins and nucleic acids [123] TREM2 signals through DNAX Activator Proteins 10 and 12 (DAP10 and DAP12). DAP10 and DAP 12 can activate several downstream pathways including PI3K/AKT and SYK, a tyrosine kinase which is involved in phagocytosis, particularly at the engulfment stage [124,125]. Peshoff et. al discovered that TREM2+ macrophages are increased in IDH wild type GBMs and its expression correlated with phagocytosis markers [121]. Unlike other cancer models where TREM2 knockout slows tumor growth, there was no effect of TREM2 ablation on GL261 or CT-2A glioma progression in-vivo syngeneic models. Similarly, Zheng et al showed a higher level of TREM2+ cells in GBM tumors and a strong association with phagocytosis however TREM2 deficiency did not have a beneficial effect on mouse survival using the GL261 model system. However Sun et.al demonstrated with different GBM cell lines (SB28 and NPA-C54B) that blockade of TREM2 increased IFNg expression, slowed tumor growth and enhanced animal survival [126]. Furthermore, this was accomplished using three different methods of TREM2 depletion including using TREM2 knockout mice, TREM2 antisense oligo (ASO) delivery and TREM2 blocking antibody administration. Whether or not this discrepancy is due to the different GBM cell lines used or some other subtle difference between model systems tested remains to be seen.

### *2.5. SIGLECs*

The pattern of glycosylated cell surface proteins and lipids is likely a strategy emplyed by vertebrates to distinguish self vs non-self [127,128]. The Sialic acid-binding immunoglobulin type lectins (Siglec) family of proteins are cell surface receptors expressed on most white blood cellscells that play a role in immune recognition and regulation [129] The extracellular region of siglec proteins contain a V-Set Ig domain which binds to sialic acid which is expressed on many glycoproteins and glycolipids. There are 14 human Siglecs and 9 murine siglecs they are thought to play an important role in immune checkpoints in several diseases. Siglecs often act as coreceptors and can activate or inhibit immune responses depending on their C-terminal domain strucure. Siglecs can modulate several cellular functions including endocytosis, antigen presentation and phagocytosis. Most siglecs contain immunosuppresive ITIM and Immunoreceptor tyrosine-based switch motif (ITSM) motifs and are therefore immunoduppressive. However a subset of siglecs do not contain ITIMs and instead have a region which can associate with the adaptor DAP12 which as noted above is involved in immune activation and phagocytosis. Hyperglycolysation of tumor cells is associated with a poor outcome [130,131] Certain siglecs are expressed primarily on macrophages and microglia. These include Siglec-7,9,10 and 15 and they have been the subject of investigation for immune checkpoint therapy [132] For example, expression of Siglec-E in TAMs was found to inhibit tumor growth [133]. Furthermore Siglec-10 engagement of CD24 on breast carcinoma cells inhibited phagocytosis [134]. In the context of GBM, Siglec-H expression is increased in IFNg treated M1 polarized microglia [135]. Interestingly, Siglec-H only binds to murine GBM cells and not normal mouse cells highlighting the hyperglycosylated aspect of cancer cells. This interaction resulted in the phagocytosis of murine GBM cell lines SMA560 and GL261 and this was dependent on DAP12. Additional siglec family members

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act as checkpoints on TAMS. Siglec-1 (also called CD169/Sialoadhesin) is expressed predominantly on macrophages and is the only member of the Siglec family seemingly which does not initiate either pro or anti immuno signaling [136,137] The role of Siglec-1/CD169 was recently investigated in a GBM model [58].Depletion of CD169 resulted in a decrease in CXCL10 expression and T cell infiltration. CD169 also played an important role in phagocytosis of GBM cells. Another Siglec that is mainly expressed on macrophages is Siglec-15. The role of Siglec-15 was investigated in a recent paper where it was discovered to be expressed primarily by peri-tumoral macrophages within GBM tumors [138]. The pattern of expression of Siglec-15 changes during the course of GBM progression as it reaches its highest expression in grade II astrocytoma. A negative correlation between Siglec-15 expression and CD3+ cell infiltration was noted. Knockout of Siglec-15 in a mouse macrophage cell line increased M1 marker expression, decreased M2 marker expression and enhanced phagocytosis of GL261 glioma cells.

### *2.6. Phagocytosis Checkpoint Cooperation with Anti-CTLA4/PD-1/PD-L1 ICIs*

There is some evidence that the immune checkpoint inhibitors currently approved for clinical use to reverse T cell inhibition may promote phagocytosis pathways. For example, IR and anti PD-L1 treatment resulted in an increase in T cell and TAM recruitment to the tumor in murine genetic models of GBM [139]. Anti PD-L1 was shown to strongly enhance macrophage phagocytosis of GBM cells and this was independent of T cells. Chen et al recently discovered that using anti CTLA4 antibodies resulted in an increase in IFNg release within GBM tumor which stimulated DC and microglia phagocytosis of tumor cells [140]. Phagocytic activity in this context was dependent on signaling from Axl and Mer RTKs.

### *2.7. Chimeric Antigen Receptor-M*

One of the approaches of modern immunotherapy involves *ex-vivo* manipulation of a patient's peripheral immune cells to be anti-tumorigenic followed by reintroduction int the patient [141,142]. Most work in this area has been with cytotoxic T cells. In particular, researchers have generated vectors which express chimeric antigen receptors whereby the ectodomain of the T cell receptor is replaced with a domain that specifically and directly interacts with target receptors on the surface of cancer cells [143] In this way, the antigen presentation steps which involve multiple immune checkpoint coreceptors (discussed above) is completely bypassed. CAR-T has been quite effective for several cancers, especially leukemias [144,145]. Unfortunately, as with other immunotherapies, CAR-T has not delivered measurable clinical responses with GBM [146]. Other leukocytes are now being tested for CAR style therapy, including macrophages. CAR-Macrophage (CAR-M) are currently receiving a lot of attention from researchers and biopharmaceutical companies [147]. Unlike T cells, which often cannot penetrate solid tumors very well, macrophages are quite good at tumor infiltration [148]. One of the major strategies for CAR-M therapy is to prevent "don't eat me" signals, such as CD47, to be conveyed to the macrophage [149]. There is some evidence in animal models that M1 polarization can improve CAR-M therapy [150]. Macrophages transduced with a HER2-Fc-Receptor chimeric antigen receptor treated with M1 polarizing stimuli IFNg and LPS and were more effective than unstimulated controls in a breast carcinoma model. Finally, purposing CAR-M to specifically target GSCs might be an effective approach for eradicating these cells as a recent paper has indicated [151]. As this technology becomes more advanced and widespread, opportunities for targeting other checkpoint targets will be exploited.

## 3. Conclusions and Perspectives

We are in an exciting era of cancer therapy as gains are being made (albeit slowly) in treating metastatic cancers. Much work remains to be done but there are clear paths delineated to the discovery of multiple immune checkpoints. GBM treatment will likely require a multi-pronged approach utilizing chemotherapy to induce GBM cell death in combination with treatments that inhibit M2 and promote M1 macrophage polarity and block phagocytosis immune checkpoints. The

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pathways and mechanisms that regulate the phagocytosis checkpoints at the glioma cell/TAM interface are summarized in Figure 1. LRIG2 is an enticing target as it upregulates CD47 and SIRPa expression in GBM cells and TAMs respectively [96]. It may also be time to revisit EGFR inhibitors for GBM treatment as this pathway enhances CD47 stability in addition to its other oncogenic activities. The "cancer glycome" also presents itself as a target rich environment for immune checkpoint blockade therapy [152,153]. Finally, to fully eradicate GBM tumors, the GSC population must be effectively eliminated. The discovery that GSC cells are more dependent on CD47 expression could greatly assist in developing treatments to eliminate these cells.

