



Challenges in glioblastoma research: focus on the tumor microenvironment

Andreas Bikfalvi, Cristine Alves da Costa, Tony Avril, Jean-Vianney Barnier, Luc Bauchet, Lucie Brisson, Pierre Francois Cartron, H  l  ne Castel, Eric Chevet, Herv   Chneiweiss, et al.

► To cite this version:

Andreas Bikfalvi, Cristine Alves da Costa, Tony Avril, Jean-Vianney Barnier, Luc Bauchet, et al.. Challenges in glioblastoma research: focus on the tumor microenvironment. Trends in Cancer, 2023, 9 (1), pp.9-27. 10.1016/j.trecan.2022.09.005 . hal-03860005

HAL Id: hal-03860005

<https://hal.science/hal-03860005>

Submitted on 24 Nov 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destin  e au d  p  t et    la diffusion de documents scientifiques de niveau recherche, publi  s ou non,   manant des   tablissements d'enseignement et de recherche fran  ais ou   trangers, des laboratoires publics ou priv  s.



Distributed under a Creative Commons Attribution - NonCommercial - NoDerivatives 4.0 International License

Feature Review

Challenges in glioblastoma research: focus on the tumor microenvironment

Andreas Bikfalvi,^{1,*,26} Cristine Alves da Costa,^{2,26} Tony Avril,^{3,26} Jean-Vianney Barnier,^{4,26} Luc Bauchet,^{5,26} Lucie Brisson,^{1,26} Pierre Francois Cartron,^{6,26} Hélène Castel,^{7,26} Eric Chevet,^{3,26} Hervé Chneiweiss,^{8,26} Anne Clavreul,^{9,26} Bruno Constantin,^{10,26} Valérie Coronas,^{10,26} Thomas Daubon,^{11,26} Monique Dontenwill,^{12,26} Francois Ducray,^{13,26} Natacha Enz-Werle,^{12,26} Dominique Figarella-Branger,^{14,26} Isabelle Fournier,^{15,26} Jean-Sébastien Frenel,^{7,26} Mathieu Gabut,^{13,26} Thierry Galli,^{16,26} Julie Gavard,^{6,26} Gilles Huberfeld,^{17,26} Jean-Philippe Hugnot,^{18,26} Ahmed Idhah,^{19,26} Marie-Pierre Junier,^{8,26} Thomas Mathivet,^{1,26} Philippe Menei,^{9,26} David Meyronet,^{20,26} Céline Mirjolet,^{21,26} Fabrice Morin,^{7,26} Jean Mosser,^{3,26} Elisabeth Cohen-Jonathan Moyal,^{22,26} Véronique Rousseau,^{4,26} Michel Salzet,^{15,26} Marc Sanson,^{19,26} Giorgio Seano,^{23,26} Emeline Tabouret,^{24,26} Aurélie Tchoghandjian,^{24,26} Laurent Turchi,^{25,26} Francois M. Vallette,^{6,26} Somya Vats,^{16,26} Maité Verreault,^{19,26} and Thierry Virolle^{25,26}

Glioblastoma (GBM) is the most deadly type of malignant brain tumor, despite extensive molecular analyses of GBM cells. In recent years, the tumor microenvironment (TME) has been recognized as an important player and therapeutic target in GBM. However, there is a need for a full and integrated understanding of the different cellular and molecular components involved in the GBM TME and their interactions for the development of more efficient therapies. In this review, we provide a comprehensive report of the GBM TME, which assembles the contributions of physicians and translational researchers working on brain tumor pathology and therapy in France. We propose a holistic view of the subject by delineating the specific features of the GBM TME at the cellular, molecular, and therapeutic levels.

