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Therapy-induced shaping of the glioblastoma microenvironment: Macrophages at play

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ABSTRACT

The intricate cross-talks between tumor cells and their microenvironment play a key role in cancer progression and resistance to treatment. In recent years, targeting pro-tumorigenic components of the tumor microenvironment (TME) has emerged as a tantalizing strategy to improve the efficacy of standard-of-care (SOC) treatments, particularly for hard-to-treat cancers such as glioblastoma. In this review, we explore how the distinct microenvironmental niches characteristic of the glioblastoma TME shape response to therapy. In particular, we delve into the interplay between tumor-associated macrophages (TAM) and glioblastoma cells within angiogenic and hypoxic niches, and interrogate their dynamic co-evolution upon SOC therapies that fuels malignancy. Resolving the complexity of therapy-induced alterations in the glioblastoma TME and their impact on disease relapse is a stepping stone to identify targetable pro-tumorigenic pathways and TAM subsets, and may open the way to efficient combination therapies that will improve clinical outcomes.

1. Introduction

In the last decade, immunotherapy has revolutionized cancer treatment and significantly improved the survival and quality of life for numerous patients. While a growing number of solid cancers respond to novel T cell-centric immunotherapeutics, several challenges need to be overcome in order to apply these therapeutic approaches for a majority of tumors, including glioblastoma. Glioblastoma is an aggressive primary brain cancer with a dismal prognosis, and an overall survival as low as 15 months despite standard-of-care (SOC) therapies [1]. While intrinsic properties of the tumor, such as genetic background and low mutational burden, are partly contributing to the low efficacy of SOC and immunotherapies, it has become evident that the tumor microenvironment (TME) plays crucial and timely roles in glioblastoma poor

Abbreviations: ANG, angiopoietin; Arg-1, arginase 1; BBB, blood-brain barrier; C/EBP-β, CCAAT/enhancer-binding protein beta; CCL2, C-C motif chemokine ligand 2; CCR2, C-C chemokine receptor type 2; CSF-1R, colony-stimulating factor 1 receptor; CXCL, chemokine (C-X-C motif) ligand; CXCR, C-X-C motif chemokine receptor; EGFR, epidermal growth factor receptor; EMAP-II, endothelial cell monocyte-activating polypeptide-II; ET-1, endothelin 1; FA, fatty acids; FADS, fatty acid desaturase; FAO, fatty acid oxidation; GEMM, genetically-engineered mouse model; GLUT1, glucose transporter 1; GPCR, G protein-coupled receptors; GSC, glioma stem-like cancer cell; HB-EGF, heparin-binding EGF-like growth factor; HIF, hypoxia-inducible factor; HILPDA, hypoxia-inducible lipid droplet-associated; ICAM-1, intercellular adhesion molecule 1; IDH1, isocitrate dehydrogenase 1; IDO1, indoleamine 2,3-dioxygenase 1; IFN, interferon; IKKβ, IkappaB Kinase; IL, interleukin; iNOS, inducible nitric oxide synthase; IR, ionizing radiation; IRF, interferon regulatory factor; JAK, janus kinase; JMDJ3, jumonji domain containing-3; LD, lipid droplet; LDH-A, lactate dehydrogenase A; LIFR, leukemia inhibitory factor receptor; LOX, oxidized low-density lipoprotein receptor 1; MAPK, mitogen-activated protein kinase; MDM, monocyte-derived macrophages; MES, mesenchymal; MIF, migration inhibitory factor; MMP9, matrix metallopeptidase 9; MMTV-PyMT, mouse mammary tumor virus-polyoma middle tumor-antigen; MRC1, mannose receptor 1; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NF1, neurofibromin 1; NK, natural killer; NRF2, Nuclear factor-erythroid factor 2-related factor 2; OLIG2, oligodendrocyte transcription factor 2; OS, overall survival; OSM, oncostatin M; OXPHOS, oxidative phosphorylation; PD-1, programmed-death receptor 1; PD-L1, programmed-death ligand 1; PDGF, platelet derived growth factor; PI3K, phosphoinositide 3-kinases; PMT, proneural-to-mesenchymal transition; PN, proneural; PPARy, peroxisome proliferator-activated receptor gamma; PVN, perivascular niche; ROS, reactive oxygen species; SDF1, stromal cell-derived factor 1; SLC1, solute carrier 1; SMP3A, semaphoring 3A; SOC, standard-of-care; SOX, SRY-box; SREBP, sterol regulator binding proteins; STAT, signal transducer and activator of transcription; TAM, tumor-associated macrophages; TAZ, tafazzin; TEC, tumor-associated endothelial cell; TEM, Tie2-expressing macrophages; TF, transcription factor; TGF-β, transforming growth factor β; TLR, toll-like receptor; TME, tumor microenvironment; TMZ, temozolomide; TNF-α, tumor necrosis factor alpha; VCAM-1, vascular cell adhesion molecule 1; VEGF, vascular endothelial growth factor; WISP1, WNT1 inducible signaling pathway; γ-H2AX, γ H2A histone family member X.

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response to treatment [2–4].

The brain microenvironment possesses unique features compared to other organs, with one key particularity being the presence of the blood-brain barrier (BBB). The BBB consists of vascular endothelial cells surrounded by pericytes and astrocytic end-feet, which form a tight diffusion barrier that excludes peripheral immune cells from entering the brain parenchyma under physiological conditions [5]. Therefore, at homeostasis, the immune landscape of the brain is limited and mostly consists of tissue-resident macrophages, known as microglia. Microglia are involved in specialized brain-specific processes such as synaptic pruning, phagocytosis of apoptotic cells and regulation of neuronal plasticity, as well as immune surveillance [6–8].

The integrity of the BBB is compromised during glioblastoma progression, and its partial disruption facilitates immune infiltration in the brain parenchyma, preferentially of myeloid cells [9,10]. Importantly, these infiltrated leukocytes, mostly consisting of monocyte-derived macrophages (MDMs), promote tumor outgrowth and contribute to an immunosuppressive TME [11,12]. Critically however, infiltration of adaptive immune cells, which are at the center of the current immunotherapeutic strategies applied in anti-cancer treatments, remains very limited.

Together, MDMs and microglia make up the pool of tumor-associated macrophages (TAMs) in glioblastoma. It has recently been appreciated that microglia and MDMs are macrophages of distinct ontogeny, derived from immature yolk-sac progenitors and hematopoietic progenitors respectively, a feature that contributes to their diverse and plastic phenotype within brain tumors [6,13]. Indeed, macrophages are a highly heterogeneous cell population that exist in a wide spectrum of phenotypes far beyond the proinflammatory “M1-like” and immunoregulatory “M2-like” extremes [14–17]. Over the past decade, well-described pro-tumorigenic functions have been attributed to TAMs, including their ability to fuel multiple biological processes such as angiogenesis, cell proliferation, survival, migration and immunosuppression [18].

In glioblastoma, TAMs represent up to 30% of cells within the TME, and their content is associated with enhanced tumor growth and correlates with poor patient survival [19,20]. Notably, high TAM infiltration is associated with relapse, suggesting that TAMs play a crucial role in predisposing the glioblastoma TME for tumor regrowth post-therapy [21]. Distinct TAM profiles have been reported in glioblastoma depending on the disease subtype. Indeed, glioblastoma are highly heterogeneous tumors, and can be classified into three dominant subtypes with distinct transcriptional signatures, termed proneural, classical and mesenchymal subtypes, which influence on the TME and TAM profiles has been recognized [22].

Due to their abundant presence and tumor-promoting properties, targeting TAMs in glioblastoma has been subject to extensive research. While TAM-targeting therapies have been successful in preclinical studies, therapeutic efficacy in clinical trials remains limited [23]. For instance, targeting macrophages through inhibition of the colony-stimulating factor 1 receptor (CSF-1R- a pathway required for macrophage survival and differentiation) remarkably increased overall survival (OS) in genetically-engineered mouse models (GEMM) of glioblastoma, both through its effect in limiting MDM recruitment and by inducing TAM reeducation into anti-tumorigenic actors [21]. However, a phase II clinical trial employing the CSF-1R inhibitor PLX3397 in patients with recurrent glioblastoma did not demonstrate a significant effect on OS (NCT01790503), and current trials are still ongoing regarding the efficacy of this strategy in first line glioblastoma treatment when combined with SOC. Moreover, while TAMs significantly contribute to disease progression and relapse, pan-targeting is challenged by the development of resistance [24] and remains to be successfully applied in the clinic. In light of TAMs heterogeneous content and pro- or anti-tumorigenic features dictated by their phenotype and anatomical location [4,25], the targeting of specific pro-tumorigenic TAM subsets that would spare anti-tumorigenic subsets will be essential to unleash their therapeutic potential.

Overall, despite ongoing efforts, targeting the immune TME in glioblastoma patients has led to limited therapeutic efficacy. As a result, therapy regimens consisting of maximal surgical resection combined with temozolomide (TMZ) and ionizing radiation (IR) remain the gold standard treatment, which is inevitably followed by tumor recurrence [1,26]. It has become increasingly clear that SOC treatment drastically alters the TME, promoting cancer cell plasticity that can fuel disease relapse [27]. While remodeling of the TME upon therapy is a multifactorial process, TAMs are central cellular actors in promoting a pro-tumorigenic environment, and are therefore a promising target to prevent emergence of relapse. In this review, we will explore how SOC treatment, IR in particular, remodels the brain TME and how macrophages adapt to these environmental alterations.

We postulate that understanding the dynamic co-evolution of TAMs and their surrounding niches is a major bottleneck to overcome in order to discover specific and functional subsets of TAMs involved in glioblastoma recurrence, and apply innovative therapeutic strategies which can be translated to the clinic.

2. Microenvironmental niches and glioblastoma progression

Glioblastoma is a highly heterogeneous cancer type that consists of several distinct anatomical compartments including: cellular tumor, leading edge, infiltrating tumor, hypoxic pseudopalisading regions and perivascular niches (PVNs) [28]. These microanatomical regions, or niches, can regulate major biological processes such as immune function, tumor metabolism, or cancer/stem cell maintenance [4]. Recently, an elegant study combined histological analyses, laser microdissection and RNA sequencing of clinical samples to generate gene expression profiles assigned to these dominant morphologic hallmarks of glioblastoma [28]. This work revealed the transcriptional diversity of each niche, with processes related to neuronal systems and glial differentiation characterizing leading edge and cellular tumor niches, while pseudopalisading niches were enriched with cellular stress, immune regulation and hypoxia signatures, and perivascular niches displayed enrichment of genes associated with angiogenesis, immune regulation and wound healing [28]. The high transcriptional heterogeneity observed between the different anatomical compartments illustrates the diversity of biological processes that occur within each niche, with potentially impactful consequences on tumor progression and resistance to treatment [28]. Importantly, presence of hypoxic pseudopalisading niches and microvascular density are the most informative predictors of poor prognosis among glioblastoma patients [4]. The different cell types present within each of these anatomical niches, their dynamic content pre- and post-SOC and potential role in recurrence, have only recently started to be explored using these transcriptional tools. While still in their infancy, these studies suggest that histological features underlie alterations in the cellular contexture of glioblastoma over time, thus influencing the cell state composition associated with both glioblastoma subtypes and recurrent disease [29].