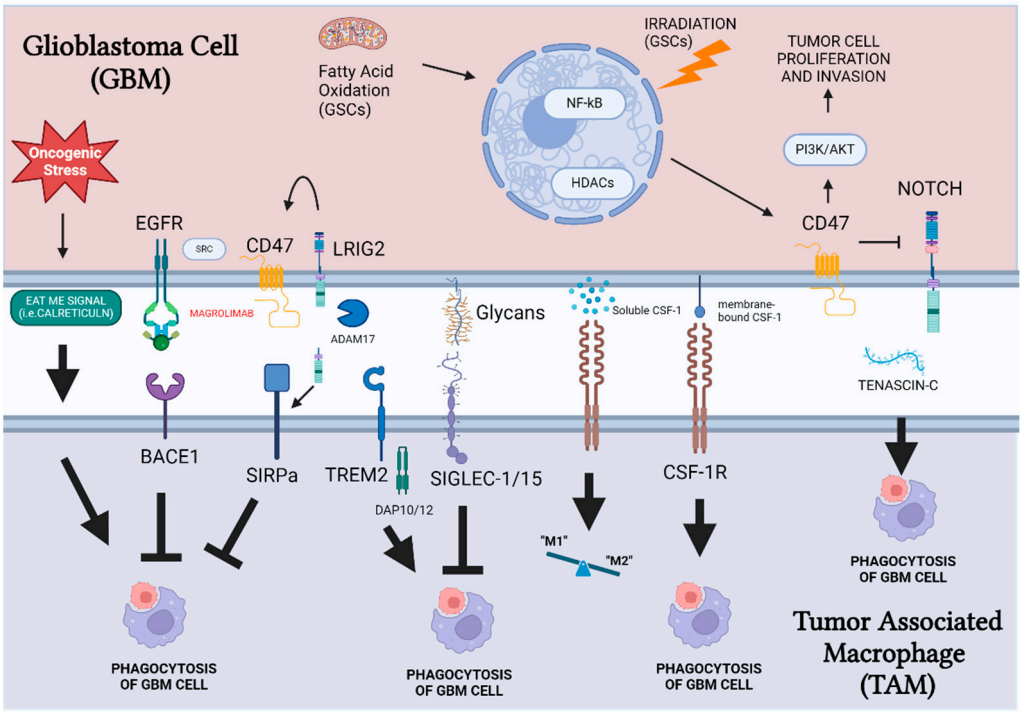


**Figure 1.** Phagocytosis Checkpoints at the Glioma/Macrophage Interface. CD47 exhibits bidirectional signaling that promotes both GBM tumor invasion and inhibition of phagocytosis by engaging SIRP receptors on the surface of TAMs. Pathways which regulate CD47 expression include EGFR/SRC, LRIG2, and NF-kB. TREM2 can also promote phagocytosis while Siglecs tend to be strongly immunosuppressive and represent another phagocytosis checkpoint along with CD47. Effective therapy for GBM will likely involve a combination of treatments with induce GBM cell death, promote an immunologically "hot" tumor by activating M1 macrophage pathways and stimulation of GBM phagocytosis by TAMs using checkpoint inhibitors.

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## References

1. Ostrom, Q.T.; Price, M.; Neff, C.; Cioffi, G.; Waite, K.A.; Kruchko, C.; Barnholtz-Sloan, J.S. CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2016-2020. *Neuro. Oncol.* **2023**, *25*, IV1–IV99. https://doi.org/10.1093/neuonc/noad149.

2. Miyazaki, T.; Ishikawa, E.; Sugii, N.; Matsuda, M. Therapeutic Strategies for Overcoming Immunotherapy Resistance Mediated by Immunosuppressive Factors of the Glioblastoma Microenvironment. *Cancers (Basel).* **2020**, *12*, 1–21. https://doi.org/10.3390/cancers12071960.
3. Sanders, S.; Debinski, W. Challenges to Successful Implementation of the Immune Checkpoint Inhibitors for Treatment of Glioblastoma. *Int. J. Mol. Sci.* **2020**, *21*. https://doi.org/10.3390/ijms21082759.
4. Yang, Y.; Tian, Y.; Li, Q.; Jiang, R.; Zhang, J. Uncovering the Immune Cell Infiltration Landscape in Low-Grade Glioma for Aiding Immunotherapy. *J. Oncol.* **2022**, *2022*. https://doi.org/10.1155/2022/3370727.
5. Joyce, J. a; Pollard, J.W. Microenvironmental Regulation of Metastasis. *Nat. Rev. Cancer* **2009**, *9*, 239–252. https://doi.org/10.1038/nrc2618.
6. Kitamura, T.; Qian, B.-Z.; Soong, D.; Cassetta, L.; Noy, R.; Sugano, G.; Kato, Y.; Li, J.; Pollard, J.W. CCL2-Induced Chemokine Cascade Promotes Breast Cancer Metastasis by Enhancing Retention of Metastasis-Associated Macrophages. *J. Exp. Med.* **2015**, *212*, 1043–1059. https://doi.org/10.1084/jem.20141836.
7. Quail, D.F.; Joyce, J.A. The Microenvironmental Landscape of Brain Tumors. *Cancer Cell* **2017**, *31*, 326–341. https://doi.org/10.1016/j.ccell.2017.02.009.
8. Gieryng, A.; Kaminska, B. Myeloid-Derived Suppressor Cells in Gliomas. *Wspolczesna Onkol.* **2016**, *20*, 345–351. https://doi.org/10.5114/wo.2016.64592.
9. Graeber, M.B.; Scheithauer, B.W.; Kreutzberg, G.W. Microglia in Brain Tumors. *Glia* **2002**, *40*, 252–259. https://doi.org/10.1002/glia.10147.
10. Locarno, C. V.; Simonelli, M.; Carenza, C.; Capucetti, A.; Stanzani, E.; Lorenzi, E.; Persico, P.; Della Bella, S.; Passoni, L.; Mavilio, D.; et al. Role of Myeloid Cells in the Immunosuppressive Microenvironment in Gliomas. *Immunobiology* **2020**, *225*, 151853. https://doi.org/10.1016/j.imbio.2019.10.002.
11. Richard, Q.; Laurenge, A.; Mallat, M.; Sanson, M.; Castro-Vega, L.J. New Insights into the Immune TME of Adult-Type Diffuse Gliomas. *Curr. Opin. Neurol.* **2022**, *35*, 794–802. https://doi.org/10.1097/wco.0000000000001112.
12. Hambardzumyan, D.; Gutmann, D.H.; Kettenmann, H. The Role of Microglia and Macrophages in Glioma Maintenance and Progression. *Nat. Neurosci.* **2016**, *19*, 20–27. https://doi.org/10.1038/nn.4185.
13. Galarneau, H.; Villeneuve, J.; Gowing, G.; Julien, J.P.; Vallières, L. Increased Glioma Growth in Mice Depleted of Macrophages. *Cancer Res.* **2007**, *67*, 8874–8881. https://doi.org/10.1158/0008-5472.CAN-07-0177.
14. Coniglio, S.J.; Eugenin, E.; Dobrenis, K.; Stanley, E.R.; West, B.L.; Symons, M.H.; Segall, J.E. Microglial Stimulation of Glioblastoma Invasion Involves Epidermal Growth Factor Receptor (EGFR) and Colony Stimulating Factor 1 Receptor (CSF-1R) Signaling. *Mol. Med.* **2012**, *18*.
15. Zhu, C.; Mustafa, D.; Zheng, P.P.; Van Der Weiden, M.; Sacchetti, A.; Brandt, M.; Chrifi, I.; Tempel, D.; Leenen, P.J.M.; Duncker, D.J.; et al. Activation of CECR1 in M2-like TAMs Promotes Paracrine Stimulation-Mediated Glial Tumor Progression. *Neuro. Oncol.* **2017**, *19*, 648–659. https://doi.org/10.1093/neuonc/now251.
16. Sielska, M.; Przanowski, P.; Pasierbińska, M.; Wojnicki, K.; Poleszak, K.; Wojtas, B.; Grzeganek, D.; Ellert-Miklaszewska, A.; Ku, M.; Kettenmann, H.; et al. Tumour-Derived CSF2/Granulocyte Macrophage Colony Stimulating Factor Controls Myeloid Cell Accumulation and Progression of Gliomas. *Br. J. Cancer* **2020**, *123*, 438–448. https://doi.org/10.1038/s41416-020-0862-2.
17. Platten, M.; Kretz, A.; Naumann, U.; Aulwurm, S.; Egashira, K.; Isenmann, S.; Weller, M. Monocyte Chemoattractant Protein-1 Increases Microglial Infiltration and Aggressiveness of Gliomas. *Ann. Neurol.* **2003**, *54*, 388–392. https://doi.org/10.1002/ana.10679.
18. Maas, S.L.N.; Abels, E.R.; Van De Haar, L.L.; Zhang, X.; Morsett, L.; Sil, S.; Guedes, J.; Sen, P.; Prabhakar, S.; Hickman, S.E.; et al. Glioblastoma Hijacks Microglial Gene Expression to Support Tumor Growth. *J. Neuroinflammation* **2020**, *17*, 1–18. https://doi.org/10.1186/s12974-020-01797-2.
19. Yuan, D.; Tao, Y.; Chen, G.; Shi, T. Systematic Expression Analysis of Ligand-Receptor Pairs Reveals Important Cell-to-Cell Interactions inside Glioma. *Cell Commun. Signal.* **2019**, *17*, 1–10. https://doi.org/10.1186/s12964-019-0363-1.
20. Schultze, J.L.; Freeman, T.; Hume, D.A.; Latz, E. A Transcriptional Perspective on Human Macrophage Biology. *Semin. Immunol.* **2015**, *27*, 44–50. https://doi.org/10.1016/j.smim.2015.02.001.
21. Xue, J.; Schmidt, S. V.; Sander, J.; Draffehn, A.; Krebs, W.; Quester, I.; De Nardo, D.; Gohel, T.D.; Emde, M.; Schmidleithner, L.; et al. Transcriptome-Based Network Analysis Reveals a Spectrum Model of Human Macrophage Activation. *Immunity* **2014**, *40*, 274–288. https://doi.org/10.1016/j.immuni.2014.01.006.
22. Brooks, L.J.; Parrinello, S. Vascular Regulation of Glioma Stem-like Cells: A Balancing Act. *Curr. Opin. Neurobiol.* **2017**, *47*, 8–15. https://doi.org/10.1016/j.conb.2017.06.008.
23. Lee, S.Y.; Kim, J.K.; Jeon, H.Y.; Ham, S.W.; Kim, H. CD133 Regulates IL-1β Signaling and Neutrophil Recruitment in Glioblastoma. *Mol. Cells* **2017**, *40*, 515–522. https://doi.org/10.14348/molcells.2017.0089.
24. Dzaye, O.D.A.; Hu, F.; Derkow, K.; Haage, V.; Euskirchen, P.; Harms, C.; Lehnardt, S.; Synowitz, M.; Wolf, S.A.; Kettenmann, H. Glioma Stem Cells but Not Bulk Glioma Cells Upregulate IL-6 Secretion in Microglia/Brain Macrophages via Toll-like Receptor 4 Signaling. *J. Neuropathol. Exp. Neurol.* **2016**, *75*, 429–440. https://doi.org/10.1093/jnen/nlw016.