Introduction

GBM is the most common type of malignant primary brain tumor, with an incidence of two to five cases per 100 000 people per year in the USA and Europe [1]. The prognosis for GBM remains poor, with a median survival of ~15 months for patients who receive standard therapy [2] and less than 1 year in population-based studies [1]. GBM is characterized by wild-type forms of *IDH1/2* [3], high proliferation rates, core necrosis, microvascular proliferation, and tumor infiltration. GBM treatment involves surgical resection and radiotherapy together with concomitant chemotherapy with temozolomide (TMZ), also known as the Stupp protocol, a treatment that has not evolved since 2005 [4]. Resistance to treatment is mainly caused by tumor cells escaping resection and/or invading normal brain parenchyma. To date, the mechanisms underlying GBM development remain incompletely understood and current experimental systems only partially recapitulate GBM properties.

The genetics of GBM have been extensively studied [5]. Recent DNA-sequencing techniques at the single cell level have documented the coexistence of different genetic subclones, while single cell RNA sequencing has unveiled different transcriptomic profiles [6]. However, this tumor cell-centric view may have underestimated the role of the TME, which has led to only incremental improvements in the therapeutic response. The TME includes components from the tumor niche as

Highlights

The tumor cell-centric view has underestimated the role of the tumor microenvironment (TME) and failed to translate into therapies that outperform Stupp treatment for glioblastomas (GBMs).

The tumor environment includes components from not only the tumor niche (TME), but also the tumor organismal milieu (macroenvironment).

The TME comprises several compartments, with intricate cellular and molecular interactions both within and between each compartment.

Neurons are recently investigated players in GBM development, which they regulate via GBM cell-neuron synapses.

Many experimental GBM models have been developed, including *in vivo/ex vivo* models in several species as well as 3D *in vitro* models.

Attempts to increase the complexity of 3D models have done so by introducing selected components of the TME.

The various therapeutic approaches in use or developed still require improvement by better defining the combination of the most promising targets within the TME.

Current therapies might also modify the TME and modulate the therapeutic response.

well as the tumor organismal milieu. A consensus review [7] listed the challenges that need to be addressed in neuro-oncology, including research on the brain TME, thereby establishing a close link with neuroscience. The TME controls both tumor growth and invasion in GBM, thus opening new possibilities for therapeutic intervention [8].

However, knowledge of the microenvironmental cues that impact GBM cell properties is sparse. The GBM microenvironment is complex, with intermingling immune cells, mainly represented by myeloid cells/microglia (one of the most abundant cellular constituents of the TME), blood vessels, astrocytes, and extracellular matrix (ECM), which varies across different tumor areas [9]. These interactions have been studied at the cellular and molecular level, such as the role of vascular endothelial growth factor (VEGF) and VEGF receptors (VEGFRs), guidance factors, and positive or negative cues between GBM cells and blood vessels [10]. More recently, an intricate interaction with neurons has been documented [11], but how all of these different elements are spatially and causally integrated is unknown.

The GBM immune landscape has also been explored and compared with brain metastasis using a combination of approaches [12]. The drawback of this work is its purely descriptive nature and the lack of information on how microglia or monocyte-derived macrophages spatially interact with the other components of the TME. Future work should investigate: (i) molecular events (and their dynamics) between tumor cells, neurons, vascular, and immune compartments; (ii) tumor regional heterogeneity; (iii) external constraints and cues regulating the TME and their impact on tumor development; and (iv) the impact of therapeutic interventions in the complex brain tumor environment (Figure 1). Given that both physicians and translational researchers working on brain tumor pathology and therapy, we provide here an extensive review that delineates the specific features of the GBM TME at the cellular, molecular, and therapeutic levels.

Glioblastoma stem cells

Cellular and molecular aspects

The concept of glioblastoma stem cells (GSCs) has provided new insights into GBM pathophysiology. This heterogeneous tumor cell population, which can be quiescent or proliferating, is essential for promoting tumor growth and heterogeneity. Although GSCs were first thought to be rare and confined to specific niches, recent studies point to a more widespread and complex distribution within tumor tissue [13].