2.1. *The perivascular niche (PVN)*

A central characteristic of the glioblastoma TME is extensive angiogenesis, a process defined by formation of new blood vessels in order to meet the oxygen and nutrient requirements needed for cancer cell proliferation [30]. However, the tumor vasculature differs from physiological vessels in multiple ways. While the physiological brain vasculature is organized in a hierarchical structure of arteries, veins and capillaries, the glioblastoma vasculature is disorganized and hemorrhagic as a result of aberrant pro-angiogenic signaling within the tumor [31]. Perivascular astrocytes and cancer cells secrete excessive amounts of pro-angiogenic growth factors such as angiopoietin (ANG) 1, ANG2 and vascular endothelial growth factor (VEGF), which causes basement membrane degradation and proliferation of tumor-associated

endothelial cells (TECs) that line the vessel walls [32]. Proliferating TECs are unable to form structured monolayers and are loosely interconnected [31]. This results in enlarged vessels which are leaky and highly susceptible to microhemorrhages, a condition known as chronic vascular hyperplasia [33]. In addition, cancer cell-derived factors, such as transforming growth factor β (TGF-β), displace pericytes and astrocytes from the blood vessels, leading to disruption of the BBB [34]. Consequently, increased vessel permeability allows fluids to leak into the tumor, leading to interstitial pressure and cerebral edema [35], and the BBB disruption permits the influx of peripheral immune cells which are normally excluded from the brain. These cells further promote tumor progression by conveying pro-survival signals to glioblastoma cancer cells, providing pro-angiogenic growth factors and maintaining an immune suppressive microenvironment [36].

The PVN plays an important role in glioblastoma progression, as increased microvessel density correlates with shorter survival in patients [37]. According to gene ontology analysis of perivascular tissues, genes upregulated within the tumor PVN are strongly associated with pro-tumorigenic processes such as immunosuppression, shielding of stem-like glioblastoma cells, angiogenesis and invasion [38,39]. Indeed, patient-derived glioblastoma cancer cells have been shown to exhibit increased growth in vitro and in xenograft models when co-cultured with human brain microvascular endothelial cells (HBMECs) [40]. This effect was mainly mediated by activation of proliferation pathways as a result of exposure to HBMEC-derived mitogens such as CXCL12/SDF1α and IL-8 [40,41]. Altogether, these findings unveil an important and direct role of the brain vasculature in promoting tumor growth.

2.2. The hypoxic niche

The aforementioned dysfunctional tumor vasculature leads to decreased blood-flow and inconsistent oxygen distribution, resulting in hypoxic areas within the tumor and eventually leading to pseudopalisading necrotic regions after further vessel collapse (Fig. 1) [4]. Several mechanisms for the formation of hypoxic niches have been proposed, including intravascular thrombosis, vascular regression and vascular collapse as a consequence of edema [42,43]. In response to necrotic cell death, hypoxic glioma cells become quiescent and align in palisade-like structures around the necrotic core, which has been recognized as a characterizing trait of glioblastoma [44]. Besides phenotypic changes, hypoxic cells also induce highly conserved signaling pathways in order to overcome hypoxic stress, resulting in the acquisition of an aggressive growth pattern [45,46].

Activation of hypoxia-induced transcription factors (TFs) such as hypoxia-inducible factor- (HIF) 1α and HIF-2α is associated with the induction and maintenance of stem cell-like characteristics in cancer cells. Indeed, HIF-2α stabilization leads to the induction of a stem cell-like genetic program, endowing glioblastoma cancer cells with self-renewal ability [47]. Furthermore, hypoxic cells plays a prominent role in sustaining the formation of new blood vessels, aiming to restore oxygen levels by secreting various pro-angiogenic growth factors,

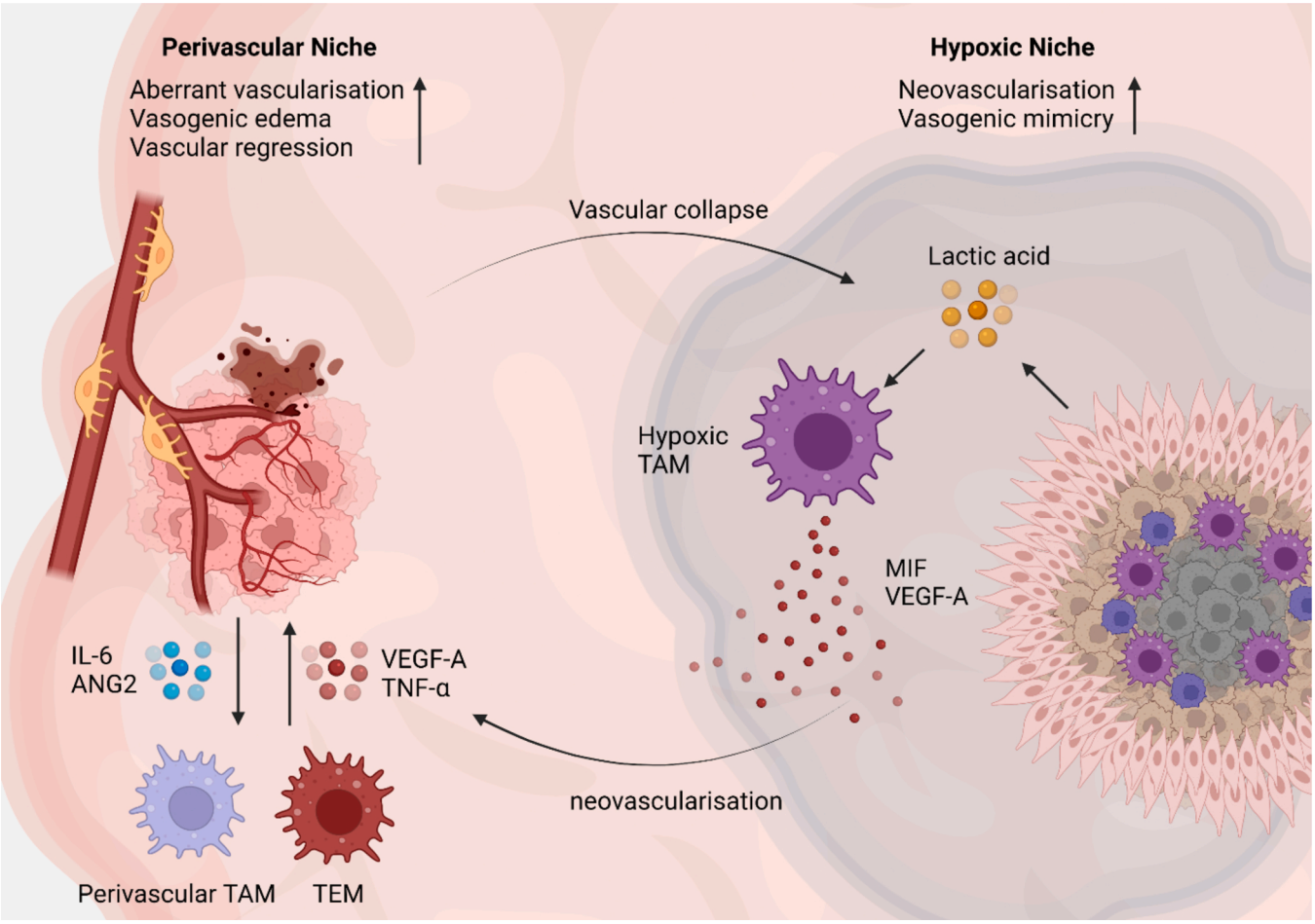

Fig. 1. Schematic representation of the dynamic interactions between the PVN and hypoxic niches. Perivascular TAMs promote the formation of aberrant vessels, leading to vasogenic edema, vascular regression and subsequent vessel collapse. As a result, dysfunctional blood perfusion promotes the formation of hypoxic pseudopalisading regions. In turn, hypoxic TAMs within these niches promotes neovascularization through secretion of MIF and VEGF-A, resulting in the formation of new PVNs.

including VEGF-A, ANG1, ANG2, TGF-β and PDGF [48]. However, this gene program in fact sustains hypoxic regions, by inducing a self-perpetuating cycle of dysfunctional vessel formation and subsequent hypoxia.

In summary, both the PVN and hypoxic niches play important roles in facilitating multiple pro-tumorigenic processes including angiogenesis, invasion and therapy resistance. Upon SOC-therapy, these niches are remodeled, leading to adaptations in the TME that can fuel recurrence [4]. It is essential to understand the biological processes underlying this therapy-induced remodeling of the TME, and how it may impact the immune compartment, in order to unravel the mechanisms leading to disease relapse. Here, we will focus on elucidating the complex and dynamic interactions between TAMs and glioblastoma cells in distinct niches of the TME, and how this cross-talk evolves in response to therapy.

3. TAMs in the perivascular niche

Perivascular niches are the prime site of recruitment of peripheric monocytes, where they differentiate into macrophages. Perivascular TAMs are characterized by expression of markers such as VEGFA, CCR2 and Tie2, and are notoriously pro-angiogenic and pro-tumorigenic [49]. Upon SOC therapy, PVN TAMs phenotype can be drastically altered, which play a pivotal role in glioblastoma recurrence.

3.1. Therapy-induced remodeling of the perivascular niche

SOC-therapy extensively remodels the TME, giving rise to resilient therapy-resistant niches which facilitate disease recurrence though multiple mechanisms. While SOC-therapy eradicates most cancer cells within the tumor bulk, specific glioblastoma cancer cell subpopulations survive within protective niches such as the PVN [39]. Moreover, PVNs shelter subpopulations of cancer cells that possess stem cell-like characteristics, referred to as glioma stem cell-like cancer cells (GSCs), which are known to resist IR and chemotherapy. For instance, the PVN-resident GSCs are less sensitive to therapy-induced DNA damage due to their low proliferation rate and enhanced DNA damage response, giving them the ability to escape therapy-induced apoptosis and mitotic catastrophe [39]. Additionally, GSCs highly express the ATP Binding Cassette Subfamily G Member 2 (ABCG2) protein, a drug efflux protein that leads to decreased cellular accumulation of chemotherapeutic drugs, resulting in chemoresistance due to rapid drug extrusion from the cytosol [50]. Altogether, this indicates that SOC treatment leads to enrichment of therapy-resistant cancer subpopulations that are able to repopulate the tumor after treatment.