25. Codrici, E.; Enciu, A.-M.; Popescu, I.-D.; Mihai, S.; Tanase, C. Glioma Stem Cells and Their Microenvironments: Providers of Challenging Therapeutic Targets. *Stem Cells Int.* **2016**, *2016*, 5728438. https://doi.org/10.1155/2016/5728438.
26. Yao, Y.; Ye, H.; Qi, Z.; Mo, L.; Yue, Q.; Baral, A.; Hoon, D.S.B.; Vera, J.C.; Heiss, J.D.; Chen, C.C.; et al. B7-H4(B7x)-Mediated Cross-Talk between Glioma-Initiating Cells and Macrophages via the IL6/JAK/STAT3 Pathway Lead to Poor Prognosis in Glioma Patients. *Clin. Cancer Res.* **2016**, *22*, 2778–2790. https://doi.org/10.1158/1078-0432.CCR-15-0858.
27. Wu, A.; Wei, J.; Kong, L.-Y.; Wang, Y.; Priebe, W.; Qiao, W.; Sawaya, R.; Heimberger, A.B. Glioma Cancer Stem Cells Induce Immunosuppressive Macrophages/Microglia. *Neuro. Oncol.* **2010**, *12*, 1113–1125. https://doi.org/10.1093/neuonc/noq082.
28. Coniglio, S.J.; Segall, J.E. Review: Molecular Mechanism of Microglia Stimulated Glioblastoma Invasion. *Matrix Biol.* **2013**, *32*, 372–380. https://doi.org/10.1016/j.matbio.2013.07.008.
29. Gao, H.; Zhang, I.Y.; Zhang, L.; Song, Y.; Liu, S.; Ren, H.; Liu, H.; Zhou, H.; Su, Y.; Yang, Y.; et al. S100B Suppression Alters Polarization of Infiltrating Myeloid-Derived Cells in Gliomas and Inhibits Tumor Growth. *Cancer Lett.* **2018**, *439*, 91–100. https://doi.org/10.1016/j.canlet.2018.07.034.
30. Wei, J.; Gabrusiewicz, K.; Heimberger, A. The Controversial Role of Microglia in Malignant Gliomas. *Clin. Dev. Immunol.* **2013**, *2013*. https://doi.org/10.1155/2013/285246.
31. AlShakweer, W.; Alwelaie, Y.; Mankung, A.M.; Graeber, M.B. Bone Marrow-Derived Microglia in Pilocytic Astrocytoma. *Front. Biosci. (Elite Ed).* **2011**, *3*, 371–379. https://doi.org/10.2741/e252.
32. Zhang, J.; Guan, M.; Wang, Q.; Zhang, J.; Zhou, T.; Sun, X. Single-Cell Transcriptome-Based Multilayer Network Biomarker for Predicting Prognosis and Therapeutic Response of Gliomas. *Brief. Bioinform.* **2020**, *21*, 1080–1097. https://doi.org/10.1093/bib/bbz040.
33. Gieryng, A.; Pszczolkowska, D.; Walentynowicz, K.A.; Rajan, W.D.; Kaminska, B. Immune Microenvironment of Gliomas. *Lab. Investig.* **2017**, *97*, 498–518. https://doi.org/10.1038/labinvest.2017.19.
34. Bronte, V.; Brandau, S.; Chen, S.H.; Colombo, M.P.; Frey, A.B.; Greten, T.F.; Mandruzzato, S.; Murray, P.J.; Ochoa, A.; Ostrand-Rosenberg, S.; et al. Recommendations for Myeloid-Derived Suppressor Cell Nomenclature and Characterization Standards. *Nat. Commun.* **2016**, *7*, 1–10. https://doi.org/10.1038/ncomms12150.
35. Wallace, P.K.; Romet-Lemonne, J.L.; Chokri, M.; Kasper, L.H.; Fanger, M.W.; Fadul, C.E. Production of Macrophage-Activated Killer Cells for Targeting of Glioblastoma Cells with Bispecific Antibody to FcγRI and the Epidermal Growth Factor Receptor. *Cancer Immunol. Immunother.* **2000**, *49*, 493–503. https://doi.org/10.1007/s002620000142.
36. Bhattacharjee, M.; Bose, I.; Sarkar, P.; Banerjee, C.; Dutta, S.; Ghosh, A.; Mukherjee, J.; Acharya, S.; Goswami, S.; Mazumdar, A.; et al. A Sequential Scanning of the Immune Efficiency in Astrocytoma (Grade I to Grade III), Meningioma and Secondary Glioma Patients with and without Therapeutic Scheduling. *Cancer Invest.* **2006**, *24*, 502–513. https://doi.org/10.1080/07357900600814839.
37. Schiltz, P.M.; Lee, G.J.; Zhang, J.G.; Hoa, N.; Wepsic, H.T.; Dillman, R.O.; Jadus, M.R. Human Allogeneic and Murine Xenogeneic Dendritic Cells Are Cytotoxic to Human Tumor Cells via Two Distinct Pathways. *Cancer Biother Radiopharm* **2007**, *22*, 672–683. https://doi.org/10.1089/cbr.2007.356.
38. Sasaki, A.; Yokoo, H.; Tanaka, Y.; Homma, T.; Nakazato, Y.; Ohgaki, H. Characterization of Microglia/Macrophages in Gliomas Developed in S-100β-v-ErbB Transgenic Rats. *Neuropathology* **2013**, *33*, 505–514. https://doi.org/10.1111/neup.12015.
39. Hamilton, L.; Astell, K.R.; Velikova, G.; Sieger, D. A Zebrafish Live Imaging Model Reveals Differential Responses of Microglia Toward Glioblastoma Cells In Vivo. *Zebrafish* **2016**, *13*, 523–534. https://doi.org/10.1089/zeb.2016.1339.
40. Gu, R.; Zhang, X.; Zhang, G.; Tao, T.; Yu, H.; Liu, L.; Dou, Y.; Li, A.; Qin, J. Probing the Bi-Directional Interaction Between Microglia and Gliomas in a Tumor Microenvironment on a Microdevice. *Neurochem. Res.* **2017**, *42*, 1478–1487. https://doi.org/10.1007/s11064-017-2204-1.
41. Persano, S.; Vicini, F.; Poggi, A.; Fernandez, J.L.C.; Rizzo, G.M.R.; Gavilán, H.; Silvestri, N.; Pellegrino, T. Elucidating the Innate Immunological Effects of Mild Magnetic Hyperthermia on U87 Human Glioblastoma Cells: An In Vitro Study. *Pharmaceutics* **2021**, *13*, 1–22. https://doi.org/10.3390/pharmaceutics13101668.
42. Chang, J.C.Y.; Wang, C.Y.; Lin, S. Interrogation of Human Microglial Phagocytosis by CRISPR Genome Editing. *Front. Immunol.* **2023**, *14*, 1–18. https://doi.org/10.3389/fimmu.2023.1169725.
43. Lecoultre, M.; Chliate, S.; Espinoza, F.I.; Tankov, S.; Dutoit, V.; Walker, P.R. Radio-Chemotherapy of Glioblastoma Cells Promotes Phagocytosis by Macrophages in Vitro. *Radiother. Oncol.* **2024**, *190*. https://doi.org/10.1016/j.radonc.2023.110049.
44. Logtenberg, M.E.W.; Scheeren, F.A.; Schumacher, T.N. The CD47-SIRPα Immune Checkpoint. *Immunity* **2020**, *52*, 742–752. https://doi.org/10.1016/j.immuni.2020.04.011.