Accumulating evidence suggests that GSCs correspond to a cell functional state rather than to a lineage, a novel and provocative concept further reinforced by the demonstration that GBM cells can reversibly adopt or lose stem-like properties in response to TME cues [13]. As such, tumor cells evolve on a continuous undifferentiated–differentiated axis, with epigenetic regulations underlying cell interconversions between these phenotypes [13–16]. Interactions with surrounding normal cells, such as subventricular zone (SVZ) cells of the neural stem cell niche, endothelial cells in the perivascular or hypoxic niche, or with tumor-activated macrophages in the immune niche, are instrumental to promote and maintain a stemness phenotype. GSCs from the SVZ display higher tumorigenicity features than those isolated from other locations [17]. Accordingly, patients with GBM cells in intimate contact with the SVZ exhibit increased multifocal recurrence and a substantial reduction in survival [18]. The crosstalk between GSCs and their non-cancerous neighbors is bidirectional and instrumental for shaping the TME and promoting therapy resistance. GSCs actively participate in perivascular niche formation mainly by the secretion of chemo-attractant and pro-angiogenic factors promoting endothelial cell (EC) proliferation and recruitment of their progenitors [19]. GSCs can also transdifferentiate into vascular/pericyte lineages and ECs [20]. Similarly, GSCs contribute to immune cell infiltration and the formation of a protumorigenic

¹Bordeaux University, INSERM, U1312 BRIC, Tumor and Vascular Biology Laboratory, F-33600, Pessac, France

²Côte d'Azur University, INSERM, CNRS, Institut de Pharmacologie Moléculaire et Cellulaire, Team "Laboratory of Excellence (LABEX) Distal", F-06560 Nice, France

³Rennes University, Inserm U1242, Centre de Lutte contre le Cancer Eugène Marquis, F-35000 Rennes, France

⁴Institute of Neuroscience Paris-Saclay, UMR9197, CNRS, Univ. Paris-Saclay, F-91191 Gif-sur-Yvette, France

⁵Montpellier University Medical Center, Department of Neurosurgery, INSERM U1191, F-34090 Montpellier, France

⁶CRCI2NA, INSERM U1307, CNRS UMR6075, Nantes Université, 44007 Nantes, France

⁷Normandie University, INSERM U1239, DC2N, Institute for Research and Innovation in Biomedicine (IRIB), F-76000 Rouen, France

⁸Sorbonne University, CNRS UMR8246, Inserm U1130, IBPS-Neuroscience Paris Seine, F-75005 Paris, France

⁹Angers University, CHU d'Angers, CRCINA, F-49000 Angers, France

¹⁰Poitiers University, CNRS UMR 6041, Laboratory Channels & Connexins in Cancers and Cell Stemness, F-86000 Poitiers, France

¹¹Bordeaux University, CNRS, IBGC, UMR 5095, F-33 077 Bordeaux, France

¹²Strasbourg University, Laboratoire de Bioimagerie et Pathologie, UMR7021 CNRS, F-67401 Illkirch-Graffenstaden, France

¹³Lyon I University, Cancer Research Centre of Lyon (CRCL) INSERM 1052&CNRS UMR5286, Centre Léon Bérard, Lyon 69008, France., F-69622 Villeurbanne, France

¹⁴Aix-Marseille University, Service d'Anatomie Pathologique et de Neuropathologie, Hôpital de la Timone, F-13385 Marseille, France

¹⁵Lille University, Inserm, CHU Lille, U1192, Laboratoire Protéomique, Réponse Inflammatoire et Spectrométrie de Masse (PRISM), F-59000 Lille, France

¹⁶Université Paris Cité, Institute of Psychiatry and Neuroscience of Paris (IPNP), INSERM U1266, Membrane Traffic in Healthy & Diseased Brain, GHU PARIS Psychiatrie & Neurosciences, F-75014 Paris, France

¹⁷College de France, Center for Interdisciplinary Research in Biology (CIRB), CNRS, INSERM, Université PSL, Paris 75005, France

¹⁸Montpellier University, Institut de Génétique Fonctionnelle, CNRS, INSERM, F-34094 Montpellier, France