Besides direct effects on cancer cells, SOC therapy also alters structural components and non-neoplastic cells within the PVN. For instance, IR directly influences the integrity of the brain vasculature and the BBB. Upon IR, the plasma membrane of TECs is destabilized, resulting in morphological changes including cell swelling, basal lamina thickening and cytoplasmic vacuolization [51,52]. Consequently, TECs undergo apoptosis within 24 h following IR due to therapy-induced DNA damage, resulting in decreased TEC density and disruption of the BBB [51,53]. Furthermore, radiotherapy activates brain-resident microglia and astrocytes, resulting in a chronic inflammatory response [54]. Together with GSCs, these PVN-resident cells release various chemoattractants including periostin, osteopontin, SDF1, CCL2 and CSF-1, resulting in the recruitment of peripheral macrophages which are able to cross the IR-disrupted BBB and infiltrate into the brain parenchyma [18,55–59]. In the course of fractioned radiotherapy, TAMs progressively accumulate in the TME in two glioblastoma GEMMs, thus altering the relative proportions of microglia and MDMs recurrent tumors post-radiotherapy, with a markedly increased infiltration of MDMs in both glioblastoma models [21].

3.2. Co-evolution of TAMs and PVNs drives tumor growth through reciprocal interactions

Due to their shared location in perivascular areas of the brain, interactions between GSCs and PV TAMs may be an additional driver of tumor recurrence after treatment [60,61]. Indeed, pro-tumorigenic perivascular TAMs and glioblastoma cancer cells initiate a complex crosstalk that sustains tumor growth. For instance, GSCs secrete WISP1, a soluble factor which plays an important role in maintaining both cancer cells and TAMs. Secreted WISP1 is able to bind to α6β1 integrin on the surface of GSCs and trigger activation of the Akt pathway, which promotes cancer cell proliferation in an autocrine manner [62]. Interestingly, α6β1 is also selectively expressed on pro-tumorigenic TAMs, allowing the GSCs to selectively expand the tumor-promoting TAM compartment [62]. In turn, pro-tumorigenic TAMs are able to support glioblastoma outgrowth through promotion of angiogenesis, immune suppression and survival signals, establishing a symbiotic relationship where TAMs and GSCs support and maintain each other [62].

Perivascular TAMs can also indirectly promote tumor outgrowth by influencing other non-neoplastic perivascular cells such as astrocytes and TECs. As mentioned above, TAMs secrete various TEC mitogens and cytokines such as bFGF and TNF-α, which leads to increased TEC proliferation and vessel density [63,64]. In turn, activated and expanded TECs can further maintain TAMs and GSCs through various mechanisms. For instance, TECs activated by either TAM-secreted cytokines or direct exposure to IR increase their secretion of IL-6 [65,66]. Together with CSF-1, EC-derived IL-6 maintains the pro-tumorigenic TAM phenotype by activating PPARγ and HIF-2α, leading to impaired antitumor immunity by increased expression of Arg-1 and IL-10 [65]. The pleiotropic effects of IL-6 further drive transcriptional upregulation of VEGF through STAT3-mediated binding of Sp1 to the VEGF promoter, resulting in the expression of VEGF-A [67]. Furthermore, IL-6 enhances glioblastoma neovascularization through induction of VEGF secretion by astrocytes and perivascular glioblastoma cells in transgenic mice [67]. In line with this study, increased number of TECs and blood vessels expanded the fraction of self-renewing GSCs in orthotopic glioblastoma xenograft models, which accelerated glioblastoma growth [68]. Importantly, vascular depletion using either Erlotinib (a small molecule inhibitor of EGFR) or bevacizumab (an anti-VEGF antibody) in an ERBB2-driven mouse model decreased the number of GSCs and slowed tumor progression in these mice [68]. Taken together, these studies illustrate that the intricate cross-talk between vasculature, TAMs and glioblastoma cancer/stem-like cells, is a key contributor to tumor malignancy that hinders the efficacy of SOC therapy.

3.3. Perivascular macrophages and tumor revascularization post-IR

Perivascular TAMs have well-described pro-angiogenic properties and have been reported to promote tumor outgrowth of various cancer types, including colorectal, breast, kidney, pancreatic, lung and brain cancer [69]. Tie2-expressing macrophages (TEMs) are an example of a pro-angiogenic TAM subset, characterized by expression of the ANG ligand Tie2, VEGF-A, mannose receptor 1 (MRC1) and CXCR4. TEMs are recruited to the PVN by ANG2, a pro-angiogenic growth factor secreted by reactive astrocytes and activated TECs upon IR [31,64,70]. Immunofluorescence microscopy analyses of orthotopic glioblastoma mice indicated that TEMs not only surround the vasculature within the PVN, but also take up a peri-endothelial location similar to pericytes. This observation indicates that TEMs are in direct contact with sprouting TECs, suggesting that they may play an important role in tumor angiogenesis [64]. Indeed, Tie2^{+} monocytes, which are precursors of TEMs, appear to be intrinsically endowed with pro-angiogenic abilities. Compared to Tie2^{-} monocytes, Tie2-expressing monocytes isolated from peripheral blood mononuclear cells (PBMCs) of healthy patients showed increased expression of various genes associated with a pro-angiogenic macrophage phenotype at baseline, including expression of *VEGFA*

and matrix metallopeptidase 9 (*MMP9)* [71]. Interestingly, their pro-angiogenic function is further enhanced by upregulation of the pro-angiogenic enzymes cathepsin B and thymidine phosphorylase after exposure to TEC-derived ANG2 in vitro. This suggests that TEMs establish a pro-angiogenic paracrine feedback loop where TEMs provide TECs with pro-angiogenic mitogens, which in turn reinforce the pro-angiogenic phenotype of TEMs through the release of ANG2 [71].

The association of TAMs with increased vascularization in glioblastoma has also been observed in preclinical mouse models [69]. Subcutaneous co-injection of Tie2^{+} monocytes with primary glioblastoma cells in nude mice leads to increased vessel density and a large profuse vascular network typical of aberrant angiogenic activity, whereas co-injection with Tie2^{-} monocytes does not lead to this effect. Interestingly, co-injection of total CD14^{+} monocytes only results in a slight increase in the overall vascular area, indicating that TEMs are specifically endowed with pro-angiogenic capabilities [69]. This observation was later confirmed in an orthotopic recurrent glioblastoma xenograft model in which TEMs are recruited into the irradiated brain through the SDF1-CXCR4 axis [72]. Once infiltrated, TEMs drive tumor recurrence by restoring the tumor blood supply after irradiation. Importantly, TEM recruitment was a prerequisite for recurrence post-therapy, highlighting the important role of this TAM subpopulation in driving recurrence by supporting tumor revascularization [72].

Perivascular macrophages are also involved in promoting glioblastoma recurrence post anti-VEGF treatment with bevacizumab. Indeed, while VEGF antagonism was deemed a favorable intervention to target the extensive vasculature of glioblastoma, anti-angiogenic therapy did not increase overall survival in newly-diagnosed glioblastoma patients, and only mildly affected progression-free survival in patients with recurrent glioblastoma [73]. Furthermore, glioblastoma that initially respond to anti-VEGF treatment inevitably recur and acquire a highly infiltrative tumor phenotype, rendering further surgical resection and chemotherapy ineffective [74]. Upon treatment with the anti-VEGF mouse analog of bevacizumab B20.4.1.1, glioblastoma cells recruit TEMs to the PVN of recurrent glioma-bearing xenograft mice by increasing secretion of ANG2 and SDF1α. TEMs then secrete MMP2 and MMP9, which enhances the invasive properties of recurrent glioma cells by remodeling the tumor ECM [75–78]. Accordingly, co-localization of MMP9 with TEMs within the PVN was also observed in recurrent human glioblastoma patients after treatment with bevacizumab, providing further evidence that MMP9-secreting TEMs facilitate glioblastoma recurrence after anti-angiogenic treatment [75,76].

Regardless of Tie2 status, perivascular TAMs can promote recurrence after anti-angiogenic treatment through secretion of TNF-α. After anti-VEGF treatment, accumulated perivascular TAMs have been shown to secrete high amounts of TNF-α due to exposure to cancer cell-derived CCL2 and IL-8 in vitro [63]. TAM-secreted TNF-α activates perivascular TECs by increasing their expression of ICAM-1, VCAM-1, CXCL10 and CXCL5, inducing TEC proliferation and correlating with worse overall survival in glioblastoma patients [63]. Importantly, TNF-α neutralization decreases TEC activation and prolongs survival of syngeneic glioblastoma-bearing mice [63]. Consistent with these findings, glioblastoma patients with low intratumoral TNF-α concentrations responded better to bevacizumab treatment compared to TNF-α high patients, and resistance to anti-VEGF treatment in orthotopic glioblastoma xenograft was associated with recruitment of TNF-α-secreting TAMs [63]. Taken together, TAM-derived TNF-α induces TEC activation, resulting in resistance to anti-VEGF treatment through activation of angiogenic pathways. In combination with anti-VEGF therapy, administration of soluble Tie2-receptors or CXCR4 inhibitors to target TEMs or perivascular TAMs respectively reduced both glioblastoma invasiveness and tumor vessel density in xenograft models, highlighting the therapeutic potential of targeting tumor angiogenesis and TAMs simultaneously to overcome macrophage-mediated resistance to anti-angiogenic treatment [77,79].

3.4. Immunosuppressive features of perivascular macrophages

In addition to their roles in angiogenesis, perivascular TAMs have well-defined immunosuppressive properties. While lymphocytes are mainly excluded from the healthy brain, both primary and recurrent glioblastoma show an increased number of tumor-infiltrating lymphocytes (TILs) [80]. However, highly immunosuppressive CD4^{+}, FoxP3^{+} regulatory T cells (Tregs) represent a large portion of these TILs, and are associated with worse overall survival and tumor recurrence in glioblastoma patients [81,82]. Upon exposure to ANG2, TEMs actively recruit and expand Tregs in a mouse mammary tumor model through secretion of CCL17. Genetic depletion of TEMs resulted not only in reduced angiogenesis, but also in reduced Treg infiltration [83]. Given that ANG2 is abundantly expressed by TECs and astrocytes in the PVN of irradiated glioblastoma tumors, it is likely that TEMs may play a similar role in the recurrent glioblastoma setting.

Although the immunosuppressive role of TAMs in glioblastoma is well established, recent studies indicate that specific TAM subsets may also promote anti-tumor immunity. Indeed, mass cytometry of human glioblastoma tumors and syngeneic murine glioblastoma models revealed a MDM subset (CD206^{+}, CD169^{+}, CD163^{+}, CD38^{+}, HLA-DR^{high}) that was positively correlated with patient survival, particularly in low-grade glioma [82]. While CD206 and CD163 are generally recognized as markers of pro-tumorigenic TAMs, CD169^{+} macrophages are known for co-expressing markers that are characteristic of both pro-tumorigenic and anti-tumorigenic macrophage phenotypes [84]. CD169^{+} TAMs are a specific subpopulation that localize near the tumor vasculature or in secondary lymphoid organs and are correlated to a better overall survival in liver and gastric cancer [85,86]. Within the tumor, CD169^{+} macrophages are recognized for their ability to activate the adaptive immune system either through antigen transfer to cross-presenting dendritic cells or by direct interaction with T cells and B cells [87–89]. While the functional implication of this TAM subset in the context of glioblastoma is still unknown, its positive correlation to patient survival suggests that the CD169^{+}/CD206^{+} TAM population may exhibit innate anti-tumorigenic properties by enhancing the anti-tumor immune response, and could be harnessed in novel immunomodulation therapeutic avenues.