45. Khalaji, A.; Yancheshmeh, F.B.; Farham, F.; Khorram, A.; Sheshbolouki, S.; Zokaei, M.; Vatankhah, F.; Soleymani-Goloujeh, M. Don't Eat Me/Eat Me Signals as a Novel Strategy in Cancer Immunotherapy. *Heliyon* **2023**, *9*, e20507. https://doi.org/10.1016/j.heliyon.2023.e20507.
46. Liu, Y.; Wang, Y.; Yang, Y.; Weng, L.; Wu, Q.; Zhang, J.; Zhao, P.; Fang, L.; Shi, Y.; Wang, P. Emerging Phagocytosis Checkpoints in Cancer Immunotherapy. *Signal Transduct. Target. Ther.* **2023**, *8*, 104. https://doi.org/10.1038/s41392-023-01365-z.
47. Barclay, A.N.; Brown, M.H. The SIRP Family of Receptors and Immune Regulation. *Nat. Rev. Immunol.* **2006**, *6*, 457–464. https://doi.org/10.1038/nri1859.
48. Brooke, G.P.; Parsons, K.R.; Howard, C.J. Cloning of Two Members of the SIRPα Family of Protein Tyrosine Phosphatase Binding Proteins in Cattle That Are Expressed on Monocytes and a Subpopulation of Dendritic Cells and Which Mediate Binding to CD4 T Cells. *Eur. J. Immunol.* **1998**, *28*, 1–11. https://doi.org/10.1002/(SICI)1521-4141(199801)28:01<1::AID-IMMU1>3.0.CO;2-V.
49. Willingham, S.B.; Volkmer, J.-P.; Gentles, a. J.; Sahoo, D.; Dalerba, P.; Mitra, S.S.; Wang, J.; Contreras-Trujillo, H.; Martin, R.; Cohen, J.D.; et al. The CD47-Signal Regulatory Protein Alpha (SIRPa) Interaction Is a Therapeutic Target for Human Solid Tumors. *Proc. Natl. Acad. Sci.* **2012**, *109*, 6662–6667. https://doi.org/10.1073/pnas.1121623109.
50. Tseng, D.; Volkmer, J.P.; Willingham, S.B.; Contreras-Trujillo, H.; Fathman, J.W.; Fernhoff, N.B.; Seita, J.; Inlay, M.A.; Weiskopf, K.; Miyanishi, M.; et al. Anti-CD47 Antibody-Mediated Phagocytosis of Cancer by Macrophages Primes an Effective Antitumor T-Cell Response. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 11103–11108. https://doi.org/10.1073/pnas.1305569110.
51. Feng, M.; Chen, J.Y.; Weissman-Tsukamoto, R.; Volkmer, J.-P.; Ho, P.Y.; McKenna, K.M.; Cheshier, S.; Zhang, M.; Guo, N.; Gip, P.; et al. Macrophages Eat Cancer Cells Using Their Own Calreticulin as a Guide: Roles of TLR and Btk. *Proc. Natl. Acad. Sci. U. S. A.* **2015**, *112*, 2145–2150. https://doi.org/10.1073/pnas.1424907112.
52. Maute, R.; Xu, J.; Weissman, I.L. CD47–SIRPα-Targeted Therapeutics: Status and Prospects. *Immuno-Oncology Technol.* **2022**, *13*. https://doi.org/10.1016/j.iotech.2022.100070.
53. Gholamin, S.; Mitra, S.S.; Feroze, A.H.; Liu, J.; Kahn, S.A.; Zhang, M.; Esparza, R.; Richard, C.; Ramaswamy, V.; Remke, M.; et al. Disrupting the CD47-SIRPα Anti-Phagocytic Axis by a Humanized Anti-CD47 Antibody Is an Efficacious Treatment for Malignant Pediatric Brain Tumors. *Sci. Transl. Med.* **2017**, *9*, 1–14. https://doi.org/10.1126/scitranslmed.aaf2968.
54. Sikic, B.I.; Lakhani, N.; Patnaik, A.; Shah, S.A.; Chandana, S.R.; Rasco, D.; Colevas, A.D.; O'Rourke, T.; Narayanan, S.; Papadopoulos, K.; et al. First-in-Human, First-in-Class Phase i Trial of the Anti-CD47 Antibody Hu5F9-G4 in Patients with Advanced Cancers. *J. Clin. Oncol.* **2019**, *37*, 946–953. https://doi.org/10.1200/JCO.18.02018.
55. Ring, N.G.; Herndler-Brandstetter, D.; Weiskopf, K.; Shan, L.; Volkmer, J.P.; George, B.M.; Lietzenmayer, M.; McKenna, K.M.; Naik, T.J.; McCarty, A.; et al. Anti-SIRPα Antibody Immunotherapy Enhances Neutrophil and Macrophage Antitumor Activity. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114*, E10578–E10585. https://doi.org/10.1073/pnas.1710877114.
56. Nickles, D.; Abschuetz, A.; Zimmer, H.; Kees, T.; Geibig, R.; Spiess, E.; Régnier-Vigouroux, A. End-Stage Dying Glioma Cells Are Engulfed by Mouse Microglia with a Strain-Dependent Efficacy. *J. Neuroimmunol.* **2008**, *197*, 10–20. https://doi.org/10.1016/j.jneuroim.2008.03.022.
57. Kees, T.; Lohr, J.; Noack, J.; Mora, R.; Gdynia, G.; Tödt, G.; Ernst, A.; Radlwimmer, B.; Falk, C.S.; Herold-Mende, C.; et al. Microglia Isolated from Patients with Glioma Gain Antitumor Activities on Poly (I:C) Stimulation. *Neuro. Oncol.* **2012**, *14*, 64–78. https://doi.org/10.1093/neuonc/nor182.
58. Kim, H.-J.; Park, J.H.; Kim, H.C.; Kim, C.W.; Kang, I.; Lee, H.K. Blood Monocyte-Derived CD169+ Macrophages Contribute to Antitumor Immunity against Glioblastoma. *Nat. Commun.* **2022**, *13*, 6211. https://doi.org/10.1038/s41467-022-34001-5.
59. Reiter, I.; Krammer, B.; Schwamberger, G. Cutting Edge: Differential Effect of Apoptotic versus Necrotic Tumor Cells on Macrophage Antitumor Activities. *J. Immunol.* **1999**, *163*, 1730–1732.
60. Wu, M.; Wu, L.; Wu, W.; Zhu, M.; Li, J.; Wang, Z.; Li, J.; Ding, R.; Liang, Y.; Li, L.; et al. Phagocytosis of Glioma Cells Enhances the Immunosuppressive Phenotype of Bone Marrow-Derived Macrophages. *Cancer Res.* **2023**, *83*, 771–785. https://doi.org/10.1158/0008-5472.CAN-22-1570.
61. Nylandsted, J.; Wick, W.; Hirt, U.A.; Brand, K.; Rohde, M.; Leist, M.; Weller, M.; Jäättelä, M. Eradication of Glioblastoma, and Breast and Colon Carcinoma Xenografts by Hsp70 Depletion. *Cancer Res.* **2002**, *62*, 7139–7142.
62. Zhu, H.; Leiss, L.; Yang, N.; Rygh, C.B.; Mitra, S.S.; Cheshier, S.H.; Weissman, I.L.; Huang, B.; Miletic, H.; Bjerkvig, R.; et al. Surgical Debulking Promotes Recruitment of Macrophages and Triggers Glioblastoma Phagocytosis in Combination with CD47 Blocking Immunotherapy. *Oncotarget* **2017**, *8*, 12145–12157. https://doi.org/10.18632/oncotarget.14553.
63. Zhang, M.; Hutter, G.; Kahn, S.A.; Azad, T.D.; Gholamin, S.; Xu, C.Y.; Liu, J.; Achrol, A.S.; Richard, C.; Sommerkamp, P.; et al. Anti-CD47 Treatment Stimulates Phagocytosis of Glioblastoma by M1 and M2

Polarized Macrophages and Promotes M1 Polarized Macrophages In Vivo. *PLoS One* **2016**, *11*, e0153550. https://doi.org/10.1371/journal.pone.0153550.