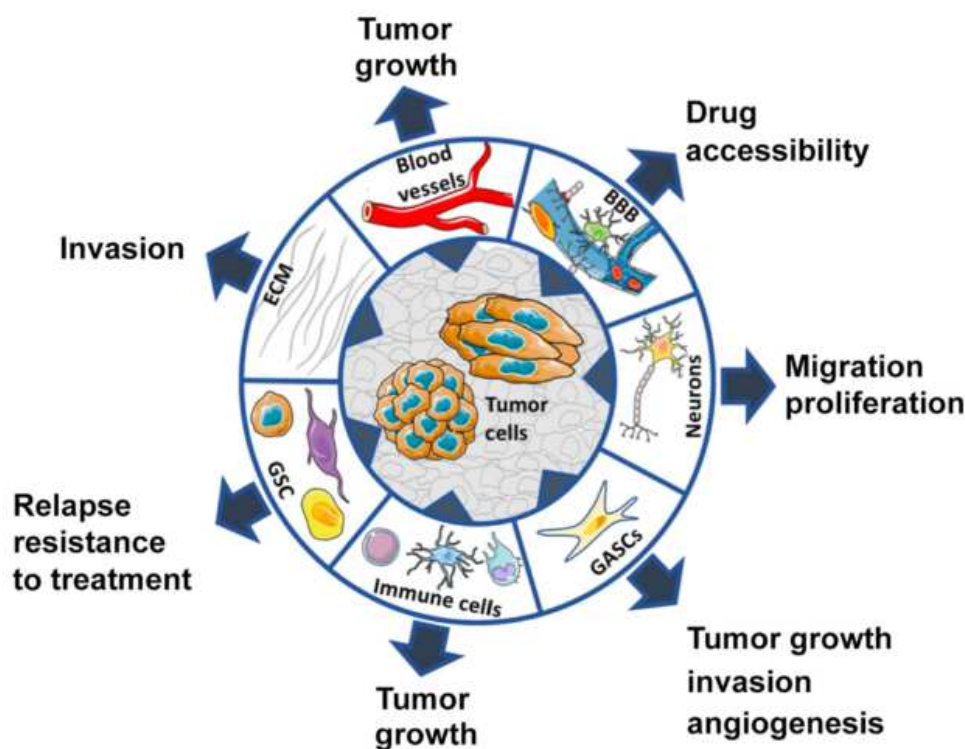


Figure 1. Schematic of the interaction of glioblastoma (GBM) cells with their microenvironment and functional consequences for tumor progression and response to treatment. Different representations of GBM tumor cells (sphere/stem versus adherent/mesenchymal) at the center of the cartoon are indicative of tumor cell heterogeneity. Abbreviations: BBB, blood-brain barrier; ECM, extracellular matrix; GASC, glioma-associated stromal cell; GSC, glioblastoma stem cell (GSC)-specific niches.

¹⁹Sorbonne University, AP-HP, Institut du Cerveau - Paris Brain Institute - ICM, Inserm, CNRS, Hôpitaux Universitaires La Pitié Salpêtrière - Charles Foix, F-75013, Paris, France

²⁰Institute of Neuropathology, Hospices Civils de Lyon, F-69008, Lyon, France

²¹Centre Georges-François Leclerc, UNICANCER, Dijon, France. Inserm U1231, Equipe Cadir, F-21000 Dijon, France

²²Institut Claudius Regaud, NSERM 1037, CRCT Team RADOPT, Département de Radiothérapie, IUCT-Oncopole, F-31100 Toulouse, France

²³Curie Institute Research Center, Tumor Microenvironment Laboratory, PSL Research University, Inserm U1021, CNRS UMR3347, F-91898 Orsay, France

²⁴Aix-Marseille University, CNRS, INP, Inst Neurophysiopathol, F-13005 Marseille, France

²⁵Côte D'Azur University, CNRS, INSERM, Institut de Biologie Valrose, Team INSERM "Cancer Stem Cell Plasticity and Functional Intra-tumor Heterogeneity", F-06108 Nice, France

²⁶The authors belong to the French preclinical brain tumor study group "GrOupe Préclinique de l'Anocé" (GOPA)

Trends in Cancer

*Correspondence: andreas.bikfalvi@u-bordeaux.fr (A. Bikfalvi).

inflammatory environment through the secretion of cytokines and growth factors [21]. In this context, GSCs induce tumor-associated macrophages (TAMs) to adopt an immuno-suppressive phenotype, whereas TAMs secrete factors contributing to GSC maintenance [22]. Therefore, the TME appears to be a key regulator of stemness, whereas GSCs affect the microenvironment. This complex dynamic is instrumental for tumor aggressiveness and, thus, its targeting could improve therapeutic strategies.