It is thus well established that the PVN fuels tumor recurrence, partly through recruiting TAMs that can promote cancer cell proliferation, angiogenesis and immunosuppression post-therapy. While perivascular TAMs represent attractive therapeutic targets, as discussed below, further investigations of macrophage subpopulations present in the PVN and their respective functions will be essential to specifically target pro-tumorigenic macrophages while sparing anti-tumorigenic ones.

3.5. Overcoming therapeutic resistance by targeting perivascular macrophages

As discussed previously, targeting the PVN with anti-angiogenic treatment has led to limited clinical efficacy, in part due to the accumulation of pro-angiogenic TAMs that counteract the effects of these therapies. Therefore, combination therapies which simultaneously target the tumor vasculature and pro-angiogenic TAMs might enhance therapeutic efficacy. For instance, a preclinical study indicated that dual targeting of VEGF and ANG2 with a bispecific antibody reprograms perivascular TAMs towards a CD206^{low}/CD11c^{high} anti-tumoral phenotype, resulting in decreased vessel density, delayed tumor growth and increased survival in glioblastoma syngeneic and xenograft mice compared to anti-VEGF alone [90].

Another tantalizing strategy to reprogram perivascular TAMs into anti-tumorigenic phenotypes, would be through genetic modification of autologous macrophages. Since TEMs are actively recruited to the PVN upon therapy, exploiting TEMs as vehicles for the delivery of pro-inflammatory cytokines to the glioblastoma PVN could be an attractive therapeutic approach. Previous preclinical studies have indicated

that local IFNα expression by TEMs at the tumor site was associated with a general reprogramming of the immune TME towards an anti-tumorigenic phenotype and reduced tumor burden. This effect was mediated through increased antigen presentation, T cell infiltration, T cell effector functions and decreased angiogenesis and metastasis in breast cancer, liver cancer, leukemia and glioma [91–94]. These promising results have led to a currently ongoing phase I/II clinical trial in patients with newly diagnosed glioblastoma, where patients receive autologous $CD34^{+}$-enriched hematopoietic stem and progenitor cells that have been genetically engineered to selectively express IFNα at the tumor site, in addition to SOC therapy (NCT03866109). In this study, the IFNα gene is directly controlled by the Tie2 promotor, causing IFNα to be selectively expressed by TEMs. Preliminary data indicates that the therapy is well tolerated and shows the potential to reprogram the TME through activation of the adaptive immune system [95].

Altogether, concurrent targeting of the tumor vasculature and peri-vascular TAMs might induce a remodeling of the PVN with beneficial effects on patient survival. A remaining challenge will be to ensure that normalization of the vasculature is achieved in a stable and long-lasting manner. Indeed, targeting the vasculature often results in vessel destabilization, leading to formation of hypoxic pseudopalisading niches. These hypoxic niches are highly supportive of glioblastoma progression, and may represent an important hurdle for therapies targeting the PVN.

4. TAMs in the hypoxic pseudopalisading niche

As previously mentioned, vascular collapse as a result of aberrant angiogenesis results in hypoxic pseudopalisading regions, which are recognized as a hallmark of glioblastoma [27]. MDMs are recruited to these hypoxic niches by multiple HIF-1α downstream targets that act as chemoattractants (Fig. 2), such as oncostatin M (OSM), eotaxin, semaphoring 3A (SMP3A), endothelial cell monocyte-activating polypeptide-II (EMAP-II), endothelin and SDF1α [96–98]. Once they reach the hypoxic niche, macrophage mobility is impaired by hypoxia-induced activation of pathways that inhibit the migratory response of TAMs to chemokines, such as mitogen activated protein kinase phosphatase 1 [99]. TAMs are thus entrapped in the hypoxic niche, and reeducated towards a tumor-supporting phenotype through hypoxia-induced signaling and by tumor-derived signals, which will be discussed below. Importantly, hypoxia is further exacerbated by various treatment modalities, which drastically impacts TAM functions.

4.1. Therapy-induced hypoxia promotes macrophage recruitment and pro-tumorigenic functions

As previously discussed, IR-induced disruption of blood vessels leads to decreased vascular density and subsequently limits blood flowing into the tumor, resulting in lower oxygen pressure [27]. Anti-angiogenic therapies, such as the anti-VEGF antibody bevacizumab, have also been reported to induce hypoxia in glioblastoma [100,101]. In murine glioblastoma xenografts, bevacizumab treatment led to a reduction in large-sized vessels, decreased permeability and branching of smaller-sized vessels, but did not translate into functional vessel normalization, as indicated by decreased blood perfusion [102]. In line with these observations, bevacizumab treatment of xenograft glioma mouse models resulted in increased hypoxia and lactate levels in the tumor [103]. Altogether, these observations indicate that the hypoxic features of glioblastoma are often aggravated upon IR or anti-angiogenic

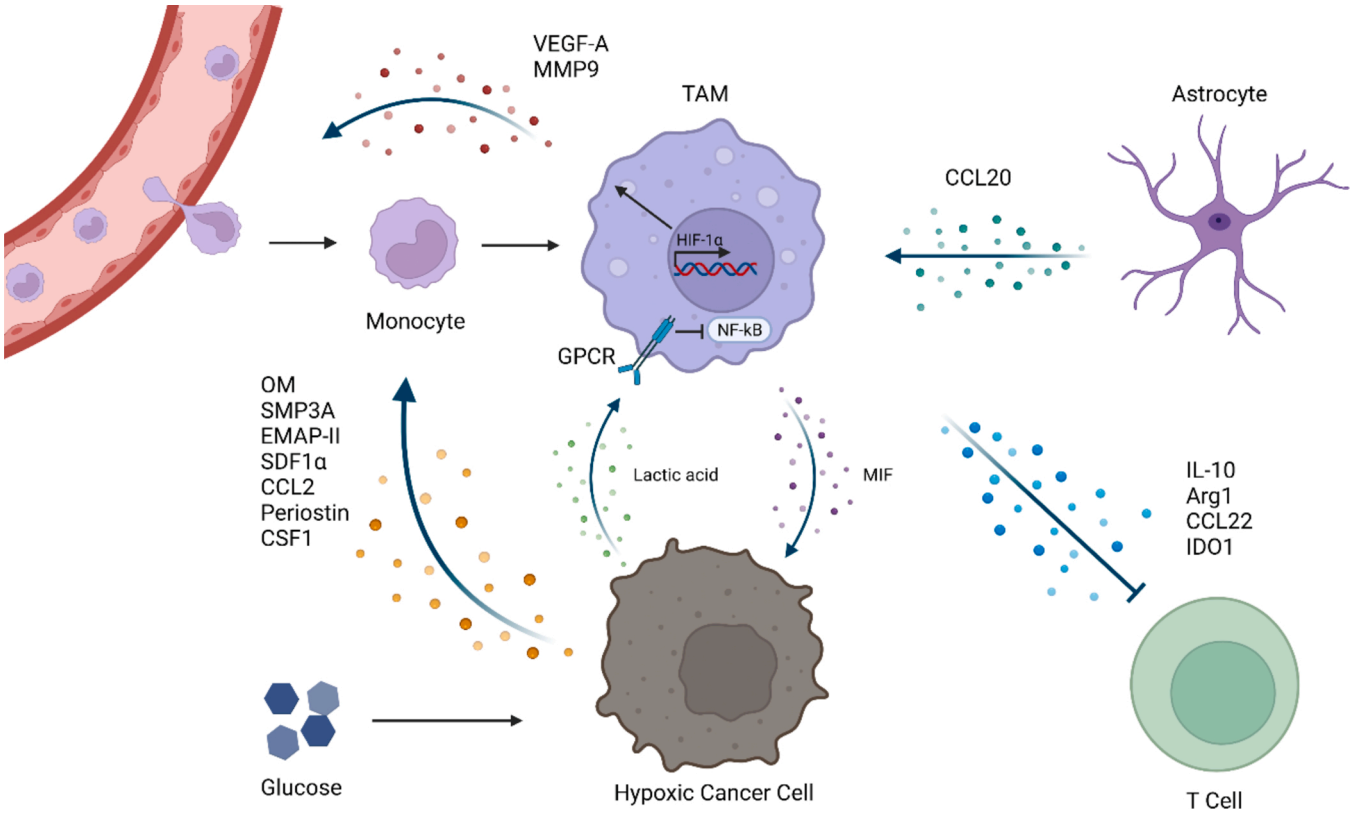

Fig. 2. Cross-talk between TAMs and glioblastoma cells within the hypoxic niche. Hypoxic cancer cells recruit and reeducate peripheral MDMs through secretion of various chemoattractants, including OM, SMP3A, EMAP-II, SDF1α, CCL2, periostin and CSF1. Once infiltrated, hypoxic TAMs promote angiogenesis by secretion of VEGF-A and MMP9 in a HIF-1α specific manner. Additionally, hypoxic TAMs promote angiogenesis by secreting MIF, a cytokine that promotes vasculogenic mimicry. In combination with astrocyte-derived CCL20, HIF-1α expression in TAMs results in the expression of immunosuppressive molecules such as IL-10, Arg-1, CCL22 and IDO1, dampening the T cell response. Hypoxic cancer cells convert glucose into lactic acid. Subsequently, hypoxic TAMs sense lactate through GPCRs, which prevents macrophage pro-inflammatory phenotype by inhibiting NF-κB.

treatment, and highlight the importance of vasculature normalization in dictating the outcome of therapy.

Therapy-induced hypoxia also participates to SOC treatment resistance and glioblastoma recurrence through blunting the effects of chemo-radiotherapy. For instance, hypoxia-induced signaling pathways activate the transcription of various antioxidants such as glutathione, which decrease intracellular ROS (reactive oxygen species) thereby limiting DNA damage and cancer cell death, altogether enhancing radioresistance [104]. Furthermore, hypoxia-mediated HIF-1α has a broad range of molecular targets promoting therapy resistance, and its stabilization leads to the transcription of genes associated with various pro-tumorigenic processes such as altered metabolism, drug efflux, angiogenesis and recruitment of immune cells – mainly macrophages as described above [105,106].

Once recruited in the hypoxic niches, TAMs are reeducated by glioblastoma cancer cells in a hypoxia-specific manner. For instance, exosomes derived from hypoxic, but not normoxic glioma cells, promote a pro-tumorigenic phenotype in TAMs [107]. Exosome-derived IL-6 from hypoxic glioma cells leads to increased expression of CD163 and immunoregulatory IL-10 in vitro through activation of STAT3, and resulted in unfavorable survival outcomes in mouse models of glioblastoma. This effect was mainly mediated by autophagy, as it was abrogated by the autophagy-inhibitor 3-MA [107].