64. Gholamin, S.; Youssef, O.A.; Rafat, M.; Esparza, R.; Kahn, S.; Shahin, M.; Giaccia, A.J.; Graves, E.E.; Weissman, I.; Mitra, S.; et al. Irradiation or Temozolomide Chemotherapy Enhances Anti-CD47 Treatment of Glioblastoma. *Innate Immun.* **2020**, *26*, 130–137. https://doi.org/10.1177/1753425919876690.
65. von Roemeling, C.A.; Wang, Y.; Qie, Y.; Yuan, H.; Zhao, H.; Liu, X.; Yang, Z.; Yang, M.; Deng, W.; Bruno, K.A.; et al. Therapeutic Modulation of Phagocytosis in Glioblastoma Can Activate Both Innate and Adaptive Antitumour Immunity. *Nat. Commun.* **2020**, *11*, 1–22. https://doi.org/10.1038/s41467-020-15129-8.
66. Zhang, X.; Wang, S.; Nan, Y.; Fan, J.; Chen, W.; Luan, J.; Wang, Y.; Liang, Y.; Li, S.; Tian, W.; et al. Inhibition of Autophagy Potentiated the Anti-Tumor Effects of VEGF and CD47 Bispecific Therapy in Glioblastoma. *Appl. Microbiol. Biotechnol.* **2018**, *102*, 6503–6513. https://doi.org/10.1007/s00253-018-9069-3.
67. Xu, B.; Tian, L.; Chen, J.; Wang, J.; Ma, R.; Dong, W.; Li, A.; Zhang, J.; Antonio Chiocca, E.; Kaur, B.; et al. An Oncolytic Virus Expressing a Full-Length Antibody Enhances Antitumor Innate Immune Response to Glioblastoma. *Nat. Commun.* **2021**, *12*, 5908. https://doi.org/10.1038/s41467-021-26003-6.
68. Song, J.; Zhang, H.; Wang, D.; Wang, J.; Zhou, J.; Zhang, Z.; Wang, J.; Hu, Y.; Xu, Q.; Xie, C.; et al. Hydrogel Loading Functionalized PAMAM/ShRNA Complex for Postsurgical Glioblastoma Treatment. *J. Control. Release* **2021**, *338*, 583–592. https://doi.org/10.1016/j.jconrel.2021.08.052.
69. Ye, L.; Lv, W.; He, W.; Li, S.; Min, Z.; Gong, L.; Zhang, Q.; Teng, C.; Sun, S.; Lv, L.; et al. Reduced Malignant Glioblastoma Recurrence Post-Resection through the Anti-CD47 Antibody and Temozolomide Co-Embedded in-Situ Hydrogel System. *J. Control. Release* **2023**, *359*, 224–233. https://doi.org/10.1016/j.jconrel.2023.05.046.
70. Tsai, R.K.; Discher, D.E. Inhibition of "Self" Engulfment through Deactivation of Myosin-II at the Phagocytic Synapse between Human Cells. *J. Cell Biol.* **2008**, *180*, 989–1003. https://doi.org/10.1083/jcb.200708043.
71. Piccione, E.C.; Juarez, S.; Tseng, S.; Liu, J.; Stafford, M.; Narayanan, C.; Wang, L.; Weiskopf, K.; Majeti, R. SIRPα-Antibody Fusion Proteins Selectively Bind and Eliminate Dual Antigen-Expressing Tumor Cells. *Clin. Cancer Res.* **2016**, *22*, 5109–5119. https://doi.org/10.1158/1078-0432.CCR-15-2503.
72. Yu, J.; Li, S.; Chen, D.; Liu, D.; Guo, H.; Yang, C.; Zhang, W.; Zhang, L.; Zhao, G.; Tu, X.; et al. SIRPα-Fc Fusion Protein IMM01 Exhibits Dual Anti-Tumor Activities by Targeting CD47/SIRPα Signal Pathway via Blocking the "Don't Eat Me" Signal and Activating the "Eat Me" Signal. *J. Hematol. Oncol.* **2022**, *15*, 2–5. https://doi.org/10.1186/s13045-022-01385-2.
73. Shimu, A.S.; Wei, H. xing; Li, Q.; Zheng, X.; Li, B. The New Progress in Cancer Immunotherapy. *Clin. Exp. Med.* **2022**. https://doi.org/10.1007/s10238-022-00887-0.
74. Zhang, X.; Chen, W.; Fan, J.; Wang, S.; Xian, Z.; Luan, J.; Li, Y.; Wang, Y.; Nan, Y.; Luo, M.; et al. Disrupting CD47-SIRPα Axis Alone or Combined with Autophagy Depletion for the Therapy of Glioblastoma. *Carcinogenesis* **2018**, *39*, 689–699. https://doi.org/10.1093/carcin/bgy041.
75. Chen, T.T.; Brown, E.J.; Huang, E.J.; Seaman, W.E. Expression and Activation of Signal Regulatory Protein α on Astrocytomas. *Cancer Res.* **2004**, *64*, 117–127. https://doi.org/10.1158/0008-5472.CAN-3455-2.
76. Deshpande, R.P.; Chandra Sekhar, Y.B.V.K.; Panigrahi, M.; Babu, P.P. SIRP Alpha Protein Downregulates in Human Astrocytoma: Presumptive Involvement of Hsa-MiR-520d-5p and Hsa-MiR-520d-3p. *Mol. Neurobiol.* **2017**, *54*, 8162–8169. https://doi.org/10.1007/s12035-016-0302-8.
77. Price, G.; Bouras, A.; Hambardzumyan, D.; Hadjipanayis, C.G. Current Knowledge on the Immune Microenvironment and Emerging Immunotherapies in Diffuse Midline Glioma. *EBioMedicine* **2021**, *69*, 103453. https://doi.org/10.1016/j.ebiom.2021.103453.
78. Turco, V.; Pfleiderer, K.; Hunger, J.; Horvat, N.K.; Karimian-Jazi, K.; Schregel, K.; Fischer, M.; Brugnara, G.; Jähne, K.; Sturm, V.; et al. T Cell-Independent Eradication of Experimental Glioma by Intravenous TLR7/8-Agonist-Loaded Nanoparticles. *Nat. Commun.* **2023**, *14*, 771. https://doi.org/10.1038/s41467-023-36321-6.
79. Huang, Y.; Zhang, Q.; Lubas, M.; Yuan, Y.; Yalcin, F.; Efe, I.E.; Xia, P.; Motta, E.; Buonfiglioli, A.; Lehnardt, S.; et al. Synergistic Toll-like Receptor 3/9 Signaling Affects Properties and Impairs Glioma-Promoting Activity of Microglia. *J. Neurosci.* **2020**, *40*, 6428–6443. https://doi.org/10.1523/JNEUROSCI.066620.2020.
80. Zhou, Y.; Guo, Y.; Chen, L.; Zhang, X.; Wu, W.; Yang, Z.; Li, X.; Wang, Y.; Hu, Z.; Wang, Z. Co-Delivery of Phagocytosis Checkpoint and STING Agonist by a Trojan Horse Nanocapsule for Orthotopic Glioma Immunotherapy. *Theranostics* **2022**, *12*, 5488–5503. https://doi.org/10.7150/thno.73104.
81. Marquardt, V.; Theruvath, J.; Pauck, D.; Picard, D.; Qin, N.; Blümel, L.; Maue, M.; Bartl, J.; Ahmadov, U.; Langini, M.; et al. Tacedinaline (CI-994), a Class I HDAC Inhibitor, Targets Intrinsic Tumor Growth and Leptomeningeal Dissemination in MYC-Driven Medulloblastoma While Making Them Susceptible to Anti-CD47-Induced Macrophage Phagocytosis via NF-KB-TGM2 Driven Tumor Inflammat. *J. Immunother. cancer* **2023**, *11*, e005871. https://doi.org/10.1136/jitc-2022-005871.
82. Tu, J.; Fang, Y.; Han, D.; Tan, X.; Xu, Z.; Jiang, H.; Wang, X.; Hong, W.; Wei, W. MicroRNA-22 Represses Glioma Development via Activation of Macrophage-Mediated Innate and Adaptive Immune Responses. *Oncogene* **2022**, *41*, 2444–2457. https://doi.org/10.1038/s41388-022-02236-7.

83. Sick, E.; Boukhari, A.; Deramaudt, T.; Rondé, P.; Bucher, B.; André, P.; Gies, J.P.; Takeda, K. Activation of CD47 Receptors Causes Proliferation of Human Astrocytoma but Not Normal Astrocytes via an Akt-Dependent Pathway. *Glia* **2011**, *59*, 308–319. https://doi.org/10.1002/glia.21102.
84. Liu, X.; Wu, X.; Wang, Y.; Li, Y.; Chen, X.; Yang, W.; Jiang, L. CD47 Promotes Human Glioblastoma Invasion through Activation of the PI3K/Akt Pathway. *Oncol. Res.* **2019**, *27*, 415–422. https://doi.org/10.3727/096504018X15155538502359.
85. Daubon, T.; Léon, C.; Clarke, K.; Andrique, L.; Salabert, L.; Darbo, E.; Pineau, R.; Guérit, S.; Maitre, M.; Dedieu, S.; et al. Deciphering the Complex Role of Thrombospondin-1 in Glioblastoma Development. *Nat. Commun.* **2019**, *10*. https://doi.org/10.1038/s41467-019-08480-y.
86. Ma, D.; Liu, S.; Lal, B.; Wei, S.; Wang, S.; Zhan, D.; Zhang, H.; Lee, R.S.; Gao, P.; Lopez-Bertoni, H.; et al. Extracellular Matrix Protein Tenascin C Increases Phagocytosis Mediated by CD47 Loss of Function in Glioblastoma. *Cancer Res.* **2019**, *79*, 2697–2708. https://doi.org/10.1158/0008-5472.CAN-18-3125.
87. Gao, X.; McDonald, J.T.; Hlatky, L.; Enderling, H. Acute and Fractionated Irradiation Differentially Modulate Glioma Stem Cell Division Kinetics. *Cancer Res.* **2013**, *73*, 1481–1490. https://doi.org/10.1158/0008-5472.CAN-12-3429.
88. Li, F.; Lv, B.; Liu, Y.; Hua, T.; Han, J.; Sun, C.; Xu, L.; Zhang, Z.; Feng, Z.; Cai, Y.; et al. Blocking the CD47-SIRPα Axis by Delivery of Anti-CD47 Antibody Induces Antitumor Effects in Glioma and Glioma Stem Cells. *Oncoimmunology* **2018**, *7*, e1391973. https://doi.org/10.1080/2162402X.2017.1391973.
89. Sun, T.; Liu, B.; Cao, Y.; Li, Y.; Cai, L.; Yang, W. AMPK-Mediated CD47 H3K4 Methylation Promotes Phagocytosis Evasion of Glioma Stem Cells Post-Radiotherapy. *Cancer Lett.* **2024**, *583*, 216605. https://doi.org/10.1016/j.canlet.2023.216605.
90. Affronti, H.C.; Wellen, K.E. Epigenetic Control of Fatty-Acid Metabolism Sustains Glioma Stem Cells. *Cancer Discov.* **2019**, *9*, 1161–1163. https://doi.org/10.1158/2159-8290.CD-19-0733.
91. Jiang, N.; Xie, B.; Xiao, W.; Fan, M.; Xu, S.; Duan, Y.; Hamsafar, Y.; Evans, A.C.; Huang, J.; Zhou, W.; et al. Fatty Acid Oxidation Fuels Glioblastoma Radioresistance with CD47-Mediated Immune Evasion. *Nat. Commun.* **2022**, *13*, 1511. https://doi.org/10.1038/s41467-022-29137-3.
92. Simion, C.; Cedano-Prieto, M.E.; Sweeney, C. The LRIG Family: Enigmatic Regulators of Growth Factor Receptor Signaling. *Endocr. Relat. Cancer* **2014**, *21*, R431–R443. https://doi.org/10.1530/ERC-14-0179.
93. Mao, F.; Wang, B.; Xiao, Q.; Cheng, F.; Lei, T.; Guo, D. LRIG Proteins in Glioma: Functional Roles, Molecular Mechanisms, and Potential Clinical Implications. *J. Neurol. Sci.* **2017**, *383*, 56–60. https://doi.org/10.1016/j.jns.2017.10.025.
94. Xie, R.; Yang, H.; Xiao, Q.; Mao, F.; Zhang, S.; Ye, F.; Wan, F.; Wang, B.; Lei, T.; Guo, D. Downregulation of LRIG1 Expression by RNA Interference Promotes the Aggressive Properties of Glioma Cells via EGFR/Akt/c-Myc Activation. *Oncol. Rep.* **2013**, *29*, 177–184. https://doi.org/10.3892/or.2012.2102.
95. Rondahl, V.; Holmlund, C.; Karlsson, T.; Wang, B.; Faraz, M.; Henriksson, R.; Hedman, H. Lrig2-Deficient Mice Are Protected against PDGFB-Induced Glioma. *PLoS One* **2013**, *8*, 1–18. https://doi.org/10.1371/journal.pone.0073635.
96. Hu, J.; Dong, F.; He, Y.; Xia, X.; Cheng, F.; Chen, S.; Hou, X.; Zhang, P.; Liu, G.; Li, Y.; et al. LRIG2 Promotes Glioblastoma Progression by Modulating Innate Antitumor Immunity through Macrophage Infiltration and Polarization. *J. Immunother. cancer* **2022**, *10*, 1–13. https://doi.org/10.1136/jitc-2021-004452.
97. Gowda, P.; Patrick, S.; Singh, A.; Sheikh, T.; Sen, E. Mutant Isocitrate Dehydrogenase 1 Disrupts PKM2-β-Catenin-BRG1 Transcriptional Network-Driven CD47 Expression. *Mol. Cell. Biol.* **2018**, *38*. https://doi.org/10.1128/MCB.00001-18.
98. de Groot, J.F.; Gilbert, M.R.; Aldape, K.; Hess, K.R.; Hanna, T. a; Ictech, S.; Groves, M.D.; Conrad, C.; Colman, H.; Puduvalli, V.K.; et al. Phase II Study of Carboplatin and Erlotinib (Tarceva, OSI-774) in Patients with Recurrent Glioblastoma. *J. Neurooncol.* **2008**, *90*, 89–97. https://doi.org/10.1007/s11060-008-9637-y.
99. Du, L.; Su, Z.; Wang, S.; Meng, Y.; Xiao, F.; Xu, D.; Li, X.; Qian, X.; Lee, S. Bin; Lee, J.H.; et al. EGFR-Induced and c-Src-Mediated CD47 Phosphorylation Inhibits TRIM21-Dependent Polyubiquitylation and Degradation of CD47 to Promote Tumor Immune Evasion. *Adv. Sci.* **2023**, *2206380*, 1–15. https://doi.org/10.1002/advs.202206380.
100. Stanley, E.R.; Chitu, V. CSF-1 Receptor Signaling in Myeloid Cells. *Cold Spring Harb. Perspect. Biol.* **2014**, *6*, 1–22. https://doi.org/10.1101/cshperspect.a021857.
101. Guerriero, J.L. Macrophages: The Road Less Traveled, Changing Anticancer Therapy. *Trends Mol. Med.* **2018**, *24*, 472–489. https://doi.org/10.1016/j.molmed.2018.03.006.
102. Ulland, T.K.; Wang, Y.; Colonna, M. Regulation of Microglial Survival and Proliferation in Health and Diseases. *Semin. Immunol.* **2015**, *27*, 410–415. https://doi.org/10.1016/j.smim.2016.03.011.
103. Di Nunno, V.; Aprile, M.; Gatto, L.; Tosoni, A.; Ranieri, L.; Bartolini, S.; Franceschi, E. Tumor Microenvironment in Gliomas: A Treatment Hurdle or an Opportunity to Grab? *Cancers (Basel).* **2023**, *15*, 1–19. https://doi.org/10.3390/cancers15041042.
104. Condeelis, J.; Pollard, J.W. Macrophages: Obligate Partners for Tumor Cell Migration, Invasion, and Metastasis. *Cell* **2006**, *124*, 263–266. https://doi.org/10.1016/j.cell.2006.01.007.