Altogether, these studies show that hypoxia-induced signaling, which is amplified upon therapy, promotes an influx of macrophages into hypoxic niches, where they acquire pro-tumorigenic functions through complex cross-talks with hypoxic glioblastoma cells.

4.2. Hypoxic TAMs and the immune TME

While hypoxic glioma cells are able to reeducate TAMs, the hypoxic niche environment also polarizes TAMs towards a pro-tumorigenic phenotype independently of glioblastoma cancer cells (Fig. 2). For instance, hypoxic TAMs upregulate HIF-1α, which is a well-known regulator of immune functions. HIF-1α stabilization in TAMs results in upregulation of CSF-1R, which increases their consumption of cancer cell-derived CSF-1, fueling their immunosuppressive phenotype [108]. Indeed, hypoxic TAMs display increased secretion of immunoregulatory cytokines IL-10 and CCL22 compared to normoxic TAMs in vitro through enhanced CSF-1R signaling [108], suggesting an aggravation of TAMs immunosuppressive features in hypoxia.

Additionally, hypoxic TAMs also modulate effector functions of other TME-resident immune cells. For instance, TAMs regulate CD8 T cell activity through upregulation of the immune checkpoint programmed-death ligand 1 (PD-L1), which is directly induced by HIF-1α. PD-L1 interactions with programmed-death receptor 1 (PD-1) expressed on T cells, leads to a decrease in T cell effector functions and proliferation [109]. Importantly, coculture of PD-L1-expressing macrophages with autologous activated T cells induced T cell anergy, indicating that PD-L1 expression on macrophages is sufficient to suppress T cell activity in vitro [110]. In line with these observations, tumor-infiltrating macrophages in human glioblastoma showed increased PD-L1 expression [111,112]. Taken together, these studies suggest that hypoxia exacerbation caused by IR may further increase PD-L1 expression in TAMs, and support an immunosuppressive phenotype in recurrent glioblastoma. However, further confirmation of these findings in mouse models of recurrent glioblastoma will be required.

MDMs particularly were shown to modulate CD8 T cell effector functions by upregulating indoleamine 2,3-dioxygenase 1 (IDO1) in response to hypoxia-induced expression of CCL20 [113]. In hepatocellular carcinoma, MDM-secreted IDO1 leads to dysfunctional T cells with significantly decreased proliferation and IFN-γ secretion of both CD4 and CD8 T cells in vitro. Although the association of CCL20-mediated IDO1 expression in glioblastoma remains to be elucidated, CCL20 and IDO1 are both highly expressed in glioblastoma tissues and are associated with poor patient survival [114]. This suggests that brain TAMs might suppress T cell functions through the same mechanism. Interestingly, tumor-associated astrocytes have been shown to abundantly secrete CCL20 in hypoxic glioblastoma regions [115]. In light of these studies, it is conceivable that astrocytes may be the primary source of CCL20 and contribute to promoting an immunosuppressive environment in glioblastoma, by interacting with TAMs through the CCL20/HIF-1α /IDO-axis.

Given that hypoxia endows TAMs with a characteristic immunosuppressive phenotype, selectively targeting hypoxic TAMs through these hypoxia-induced markers represents an interesting therapeutic opportunity. For instance, treatment of macrophages with HIF-inhibitor acriflavine (ACF) partially reversed their pro-tumorigenic polarization by downregulating HIF target genes CSF-1R and TGF-β [108]. Upon treatment with ACF in vitro, macrophages decreased their secretion of IL-10 and CCL-22, thus attenuating their immunosuppressive capabilities. Additionally, targeting MRC1 (CD206), a pro-tumorigenic marker overexpressed by hypoxic TAMs in glioma, can also be beneficial to revert their pro-tumorigenic phenotype. In syngeneic murine pancreatic ductal adenocarcinoma models, CD206 neutralization with the long peptide RP-182 reeducated hypoxic TAMs into expressing anti-tumorigenic markers such as CD86, IL-1β, IL-12, TNF-α and iNOS and led to enhanced phagocytic properties and increased CD8 T cell function [116]. Moreover, $CD206^{+}$ TAMs internalized the CD206-specific peptide CSPGAKVRC in multiple mouse models, including an orthotopic glioblastoma model [117]. These studies suggest that the ability of hypoxic TAMs to internalize CD206-targeting compounds can be used as a reeducation strategy in glioblastoma to selectively deliver therapeutically relevant drugs. Proof of concept of this approach have been established using nanoparticles targeting CD206 to selectively deliver in vitro-transcribed IRF5/IKKβ mRNA to pro-tumorigenic TAMs in a transgenic PDGFβ-driven glioblastoma model [118]. Delivery of IRF5/IKKβ skewed TAMs towards an anti-tumorigenic phenotype and when combined with IR, drastically reduced tumor growth and doubled the OS of tumor-bearing mice compared to monotherapies. Altogether, these studies highlight the therapeutic potential of combining SOC therapy with targeting of hypoxic TAMs.

4.3. Effect of hypoxic TAMs on angiogenesis

In addition to its role in immune regulation, HIF-1α is widely recognized as a key regulator of angiogenesis. For instance, upregulation of HIF-1 and HIF-2 by hypoxic TAMs stimulate angiogenesis through secretion of pro-angiogenic growth factor VEGF-A [119–121]. Additionally, HIF-1α upregulates MMP9, an enzyme that releases latent VEGF sequestered by extracellular matrix proteins [122].

Another way hypoxic TAMs stimulate angiogenesis is through macrophage migration inhibitory factor (MIF). Hypoxic TAMs secrete high amounts of MIF, which binds to hypoxia-induced CXCR4 on glioblastoma cancer cells. Subsequently, CXCR4 activation on tumor cells leads to the induction of a mesenchymal transcriptional program through activation of the Akt pathway [123]. MIF-dependent Akt signaling in glioblastoma cancer cells results in upregulation of mesenchymal markers N-cadherin and vimentin. Strikingly, instead of promoting TEC proliferation, glioblastoma cancer cells treated with MIF formed a network of vessels themselves in vitro. This process, known as vascular mimicry, is an alternative angiogenic process where *de novo* vasculature is formed by trans-differentiation of malignant cells [124]. These findings were confirmed in a preclinical study where subcutaneous injection of MIF induces vasculogenic mimicry in murine xenograft models as a result of MIF-induced epithelial-to-mesenchymal transition. Inhibition of the CXCR4/Akt pathway abrogated this effect, supporting the hypothesis that vasculogenic mimicry is mediated by reciprocal interactions of macrophages and glioma cells through MIF-CXCR4-Akt signaling under hypoxic conditions [123].

Taken together, these studies illustrate that hypoxia induces a pro-

angiogenic response to counteract the low oxygen levels (Fig. 1), leading to neovascularization not only through stimulation of TEC proliferation, but also through trans-differentiation of glioblastoma cancer cells.

4.4. TAM polarization in response to hypoxia-induced lactic acidosis

Angiogenesis and immunosuppression are key features of tumor hypoxia and have major implications on tumor outgrowth. Interestingly, gene expression analysis of glioblastoma xenografts indicated that the angiogenic switch in hypoxic tumor areas resulted in metabolic reprogramming of cancer cells [125]. Indeed, in vitro culture of glioblastoma xenograft-derived spheroids significantly enhanced glycolysis under hypoxia compared to normoxia, resulting in high secretion of lactate [125]. Likewise, hypoxic areas in patient biopsies exhibit higher expression of the glycolytic enzyme LDH-A and glucose transporter GLUT1 compared to non-hypoxic glioblastoma regions and low-grade gliomas, illustrating hypoxia-induced metabolic rewiring [125]. Taken together, these observations indicate that hypoxia rewires metabolic pathways in glioblastoma cancer cells, resulting in increased glycolysis and lactification of the glioblastoma TME, also known as lactic acidosis [126].

Tumor-derived lactic acid has been reported to induce a tumor-promoting phenotype in TAMs located in hypoxic regions – another mechanism through which hypoxia mediates macrophage reeducation [127]. Macrophages express multiple receptors that can sense the acidification of the TME. For instance, detection of an acidic environment by G protein-coupled receptors (GPCRs) leads to expression of inducible cyclic AMP early repressor (ICER) by macrophages in vitro [128]. ICER limits the induction of pro-inflammatory macrophages through inhibition of TLR-dependent NF-κB activation [129]. Importantly, expression of lactic acid-induced ICER in a melanoma mouse model leads to the expression of immunosuppressive markers such as Arg-1, which was inversely correlated with the expression of the pro-inflammatory marker TNF-α [127]. While the role of ICER-mediated TAM polarization in the context of glioblastoma remains to be elucidated, this study illustrates that macrophages can be influenced by direct sensing of their metabolic environment.

Additionally, macrophage phenotype is known to be influenced by direct uptake of lactic acid. Murine macrophages uptake lactic acid through mono-carboxylate transporters (MCT), leading to inhibition of prolyl hydroxylases and subsequent stabilization of HIF-1α [130]. Lactic acid-mediated HIF-1α stabilization resulted in increased expression of genes associated with TAM pro-tumorigenic phenotype, including *Arg1, Fizz1, Mgl1, Mgl2 and Vegf*. Treatment of macrophages with MCT inhibitor α-cyano-4-hydroxycinnamate in vitro abrogated this effect, supporting the hypothesis that TAM reeducation is mediated by uptake of lactic acid [130].

A more recent study indicated that HIF-1α stabilization upon MCT-mediated uptake of lactic acid was also occurring in human MDMs derived from healthy patients. In combination with CSF-1, lactic acid-mediated HIF-1α stabilization induces an alternative tumor-promoting gene program in vitro, characterized by the secretion of a variety of pro-tumorigenic growth factors and pro-inflammatory cytokines in macrophages including TNF-α, VEGF-A, OSM, IL-1β, ET-1, EGFR ligand HB-EGF and TGF-α [131]. Interestingly, the lactate-induced macrophage phenotype differed from the classical tumor-promoting TAM phenotype which are typically induced by IL-4 or IL-10 [132]. Compared to classic pro-tumorigenic TAMs, lactate-treated macrophages produced larger amounts of HB-EGF, ET-1 and TGF-α, and lower amounts of VEGF-A. An explanation may be that hypoxia-induced CSF-1R upregulation enhances the secretion of pro-inflammatory cytokines through CSF-1 signaling. The combined effects of lactic acid and enhanced CSF-1 signaling results in an alternative TAM phenotype which is characterized by secretion of pro-inflammatory cytokines [133, 134]. A later study indicated that TAMs can determine the distance from the tumor vasculature by sensing the oxygen and lactate gradient within the tumor, resulting in phenotypic diversity based on their position in respect to nutrient-rich normoxic regions [135]. The distance of TAMs from normoxic regions within the tumor was strongly correlated with MAPK signaling, which was gradually increased towards hypoxic environments and required for the phenotypic switch of TAMs. As a result, TAMs located in hypoxic regions showed increased expression of Arg-1 and VEGF-A compared to TAMs in normoxic regions in MMTV-PyMT mouse models of breast cancer [135]. Although the impact of lactate-induced macrophage polarization on glioblastoma progression remains to be elucidated, these observations highlight the plasticity of macrophages in response to extracellular metabolites.