105. Cannarile, M.A.; Weisser, M.; Jacob, W.; Jegg, A.-M.; Ries, C.H.; Rüttinger, D. Colony-Stimulating Factor 1 Receptor (CSF1R) Inhibitors in Cancer Therapy. *J. Immunother. cancer* **2017**, *5*, 53. https://doi.org/10.1186/s40425-017-0257-y.
106. Coniglio, S.J.; Segall, J.E. Microglial-Stimulation of Glioma Invasion Involves the EGFR Ligand Amphiregulin. *PLoS One* **2021**, *16*, e0260252. https://doi.org/10.1371/journal.pone.0260252.
107. Zeren, N.; Afzal, Z.; Morgan, S.; Marshall, G.; Uppiliappan, M.; Merritt, J.; Coniglio, S.J. The Chemokine Receptor CCR1 Mediates Microglia Stimulated Glioma Invasion. *Int. J. Mol. Sci.* **2023**, *24*, 5136. https://doi.org/10.3390/ijms24065136.
108. Pyonteck, S.M.; Akkari, L.; Schuhmacher, A.J.; Bowman, R.L.; Sevenich, L.; Quail, D.F.; Olson, O.C.; Quick, M.L.; Huse, J.T.; Teijeiro, V.; et al. CSF-1R Inhibition Alters Macrophage Polarization and Blocks Glioma Progression. *Nat. Med.* **2013**, *19*, 1264–1272. https://doi.org/10.1038/nm.3337.
109. Rougerie, P.; Miskolci, V.; Cox, D. Generation of Membrane Structures during Phagocytosis and Chemotaxis of Macrophages: Role and Regulation of the Actin Cytoskeleton. *Immunol. Rev.* **2013**, *256*, 222–239. https://doi.org/10.1111/imr.12118.
110. Fermi, V.; Warta, R.; Wöllner, A.; Lotsch, C.; Jassowicz, L.; Rapp, C.; Knoll, M.; Jungwirth, G.; Jungk, C.; Dao Trong, P.; et al. Effective Reprogramming of Patient-Derived M2-Polarized Glioblastoma-Associated Microglia/Macrophages by Treatment with GW2580. *Clin. Cancer Res.* **2023**, *29*, 4685–4697. https://doi.org/10.1158/1078-0432.CCR-23-0576.
111. Butowski, N.; Colman, H.; De Groot, J.F.; Omuro, A.M.; Nayak, L.; Wen, P.Y.; Cloughesy, T.F.; Marimuthu, A.; Haidar, S.; Perry, A.; et al. Orally Administered Colony Stimulating Factor 1 Receptor Inhibitor PLX3397 in Recurrent Glioblastoma: An Ivy Foundation Early Phase Clinical Trials Consortium Phase II Study. *Neuro. Oncol.* **2016**, *18*, 557–564. https://doi.org/10.1093/neuonc/nov245.
112. Eda, H.; Zhang, J.; Keith, R.H.; Michener, M.; Beidler, D.R.; Monahan, J.B. Macrophage-Colony Stimulating Factor and Interleukin-34 Induce Chemokines in Human Whole Blood. *Cytokine* **2010**, *52*, 215–220. https://doi.org/10.1016/j.cyto.2010.08.005.
113. Pixley, F.J.; Stanley, E.R. CSF-1 Regulation of the Wandering Macrophage: Complexity in Action. *Trends Cell Biol.* **2004**, *14*, 628–638. https://doi.org/10.1016/j.tcb.2004.09.016.
114. Jadus, M.R.; Irwin, M.C.; Irwin, M.R.; Horansky, R.D.; Sekhon, S.; Pepper, K. a; Kohn, D.B.; Wepsic, H.T. Macrophages Can Recognize and Kill Tumor Cells Bearing the Membrane Isoform of Macrophage Colony-Stimulating Factor. *Blood* **1996**, *87*, 5232–5241.
115. Jadus, M.R.; Williams, C.C.; Avina, M.D.; Ly, M.; Kim, S.; Liu, Y.; Narasaki, R.; Lowell, C.A.; Wepsic, H.T. Macrophages Kill T9 Glioma Tumor Cells Bearing the Membrane Isoform of Macrophage Colony Stimulating Factor through a Phagocytosis-Dependent Pathway. *J Immunol* **1998**, *160*, 361–368.
116. Jadus, M.R.; Chen, Y.; Boldaji, M.T.; Delgado, C.; Sanchez, R.; Douglass, T.; Al-Atar, U.; Schulz, W.; Lloyd, C.; Wepsic, H.T. Human U251MG Glioma Cells Expressing the Membrane Form of Macrophage Colony-Stimulating Factor (MM-CSF) Are Killed by Human Monocytes in Vitro and Are Rejected within Immunodeficient Mice via Paraptosis That Is Associated with Increased Expression of Thre. *Cancer Gene Ther.* **2003**, *10*, 411–420. https://doi.org/10.1038/sj.cgt.7700583.
117. Miners, J.S.; Baig, S.; Palmer, J.; Palmer, L.E.; Kehoe, P.G.; Love, S. Aβ-Degrading Enzymes in Alzheimer's Disease. *Brain Pathol.* **2008**, *18*, 240–252. https://doi.org/10.1111/j.1750-3639.2008.00132.x.
118. Zhai, K.; Huang, Z.; Huang, Q.; Tao, W.; Fang, X.; Zhang, A.; Li, X.; Stark, G.R.; Hamilton, T.A.; Bao, S. Pharmacological Inhibition of BACE1 Suppresses Glioblastoma Growth by Stimulating Macrophage Phagocytosis of Tumor Cells. *Nat. cancer* **2021**, *2*, 1136–1151. https://doi.org/10.1038/s43018-021-00267-9.
119. Lu, J.; Wang, Z.; He, Z.; Hu, Y.; Duan, H.; Liu, Z.; Li, D.; Zhong, S.; Ren, J.; Zhao, G.; et al. Oligomer-Aβ42 Suppress Glioma Progression via Potentiating Phagocytosis of Microglia. *CNS Neurosci. Ther.* **2024**, *30*, 1–14. https://doi.org/10.1111/cns.14495.
120. Cappellano, G.; Vecchio, D.; Magistrelli, L.; Clemente, N.; Raineri, D.; Barbero Mazzucca, C.; Virgilio, E.; Dianzani, U.; Chiocchetti, A.; Comi, C. The Yin-Yang of Osteopontin in Nervous System Diseases: Damage versus Repair. *Neural Regen. Res.* **2021**, *16*, 1131–1137. https://doi.org/10.4103/1673-5374.300328.
121. Peshoff, M.M.; Gupta, P.; Trivedi, R.; Oberai, S.; Chakrapani, P.; Dang, M.; Milam, N.; Maynard, M.E.; Vaillant, B.D.; Huse, J.T.; et al. Triggering Receptor Expressed on Myeloid Cells 2 (TREM2) Regulates Phagocytosis in Glioblastoma. *bioRxiv Prepr. Serv. Biol.* **2023**, *2*. https://doi.org/10.1101/2023.04.05.535792.
122. Zheng, J.; Wang, L.; Zhao, S.; Zhang, W.; Chang, Y.; Dheer, A.; Gao, S.; Xu, S.; Ayasoufi, K.; Al-Kharboosh, R.; et al. TREM2 Mediates MHCII-Associated CD4 + T Cell Response against Gliomas. *bioRxiv Prepr. Serv. Biol.* **2023**, 31–41. https://doi.org/10.1101/2023.04.05.535697.
123. Wolf, E.M.; Fingleton, B.; Hasty, A.H. The Therapeutic Potential of TREM2 in Cancer. *Front. Oncol.* **2022**, *12*, 1–10. https://doi.org/10.3389/fonc.2022.984193.
124. Luo, Y.; Pollard, J.W.; Casadevall, A. Fcgamma Receptor Cross-Linking Stimulates Cell Proliferation of Macrophages via the ERK Pathway. *J. Biol. Chem.* **2010**, *285*, 4232–4242. https://doi.org/10.1074/jbc.M109.037168.