5. Impact of SOC therapy on TAM metabolism

As discussed above, SOC or anti-angiogenic treatment often results in the formation of tumor-promoting hypoxic niches, in which TAM recruitment and reeducation participate to emergence of glioblastoma recurrence. Hypoxia alters tumor cell glycolytic activity and further disrupt cellular metabolism, for instance by depriving the TME from glucose and glutamate which fuels the anti-tumor response of several immune cells. Conversely, tumor-derived metabolites benefit immunosuppressive immune cells, highlighting the complex metabolic symbiosis occurring between cancer and immune cells in the glioblastoma TME [136]. In the sections below, we will discuss how metabolic rewiring influences the reciprocal communication between cancer cells and diverse component of the immune landscape of glioblastoma, with a particular focus on TAMs.

5.1. Metabolic rewiring of glioblastoma cells and impact on the TME

glioblastoma cancer cells employ a wide range of metabolic programs to sustain their rapid growth and proliferation [137]. As mentioned before, glioblastoma cancer cells engage into anaerobic glycolysis under hypoxic conditions. However, this is not limited to hypoxic areas, since cancer cells generally favor the processing of glucose into lactic acid over oxidative phosphorylation (OXPHOS) regardless of oxygen availability, a process also known as the Warburg effect [138]. This altered metabolic pathway provides cancer cells with fast ATP generation and the biosynthesis of building blocks needed for rapid proliferation. In addition to glycolysis, glioblastoma cancer cells maintain energetic homeostasis by utilizing lipid metabolism [139]. In glioblastoma, cancer cells accumulate fatty acids (FA), which are stored as triglycerides in lipid droplets (LDs) [140]. glioblastoma cancer cells use these LDs as energy reserves, which can be utilized during metabolic stress. For instance, glucose starvation results in the release of FA from LDs through autophagy and are trafficked to the mitochondria, promoting cancer cell survival despite nutrient deprivation [139,140]. Interestingly, a recent study using organoid cultures, xenografts and glioblastoma patient samples, revealed a difference in lipid handling between anatomically distinct glioblastoma regions. Accumulation of LDs was observed in cancer cells localized in hypoxic pseudopalisading regions, which was due to differential expression of hypoxia-inducible lipid droplet-associated (HILPDA), a protein needed for lipid trafficking in cytosolic LDs [141]. Taken together, these observations highlight that glioblastoma cancer cells rewire major metabolic pathways to promote tumor outgrowth, and that specific metabolic alterations are dependent on the anatomical location of cancer cells. While cancer cells modulate their own metabolome to support their rapid growth, they also drastically impact the metabolite composition within the TME. The altered metabolic TME acts as a powerful evolutionary force which shapes the metabolic programs and functions of nearby immune cells, including macrophages.

5.2. Metabolic programs dictate the functional phenotype of macrophages

Macrophages react to extracellular metabolites and cytokines by

rewiring core metabolic programs, which has major implications on their functional phenotype. For instance, classically activated M1-like macrophages are characterized by high glycolytic activity, lactate secretion and FA synthesis, which promotes production of ROS and pro-inflammatory cytokines. In contrast, the metabolic profile of M2-like macrophages is characterized by OXPHOS and high FA oxidation (FAO) [142]. While these in vitro studies highlight the metabolic plasticity of macrophages in the context of inflammation, the influence of metabolism on the functional phenotype of macrophages is far more complex. To address the intricacies of macrophage response to metabolic cues, a metabolic axis of macrophage polarization has recently been proposed, whereby metabolic stimuli can modulate the immunophenotype of macrophages within a spectrum that transcends the traditional M1/M2-like inflammatory axis [143].

It is generally assumed that the high energetic demand of cancer cells deprives TAMs from nutrients, forcing them to shift to an alternative fuel source and thus altering their metabolic programs. As a result of nutrient competition between TAMs and cancer cells, TAMs lack the fuel needed to execute their pro-inflammatory functions, which is highly dependent on the presence of extracellular glucose [144]. Of note, recent studies indicate that the TME is not always deprived of nutrients and that immune cell metabolism may be dysregulated by cell-intrinsic mechanisms that are independent of nutrient availability, adding another layer of complexity to the metabolic rewiring that occurs in the context of cancer [145].

5.3. Metabolic rewiring of TAMs within the glioblastoma TME

The metabolic activity of glioblastoma cancer cells leads to secretion of various metabolic intermediates in the TME. As a result, TAMs adapt their metabolic programs depending on the nutrients made available by cancer cells, such as glutamate, ketone bodies, lactate and lipids [146–149]. Upon co-culture with patient-derived glioblastoma cancer cells, TAMs isolated from surgical glioblastoma sections showed an upregulation of glutamate transporter genes *GRIA2, SLC1A2* and *SLC1A3*, suggesting increased glutamate uptake by TAMs [150]. Additionally, TAMs upregulated glutamine synthase (GS), which converts glutamate into glutamine in order to fuel the tricarboxylic acid (TCA) cycle [150]. Consequently, enhanced glutamine metabolism leads to the accumulation of α-ketoglutarate (αKG), a metabolic intermediate that polarizes macrophages into a pro-tumorigenic phenotype through multiple mechanisms. For instance, αKG activates the JMDJ3 demethylase, an epigenetic regulator that induces an M2 gene program through the activation of the IRF4 TF [151,152]. Furthermore, αKG also prevents the transition into pro-inflammatory phenotypes by inhibiting the nuclear translocation of NF-κB, a crucial TF needed for the induction of the pro-inflammatory gene program of macrophages [152]. Interestingly, inhibiting GS activity with methionine sulfoximine reeducated macrophages into an anti-tumorigenic phenotype, as shown by increased expression of TNF-α and SOX2, which was inversely correlated with pro-tumorigenic markers MRC1, CCL17 and CCL18 [153]. The switch to glutamine metabolism illustrates how cancer-derived oncometabolites are able to rewire TAM metabolism, resulting in a functional transition of TAMs into a tumor-promoting phenotype.

In recent years, the role of lipid metabolism on cancer progression has drawn increasing interest. While it is evident that lipid metabolism directly impacts cancer cell growth, the role of lipid metabolism on TAM functions and its impact on cancer progression remains poorly understood, particularly in glioblastoma. TAMs derived from human multiple myeloma and prostate, breast and colon cancer exhibit increased intracellular lipid content, which correlated with accelerated cancer progression [154]. TAMs are able to take up lipids through surface receptors such as LOX1, CD204 or scavenger receptor CD36, resulting in increased FAO and lipid storage in LDs [155]. The LD-derived FA are used by TAMs to fuel OXPHOS through mTOR signaling, which coincidentally upregulates expression of genes associated with tumor-promoting TAMs such as CD206, IL-6, VEGFα, CCL6, MMP9 and Arg-1 [155,156]. Importantly, blocking either LD-derived FA release or FA-uptake in TAMs decreases tumor growth in murine models for fibrosarcoma, colon cancer and prostate cancer, revealing an important role of lipid-loaded TAMs in tumor progression [154–156]. In glioblastoma, a recent study investigating the heterogenic transcriptional profiles of TAMs identified specific subsets that were enriched for genes associated with lipid metabolism, in both human and murine glioblastoma samples [25]. Strikingly, further analysis also indicated that genes associated with lipid metabolism in TAMs were enriched in recurrent glioblastoma compared to newly diagnosed tumors. This suggests that TAMs may exhibit an increase in lipid metabolism upon therapy, a metabolic switch that is associated with a pro-tumorigenic phenotype in multiple other tumor types as described before. However, the functional implication of this lipid-rich TAM subset in glioblastoma recurrence, and its potential role in favoring specific subtype of glioblastoma outgrowth that may participate to SOC resistance, remains to be explored.

5.4. The role of tumor metabolism in therapy response

In addition to its role in cancer progression, metabolic rewiring also plays a major role in therapy response. While glioblastoma cancer cells normally use high glycolytic activity to generate fast ATP, IR often induces a glycolysis-to-OXPHOS transition [157]. As a result, treated cancer cells often adopt a lipogenic phenotype, which drives therapy resistance and cancer cell survival [158]. For example, GSCs that localize in the hypoxic niche employ lipid metabolism to enhance resistance against SOC therapy. As described earlier, GSCs are slow-cycling cells that are endowed with intrinsic radioresistance and speculated to be the source of disease relapse in glioblastoma patients. Recent studies have indicated that metabolic programming in GSCs differs from non-GSCs, promoting therapy resistance and subsequent tumor growth post-therapy. While xenograft-derived $CD133^{-}$ cancer cells stored diacylglycerol and triacylglycerol in cytosolic LDs, $CD133^{+}$ GSCs preferentially shuttled *de novo* synthesized Fas into phospholipids, which was mediated by upregulation of FA desaturase- (FADS) 1 and FADS2, to maintain their stem-like state and survival [141]. In line with these in vitro observations, orthotopic xenografts of slow-cycling GSCs showed increased resistance to TMZ treatment compared to mice bearing fast-cycling non-GSC tumors, which was due to enhanced lipid metabolism [159]. Importantly, RNA sequencing analysis of recurrent human glioblastoma tumors indicated that lipid metabolism was highly upregulated compared to primary tumors. This metabolic signature was comparable to that of GSCs, further highlighting their role in therapy resistance and disease recurrence [159].

Despite indications that TAM metabolism is altered in recurrent tumors, knowledge on therapy-induced metabolic rewiring of TAMs is scarce. Recent insights indicate that, similar to cancer cells, TAMs switch from glycolysis to OXPHOS through lipid metabolism. For instance, both microglia and MDM-derived TAMs in recurrent glioblastoma samples were shown to upregulate expression of cholesterol 25-hydroxylase (CH25H), an enzyme that catalyzes cholesterol into 25-hydroxycholesterol [25]. 25-Hydroxycholesterol (25-H) is a potent inhibitor of sterol regulator binding proteins (SREBP), a master regulator which promotes fatty acid and cholesterol synthesis [160]. Coincidentally, SREBP also regulates the inflammatory response in macrophages by promoting phagocytic activity and assembly of the inflammasome [161,162]. Additionally, secreted 25-H promotes an immunosuppressive TME by recruiting TAMs and inhibiting cytotoxicity of NK cells in vitro [163,164]. While the functional role of 25-H on glioblastoma tumor recurrence remains unclear, the upregulation of CH25H in recurrent glioblastoma TAMs suggests a role for lipid metabolism in promoting immunosuppression post-therapy.