125. Gordon, S. Phagocytosis: An Immunobiologic Process. *Immunity* **2016**, *44*, 463–475. https://doi.org/10.1016/j.immuni.2016.02.026.
126. Sun, R.; Han, R.; McCornack, C.; Khan, S.; Tabor, G.T.; Chen, Y.; Hou, J.; Jiang, H.; Schoch, K.M.; Mao, D.D.; et al. TREM2 Inhibition Triggers Antitumor Cell Activity of Myeloid Cells in Glioblastoma. *Sci. Adv.* **2023**, *9*, eade3559. https://doi.org/10.1126/sciadv.ade3559.
127. Maverakis, E.; Kim, K.; Shimoda, M.; Gershwin, M.E.; Patel, F.; Wilken, R.; Raychaudhuri, S.; Ruhaak, L.R.; Lebrilla, C.B. Glycans in the Immune System and The Altered Glycan Theory of Autoimmunity: A Critical Review. *J. Autoimmun.* **2015**, *57*, 1–13. https://doi.org/10.1016/j.jaut.2014.12.002.
128. Qin, R.; Mahal, L.K. The Host Glycomic Response to Pathogens. *Curr. Opin. Struct. Biol.* **2021**, *68*, 149–156. https://doi.org/10.1016/j.sbi.2020.12.011.
129. Duan, S.; Paulson, J.C. Siglecs as Immune Cell Checkpoints in Disease. *Annu. Rev. Immunol.* **2020**, *38*, 365–395. https://doi.org/10.1146/annurev-immunol-102419-035900.
130. Grabenstein, S.; Barnard, K.N.; Anim, M.; Armoo, A.; Weichert, W.S.; Bertozzi, C.R.; Parrish, C.R.; Willand-Charnley, R. Deacetylated Sialic Acids Modulates Immune Mediated Cytotoxicity via the Sialic Acid-Siglec Pathway. *Glycobiology* **2021**, *31*, 1279–1294. https://doi.org/10.1093/glycob/cwab068.
131. Imbert, P.R.C.; Saric, A.; Pedram, K.; Bertozzi, C.R.; Grinstein, S.; Freeman, S.A. An Acquired and Endogenous Glycocalyx Forms a Bidirectional "Don't Eat" and "Don't Eat Me" Barrier to Phagocytosis. *Curr. Biol.* **2021**, *31*, 77-89.e5. https://doi.org/10.1016/j.cub.2020.09.082.
132. Boelaars, K.; van Kooyk, Y. Targeting Myeloid Cells for Cancer Immunotherapy: Siglec-7/9/10/15 and Their Ligands. *Trends in Cancer* **2024**, *10*, 230–241. https://doi.org/10.1016/j.trecan.2023.11.009.
133. Stanczak, M.A.; Mantuano, N.R.; Kirchhammer, N.; Sanin, D.E.; Jacob, F.; Coelho, R.; Everest-Dass, A. V.; Wang, J.; Trefny, M.P.; Monaco, G.; et al. Targeting Cancer Glycosylation Repolarizes Tumor-Associated Macrophages Allowing Effective Immune Checkpoint Blockade. *Sci. Transl. Med.* **2022**, *14*, 1–18. https://doi.org/10.1126/scitranslmed.abj1270.
134. Barkal, A.A.; Brewer, R.E.; Markovic, M.; Kowarsky, M.; Barkal, S.A.; Zaro, B.W.; Krishnan, V.; Hatakeyama, J.; Dorigo, O.; Barkal, L.J.; et al. CD24 Signalling through Macrophage Siglec-10 Is a Target for Cancer Immunotherapy. *Nature* **2019**, *572*, 392–396. https://doi.org/10.1038/s41586-019-1456-0.
135. Kopatz, J.; Beutner, C.; Welle, K.; Bodea, L.G.; Reinhardt, J.; Claude, J.; Linnartz-Gerlach, B.; Neumann, H. Siglec-h on Activated Microglia for Recognition and Engulfment of Glioma Cells. *Glia* **2013**, *61*, 1122–1133. https://doi.org/10.1002/glia.22501.
136. O'Neill, A.S.G.; Van Den Berg, T.K.; Mullen, G.E.D. Sialoadhesin - a Macrophage-Restricted Marker of Immunoregulation and Inflammation. *Immunology* **2013**, *138*, 198–207. https://doi.org/10.1111/imm.12042.
137. Angata, T.; Varki, A. Discovery, Classification, Evolution and Diversity of Siglecs. *Mol. Aspects Med.* **2023**, *90*, 101117. https://doi.org/10.1016/j.mam.2022.101117.
138. Chen, Q.; Chen, B.; Wang, C.; Hu, L.; Wu, Q.; Zhu, Y.; Zhang, Q. Dynamic Change in Siglec-15 Expression in Peritumoral Macrophages Confers an Immunosuppressive Microenvironment and Poor Outcome in Glioma. *Front. Immunol.* **2023**, *14*, 1–14. https://doi.org/10.3389/fimmu.2023.1159085.
139. Ene, C.I.; Ene, C.I.; Kreuser, S.A.; Jung, M.; Jung, M.; Zhang, H.; Arora, S.; White Moyes, K.; Szulzewsky, F.; Barber, J.; et al. Anti-PD-L1 Antibody Direct Activation of Macrophages Contributes to a Radiation-Induced Abscopal Response in Glioblastoma. *Neuro. Oncol.* **2020**, *22*, 639–651. https://doi.org/10.1093/neuonc/noz226.
140. Chen, D.; Varanasi, S.K.; Hara, T.; Traina, K.; Sun, M.; McDonald, B.; Farsakoglu, Y.; Clanton, J.; Xu, S.; Garcia-Rivera, L.; et al. CTLA-4 Blockade Induces a Microglia-Th1 Cell Partnership That Stimulates Microglia Phagocytosis and Anti-Tumor Function in Glioblastoma. *Immunity* **2023**, *56*, 2086-2104.e8. https://doi.org/10.1016/j.immuni.2023.07.015.
141. Lim, W.A.; June, C.H. The Principles of Engineering Immune Cells to Treat Cancer. *Cell* **2017**, *168*, 724–740. https://doi.org/10.1016/j.cell.2017.01.016.
142. Luo, C.; Wang, P.; He, S.; Zhu, J.; Shi, Y.; Wang, J. Progress and Prospect of Immunotherapy for Triple-Negative Breast Cancer. *Front. Oncol.* **2022**, *12*, 919072. https://doi.org/10.3389/fonc.2022.919072.
143. Brandt, L.J.B.; Barnkob, M.B.; Michaels, Y.S.; Heiselberg, J.; Barington, T. Emerging Approaches for Regulation and Control of CAR T Cells: A Mini Review. *Front. Immunol.* **2020**, *11*, 326. https://doi.org/10.3389/fimmu.2020.00326.
144. Bonte, S.; De Munter, S.; Goetgeluk, G.; Ingels, J.; Pille, M.; Billiet, L.; Taghon, T.; Leclercq, G.; Vandekerckhove, B.; Kerre, T. T-Cells with a Single Tumor Antigen-Specific T-Cell Receptor Can Be Generated in Vitro from Clinically Relevant Stem Cell Sources. *Oncoimmunology* **2020**, *9*. https://doi.org/10.1080/2162402X.2020.1727078.
145. Zhang, D.K.Y.; Adu-Berchie, K.; Iyer, S.; Liu, Y.; Cieri, N.; Brockman, J.M.; Neuberg, D.; Wu, C.J.; Mooney, D.J. Enhancing CAR-T Cell Functionality in a Patient-Specific Manner. *Nat. Commun.* **2023**, *14*, 1–13. https://doi.org/10.1038/s41467-023-36126-7.
146. Sharma, P.; Debinski, W. Receptor-Targeted Glial Brain Tumor Therapies. *Int. J. Mol. Sci.* **2018**, *19*, 3326. https://doi.org/10.3390/ijms19113326.

147. Sloas, C.; Gill, S.; Klichinsky, M. Engineered CAR-Macrophages as Adoptive Immunotherapies for Solid Tumors. *Front. Immunol.* **2021**, *12*, 1–10. https://doi.org/10.3389/fimmu.2021.783305.
148. Chen, Y.; Yu, Z.; Tan, X.; Jiang, H.; Xu, Z.; Fang, Y.; Han, D.; Hong, W.; Wei, W.; Tu, J. CAR-Macrophage: A New Immunotherapy Candidate against Solid Tumors. *Biomed. Pharmacother.* **2021**, *139*, 111605. https://doi.org/10.1016/j.biopha.2021.111605.
149. Qu, T.; Li, B.; Wang, Y. Targeting CD47/SIRP$\alpha$ as a Therapeutic Strategy, Where We Are and Where We Are Headed. *Biomark. Res.* **2022**, *10*, 20. https://doi.org/10.1186/s40364-022-00373-5.
150. Huo, Y.; Zhang, H.; Sa, L.; Zheng, W.; He, Y.; Lyu, H.; Sun, M.; Zhang, L.; Shan, L.; Yang, A.; et al. M1 Polarization Enhances the Antitumor Activity of Chimeric Antigen Receptor Macrophages in Solid Tumors. *J. Transl. Med.* **2023**, *21*, 225. https://doi.org/10.1186/s12967-023-04061-2.
151. Chen, C.; Jing, W.; Chen, Y.; Wang, G.; Abdalla, M.; Gao, L.; Han, M.; Shi, C.; Li, A.; Sun, P.; et al. Intracavity Generation of Glioma Stem Cell–Specific CAR Macrophages Primes Locoregional Immunity for Postoperative Glioblastoma Therapy. *Sci. Transl. Med.* **2022**, *14*, 1–12. https://doi.org/10.1126/scitranslmed.abn1128.
152. Flynn, R.A.; Pedram, K.; Malaker, S.A.; Batista, P.J.; Smith, B.A.H.; Johnson, A.G.; George, B.M.; Majzoub, K.; Villalta, P.W.; Carette, J.E.; et al. Small RNAs Are Modified with N-Glycans and Displayed on the Surface of Living Cells. *Cell* **2021**, *184*, 3109-3124.e22. https://doi.org/10.1016/j.cell.2021.04.023.
153. Pedram, K.; Shon, D.J.; Tender, G.S.; Mantuano, N.R.; Northey, J.J.; Metcalf, K.J.; Wisnovsky, S.P.; Riley, N.M.; Forcina, G.C.; Malaker, S.A.; et al. Design of a Mucin-Selective Protease for Targeted Degradation of Cancer-Associated Mucins. *bioRxiv* **2022**, 2022.05.20.492748. https://doi.org/10.1038/s41587-023-01840-6.