Besides driving immunosuppression, TAMs presenting enhanced lipid metabolism may also play a protective role in GSC niches, which include the PVN and hypoxic environments. During embryogenesis of

drosophila, glial LDs protect neuronal stem cells from oxidative stress [165]. Indeed, glial-derived LDs inhibited the peroxidation of polyunsaturated FAs in neuroblasts during hypoxia, acting as an antioxidant by reducing peroxidation-mediated oxidative stress and promoting neuroblast proliferation and survival during hypoxia [165]. As previously mentioned, TAMs and GSCs accumulate LDs through activation of HILPDA in the hypoxic pseudopalisading niches. These observations thus allow us to speculate that LDs in hypoxic TAMs may play a similar role in protecting GSCs from radiotherapy-induced ROS, although further studies are warranted to confirm these findings in the glioblastoma setting.

In sum, the evolution of cancer cell metabolism during glioblastoma progression greatly impacts the TME, shaping response to therapy and driving TAMs to alter their metabolic programs in favor of a tumor-promoting phenotype. Metabolic properties of both TAMs and cancer cells may differ across the distinct glioblastoma niches. Hypoxic gradients dictate the metabolic status of tumor cells and highly influence their abilities to process lipids [141], which is likely to impact the metabolism of adjacent TAMs. In contrast, angiogenic niches are often associated with a metabolic shift towards increased glycolytic activity of glioblastoma cells [125]. Interestingly, this metabolic shift in PVN correlates with a switch of glioblastoma cell molecular signature towards a mesenchymal phenotype [125,166], highlighting the powerful impact of environmental cues on cancer cell metabolism and intrinsic features. Altogether, global and niche-specific changes in metabolite composition of the TME orchestrate a metabolic co-evolution of TAMs and glioblastoma cells which play an important role in therapy resistance.

6. Impact of macrophages on therapy-induced glioblastoma subtype transition

As mentioned previously, glioblastoma can be classified into three different subtypes known as classical, proneural (PN) and mesenchymal (MES), which are characterized by distinct transcriptional state and aberrant expression of genes such as *EGFR, PDGFRA/IDH1* and *NF1* respectively [167]. Importantly, these different transcriptional subtypes can occur at regional or cellular level within one tumor [168,169]. Glioblastoma subtypes have proven to be clinically relevant as an overall PN signature is generally associated with a better prognosis compared to a MES signature [170,171].

Glioblastoma tumors are known to transition between subtypes during therapeutic intervention, resulting in changes in cellular behavior and sensitivity to treatment. It has now been well established for instance that primary PN tumors often adopt a MES gene signature upon disease recurrence, resulting in therapy resistance, tumor outgrowth and cancer relapse [29,171]. It is now appreciated that changes in the PN-MES transition (PMT) are largely occurring through plasticity rather cancer cell-intrinsic genetic events [29,172]. Meanwhile, the associated changes in the TME remain poorly understood, so does the potential role of immune cells in modulating glioblastoma subtype changes, which we will discuss in this section.

6.1. TAM-derived signals drive PMT upon SOC-therapy

As described earlier, a large portion of the irradiated glioblastoma TME is comprised of TAMs, which play various roles in tumor progression [19]. Strikingly, macrophage depletion in MES glioblastoma-bearing mice results in the loss of the MES signature, suggesting that TAMs are directly involved in the maintenance of the MES phenotype of glioblastoma cancer cells [173]. Indeed, the macrophage-derived cytokine OSM drives PMT in both patient-derived gliomas spheroid cultures and preclinical mouse models [173]. Mechanistically, macrophage-secreted OSM interacts with its ligand OSMR or LIFR on cancer cells, leading to activation of the STAT3 pathway, one of the main inducers of the MES gene program [174]. Notably, macrophages associated with MES-like cancer cells adopted a distinct gene signature independent of the traditional M1/M2-like polarization axis, which might be induced by MES-like cancer cells themselves or by general environmental factors within MES glioblastoma, such as hypoxia or necrosis [173]. Several potential MES cancer-secreted factors have been put forward as inducers of the macrophage-mesenchymal gene program, such as CSF-1, CSF3, CX3CL1, TGFB2, TGFB3 and CCL7. While these factors are highly expressed by MES cancer cells, and their corresponding receptors (CSF-1R, CSF3R, CX3CR1, TGFBR2, CCR2 and CCR5 respectively) are present in TAMs exhibiting the mesenchymal signature, further studies are required to elucidate whether MES cancer cells are directly reprogramming macrophages in this context. Altogether, these observations strongly suggest that TAMs may play an important role in facilitating mesenchymal transition.

Another macrophage-derived cytokine involved in PMT is IL-6, a cytokine usually associated with a pro-inflammatory response. Cancer cell-derived CCL2 was found to upregulate IL-6 in pro-tumorigenic TAMs [175,176]. TAM-secreted IL-6 then binds to IR-induced IL-6 receptor alpha (IL-6Rα) on glioblastoma cancer cells, leading to downstream activation of PMT regulator STAT3 in vitro and in xenograft mouse models [177,178]. Indeed, transcriptome analyses indicated that IL-6-mediated JAK-STAT3 signaling resulted in a MES gene expression program which was paired with increased radioresistance in primary glioblastoma cultures [177,179]. Interestingly, inhibition of the IL-6/STAT3 axis in glioblastoma cells decreased the expression of MES markers CD44 and YKL-40, which was inversely correlated with the expression of PN markers OLIG2 and SOX2. Furthermore, inhibition of the IL-6/STAT3 axis suppressed glioblastoma growth, leading to increased survival of glioblastoma-bearing xenograft mice [179]. Altogether, these observations show that cancer cells facilitate therapy-induced PMT through CCL2-mediated upregulation of IL-6 in TAMs, which results in the activation of master regulators of PMT in glioblastoma cells.

Independently of its effects on glioblastoma cells, radiotherapy may also promote PMT through direct modulation of TAM phenotypes. Previous studies showed that cranial IR of healthy mice directly induced a pro-inflammatory phenotype in TAMs, which increased production of NO, ROS and pro-inflammatory cytokines [180]. Upon IR, TAMs abundantly express the pro-inflammatory cytokine TNF-α, which was shown to induce PMT in patient-derived PN GSCs in vitro [181]. In the context of TNF-α treatment, PN GSCs increased expression of YKL-40 and CD44 and adopted a gene signature similar to MES glioblastoma through NF-κB-mediated STAT3, C/EBP-β and TAZ activation. Immunohistochemical analysis of MES glioblastoma tissue sections revealed that NF-κB-expressing cancer cells were in close contact with TAMs, further supporting the hypothesis that TAMs drive PMT through expression of TNF-α. Notably, TNF-α-mediated PMT is associated with acquired radioresistance, as pretreatment of PN GSCs with TNF-α greatly reduced γ-H2AX foci and G2/M accumulation upon IR in gliomasphere cultures [181]. In line with these findings, intracranial injection of TNF-α combined with IR resulted in a significant increase in tumor growth compared to TNF-α or IR alone in preclinical orthotopic mouse models. Addition of NF-κB inhibitor abrogated this effect, indicating that TAM-secreted TNF-α induces PMT and radioresistance through NF-κB activation.

TAM-secreted TNF-α also increases intracellular ROS levels of glioblastoma cancer cells [182]. Intracellular ROS has a dual role in cancer progression as it can exert pro-tumorigenic functions as well as anti-tumorigenic functions. An excess level of cellular ROS in cancer cells can directly lead to damage in DNA, proteins, lipids and organelles which subsequently leads to cancer cell death through apoptosis [183]. On the other hand, low levels of ROS can promote tumorigenesis by promoting cell proliferation through activation of the MAPK and PI3K pathways [184]. Interestingly, PN GSCs increase their expression of the antioxidant response gene NRF2 in response to high intracellular ROS levels. Coincidentally, NRF2 also induces a mesenchymal genetic program as indicated by increased expression of CD44, C/EBP- β and

TWIST1 [185]. This observation suggests that increased intracellular ROS levels in response to IR-induced TNF-α secretion by TAMs might promote PMT through activation of an antioxidant gene program.

Upon IR, glioblastoma cancer cells increase secretion of microRNAs (miR) through small extracellular vesicles (EVs) which are taken up by macrophages, leading to alterations in their transcriptomic programs and phenotype [186,187]. Multiple studies have shown that tumor-derived EVs are able to promote TAM proliferation and induce a tumor-promoting phenotype, especially in MDMs which often accumulate in the irradiated glioblastoma TME [188,189]. In turn, macrophage-derived small EVs (MDE) modulate various tumor-promoting processes, including PMT. *In vitro* studies showed that MDE-mediated delivery of miR-27a-3p, miR-22–3p and miR- 221–3p to PN GSCs significantly enhanced expression of MES markers CD44 and YKL-40 while downregulating PN marker SOX2. Mechanistically, downregulation of CHD7, the mutual target for these miRNAs, leads to activation of downstream STAT3 and the RelB/P50 pathway, driving PMT and radioresistance [190]. This cross-talk between glioblastoma cancer cells and TAMs through exchange of EVs is another example that illustrates how glioblastoma cancer cells can benefit from these reciprocal interactions, by nurturing a specific phenotype in TAMs that can induce and maintain a MES signature, thus contributing greatly to glioblastoma malignancy.

7. Conclusion and future therapeutic perspectives

In this review, we provided insights into the impact of therapy on biologically relevant niches in glioblastoma, and discussed how large-scale remodeling of the TME heavily influences TAM function, consequentially promoting disease relapse. SOC-therapy drastically alters the TME, leading to enhanced infiltration of TAMs into perivascular and hypoxic niches. Once infiltrated, TAMs are reeducated by tumor-derived factors and environmental cues to promote various pro-tumorigenic processes, including angiogenesis, immunosuppression, cancer cell proliferation and PMT.

Given their prominent role in glioblastoma progression and disease relapse upon therapy, extensive efforts have gone into targeting TAMs as a monotherapy or in combination with other treatment modalities (Table 1). Current approaches include TAM depletion, TAM repolarization and inhibition of TAM recruitment [191]. However, clinical efficacy of these therapeutic approaches in glioblastoma remains limited, mainly due to acquired resistance mechanisms as a result of the exquisite plasticity of TAMs [24,191]. Additionally, most of these therapeutic approaches target the TAM population as a whole, leading to the elimination of both pro-and anti-tumorigenic TAM subpopulations. While TAMs are typically associated with accelerated tumor growth, some reports indicate that TAMs may correlate with a positive prognostic factor in some cancer types, such as lung and colon cancer [192,193]. In glioblastoma, TAMs are comprised of both pro-tumorigenic and anti-tumorigenic subsets that localize in distinct anatomical locations within the TME [25,38]. Therefore, we surmise that selectively targeting tumor-promoting TAM subsets in their biologically relevant niche is a tantalizing strategy to prevent glioblastoma recurrence and improve patient survival [25]. Furthermore, in light of the influence of TAM ontogeny on their transcriptional education and plasticity [13,21], it will be essential to address the functional potential of targeting tissue resident microglia or MDM recruitment.

Due to the central role of angiogenesis in glioblastoma malignancy, the PVN has been extensively investigated as a source of therapeutic targets, with the aim to reduce neovascularization and sensitize glioblastoma cancer cells to chemoradiation [95,194]. However, anti-angiogenic drugs such as bevacizumab have not demonstrated significant clinical benefits, partly due to the accumulation of pro-angiogenic TAMs in the PVN [74,75,100,102]. Thus, targeting perivascular TAMs in combination with anti-VEGF therapy may be a valuable therapeutic strategy to prevent acquired resistance to bevacizumab. Indeed, targeting TEMs, the most characterized pro-angiogenic TAM subset in the PVN, has already shown some efficacy in preclinical and clinical studies [90,95] (NCT03866109). However, TEM-targeting strategies only affect one specific subset of angiogenic TAMs ($Tie2^{+}$), and untargeted $Tie2^{-}$ perivascular TAMs exhibiting pro-angiogenic functions may eventually lead to therapy resistance through resumption of aberrant neovascularization. Dissecting the diversity of perivascular TAM subsets may lead to identification of novel pro-tumorigenic pathways that could be used to reeducate all PVN-resident TAMs, leading to better therapy outcomes.

The persistence of "leaky" and disorganized vessels following anti-angiogenic treatment, together with radiotherapy-induced vasculopathy, contributes to the expansion of hypoxic pseudopalisading niches [27]. In response, TAMs are actively recruited to the hypoxic niche where they are metabolically and functionally reeducated to promote tumor outgrowth and facilitate tumor recurrence [125]. Targeting hypoxia-induced chemoattractants represents an appealing strategy to prevent TAM accumulation in hypoxic niches, and has been subject of extensive research. Inhibition of SDF-1α or its ligand CXCR4 impeded TAM recruitment in the hypoxic niche of orthotopic glioblastoma models, decreasing tumor progression [79,195]. Orthogonally, multiple pre-clinical studies have shown that reprogramming hypoxic TAMs towards an anti-tumorigenic phenotype through HIF inhibition or by targeting MRC1 with nanocarriers results in decreased tumor progression and a modest increase in OS of tumor-bearing mice [116–118]. While the functional impact of targeting hypoxic TAMs on tumor progression is modest, combining these strategies with SOC therapy substantially increases the therapeutic effect. This further highlights the role of specific TAM subsets on tumor recurrence post-therapy and the need to simultaneously target TAMs during SOC treatment in order to overcome therapeutic resistance in glioblastoma patients.

Since TAM metabolism is tightly linked to their functional phenotypes, metabolic reprogramming of tumor-promoting TAMs might be an elegant, albeit challenging avenue to reeducate TAMs towards an anti-tumoral phenotype. When combined with SOC therapy, targeting the heightened lipid metabolism of glioblastoma cancer cells significantly inhibits tumor outgrowth in preclinical mouse models [196], suggesting that sensitizing cancer cells to metabolic stress enhances SOC effects. Dietary interventions could be another way to systemically target tumor metabolism, impacting not only the cancer cells but also components of the TME. For instance, ketogenic diets have been shown to reduce angiogenesis and acted synergistically with IR in glioblastoma xenografts [197]. In light of its effect on polarizing TAMs towards an anti-tumorigenic phenotype, ketogenic diets represent an attractive systemic approach to target aberrant lipid metabolism in glioblastoma [198]. So far however, clinical evidence of ketogenic diets efficacy in glioblastoma patients is limited to case studies [197,199–203] and the implications of therapy-induced metabolic reprogramming in TAMs and in the TME will require further investigation. Indeed, the functional changes associated with the metabolic co-evolution of TAMs and glioblastoma during therapy may shed light into novel targets that can be harnessed to reprogram pro-tumorigenic TAMs.

In conclusion, during disease progression and therapy, glioblastoma microenvironmental niches undergo drastic alterations that foster malignancy and promote disease recurrence. Glioblastoma niches are highly dynamic and can easily transition from one tumor-promoting niche to another, as illustrated by PVN progressing to hypoxic pseudopalisading regions upon therapy (Fig. 1). Therefore, targeting common denominators of these niches will be key to disrupt the nurturing interplay between glioblastoma cells and their environment. TAM abundance is a recurrent feature within both the PVN and hypoxic niches, and we can envision that using niche-based therapies to reeducate TAMs into anti-tumorigenic agents will represent a compelling strategy to improve the efficacy of SOC therapies. In order to devise such tailored therapeutic approaches, identification and in-depth analyses of specific pro-tumorigenic TAM subpopulations within their

Table 1
List of clinical trials involving TAM-targeting therapies in multiple cancer types.

Cellular target	Functional target	Drug	Disease	Study number	Additional Intervention	Phase	Study status/description
MMP	Perivascular, Hypoxic TAMs	Minocyclin	Recurrent glioblastoma	NCT01580969	IR + bevacizumab	I	Therapy well tolerated
			Recurrent glioblastoma	NCT02770378	TMZ	I	Therapy well tolerated
			Newly diagnosed glioblastoma	NCT02272270	IR + TMZ	I	Completed, no results available
CCL2	TAM recruitment	Carlumab	Prostate cancer	NCT00992186	None	II	No significant therapeutic effect as a single agent
			Solid tumors	NCT01204996	SOC therapy	I	No added therapeutic effect compared to SOC alone
MIF	Hypoxic TAMs	Ibudilast	Newly diagnosed/ Recurrent glioblastoma	NCT03782415	TMZ	I/II	Recruiting
Arginase	Perivascular, Hypoxic TAMs	INCB001158	Advanced solid tumors	NCT02903914	None, Pembrolizumab	I	Active
			Solid tumors	NCT03314935	Chemotherapy	I/II	Active
CSF1	TAM recruitment and polarization	Lacnotuzumab	Solid tumors	NCT02807844	PDR001	I/II	Therapy was well tolerated
			Advanced triple-negative breast cancer	NCT02435680	Carboplatin + Gemcitabine	II	No added therapeutic benefit compared to Carboplatin/ Gemcitabine alone
CSF-1R	TAM recruitment and polarization	BLZ945	Newly diagnosed glioblastoma	NCT02829723	Spartalizumab	I/II	Active
		PLX3397	Recurrent glioblastoma	NCT01349036	none	II	Reduction in microglia population, but no improvement of progression-free survival
			Newly diagnosed glioblastoma	NCT01790503	SOC therapy	I/II	No added therapeutic effect compared to SOC therapy alone
			Gastrointestinal cancer	NCT03158103	MEK162	I	Significant therapeutic efficacy observed in some patients
			Metastatic breast cancer	NCT01596751	Eribulin	Ib/II	Completed, no results available
			Sarcoma	NCT02584647	Sirolimus	I	Recruiting
			Pancreatic and colorectal cancer	NCT02777710	Durvalumab	I	Therapy well tolerated
			Prostate cancer	NCT02472275	None	I	Completed, no results available
			Advanced solid tumors	NCT02734433	None	I	Therapy well tolerated
			Tenosynovial giant cell tumor	NCT02371369	None	III	Enhanced objective response rate compared to placebo
		ARRY-382	Advanced solid tumors	NCT02880371	Pembrolizumab	II	Completed, no results available
				NCT01316822	None	I	Completed, no results available
		Cabiralizumab	Advanced malignancy	NCT03158272	Nivolumab	I	Completed, no results available
			Advanced solid tumors	NCT02526017	Nivolumab	I	Completed, no results available
			Tenosynovial giant cell tumor	NCT02471716	None	II	Completed, no results available
			Triple-negative breast cancer	NCT04331067	Nivolumab	I/II	Recruiting
			T-cell lymphoma	NCT03927105	Nivolumab	II	Active
			Melanoma, non-small-cell lung cancer, renal cell carcinoma	NCT03502330	APX005M nivolumab	I	Recruiting
		SNDX-6532	Cholangiocarcinoma	NCT04301778	Durvalumab	II	Recruiting
			Solid tumor	NCT03238027	Durvalumab	I	Active
		TPX-0022	Advanced solid tumor	NCT03993873	None	I	Recruiting
		Q702	Solid tumor	NCT04648254	None	I	Recruiting
		Edicotinib	Prostate cancer	NCT03177460	None	I	Active
		IMC-CS4	Melanoma	NCT03101254	Vemurafenib, cobimetinib	I/II	Active
			Pancreatic ductal adeno-carcinoma	NCT03153410	Cyclophosphamide, pembrolizumab, GVAX	I	Active
			Advanced solid tumors	NCT01346358	None	I	Decreased pro-inflammatory monocyte counts in peripheral blood, limited clinical efficacy
			Advanced breast, prostate cancer	NCT02265536	None	I	Reduction in TAM counts, prolonged stable disease in specific patient population
			Solid tumor	NCT02718911	Durvalumab, tremelimumab	I	Therapy well tolerated, limited therapeutic efficacy
		Emactuzumab	Pancreatic ductal adeno-carcinoma	NCT03193190	Additional therapies	I/II	Active
			Advanced head and neck squamous cell carcinoma	NCT03708224	Atezolizumab	II	Recruiting
			Advanced solid tumors	NCT02760797	RO7009789	I	Decreased pro-inflammatory monocyte counts in peripheral blood, limited clinical efficacy
				NCT02323191	Atezolizumab	I	Completed, no results available
				NCT01494688	Paclitaxel	I	Depletion of immunosuppressive TAMs, but no clinically-relevant response

(*continued on next page*)

Table 1 (*continued*)

Cellular target	Functional target	Drug	Disease	Study number	Additional Intervention	Phase	Study status/description
		DCC-3014	Advanced malignant neoplasm	NCT03069469	None	I/II	Recruiting
			Sarcoma	NCT04242238	Avelumab	I	Recruiting
CCR2	TAM recruitment	MLN1202	Bone metastases	NCT01015560	None	II	Completed, no results available
		BMS-813160	Renal cell carcinoma	NCT02996110	Nivolumab, ipilimumab, relatlimab, BMS-986205	II	Recruiting
			Colorectal, pancreatic cancer	NCT03184870	Chemotherapy or nivolumab	I/II	Active
			Pancreatic cancer	NCT03496662	Nivolumab abraxane, gemcitabine	I/II	Recruiting
				NCT03767582	IR, nivolumab, GVAX	I/II	Recruiting
			Non-small-cell lung cancer, hepatocellular carcinoma	NCT04123379	Nivolumab, BMS-986253	II	Recruiting
Tie2	TEMs	Temferon	Newly diagnosed glioblastoma	NCT03866109	IR	I/II	Recruiting
		Regorafenib	Hepatocellular carcinoma	NCT04170556	Nivolumab	I/II	Recruiting
		CEP-11981	Advanced solid tumors	NCT00875264	None	I	Completed, no results available
			Prostate cancer	NCT04159896	Nivolumab	II	Recruiting
				NCT03456804	None	II	Active

respective niches is key.

Unraveling the complexity of TAM plasticity and dynamic co-evolution with cancer cells in distinct glioblastoma niches, using groundbreaking technologies such as advanced multiplex immunofluorescence imaging or spatial transcriptomics [204,205], may open the way to the development of efficient combination therapies direly needed for glioblastoma patients.

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