Therapeutic implications

GSCs are resistant to current therapies and have a crucial role in tumor recurrence. Indeed, tunneling nanotubes (TNTs), thin membranous open-ended channels connecting distant cells, provide alternative means of connection between GSCs, allowing mitochondrial transfer. GSCs from the metabolic area in the infiltrative zone (FLAIR) defined by MRI spectroscopy in patients treated for GBM have more TNT connections compared with GSCs from the non-metabolic area [23]. Moreover, radiotherapy can stimulate TNT formation and mitochondria transfer in GSCs from the metabolic area (Box 1). These results show the importance of plasticity in GBM cells and metabolic heterogeneity in response to radiotherapy [24]. Scientists have since tried to develop strategies directed at their elimination either by cytotoxic agents [25] or by repressing their stemness properties [26,27].

Box 1. Metabolism in GBM

Primitive brain cancers and brain cells share an affinity for glucose and glutamine [145]. Aberrant glucose and glutamine metabolism is a major metabolic hub stimulating GBM growth and inducing chemoresistance [146]. Numerous studies have shown that GBMs are metabolically plastic and can adapt to nutrient shortage by switching from one carbon source to another, or to alternative energy sources [147]. Furthermore, the microenvironment has been shown to be implicated in metabolic plasticity and reprogramming [148]. Glucose/glutamine interconnection in GBM could be reminiscent of the astrocyte–neuron metabolic interplay in which glutamate to glutamine conversion and reciprocal reaction fuels the tricarboxylic acid (TCA). By contrast, lactate produced in glycolytic cells can be released and then taken up by surrounding cancer cells for making amino acids, nucleic acids and fatty acids through anaplerosis [149]. The microenvironment is one of the major actors in cancer metabolism plasticity, which determines most glioma features, such as migration, cell survival, cell death, EMT, dormancy, and stemness. Mitochondria have a central role in ATP production and redox balance, but are also involved in many cellular processes, such as proliferation and cell death. Thus, changes induced by metabolism affect organelle functions and several cellular processes. Cancer development progression and treatment resistance in GBM is linked to distinct subsets of GSCs [150]. Metabolically, GSCs have a higher plasticity than their normal counterparts (neural stem cells) and their differentiation in proliferating GBM appears to be under the control of several metabolic enzymes, with non-canonical PKM2 activity being crucial in this process [151]. As reported above, original studies have shown that GSCs mainly depend on glycolysis, but recent articles have demonstrated that these cells might prefer mitochondrial oxidative metabolism. The slow-cycling GSCs appear to rely on both glycolysis and oxidative phosphorylation depending on the environmental situation and give rise to metabolically distinct GBM subpopulations [152]. GSC maintenance is also under the control of a methyl donor metabolism, nicotinamide *N*-methyltransferase (NNMT), implicated in epigenetic regulation [15].

GBM vascular compartment

Cellular and molecular aspects

GBM are highly vascularized tumors, in which the endothelium not only fuels tumor growth with oxygen and necessary nutrients, but also provides a protective microenvironment. As such, ECs are active components of tumor development. The formation of new blood vessels in brain tumors leads to disorganized, convoluted, and leaky vascular networks, suggesting abnormal angiogenesis in the tumor. Pro-angiogenic factors are produced by tumor cells, cancer-derived stromal cells, and inflammatory cells [28], leading to the idea that the TME provides an aberrant vascular bed [29]. In the brain tumor ecosystem, metabolic, hypoxic, and inflammatory stressors modulate the production rate and nature of pro-angiogenic factors. Notably, cells within the hypoxic environment, often at the periphery of the necrotic core, release aberrant levels of VEGF, among other pro-angiogenic paracrine factors, which might also trigger enhanced vascular permeability, exacerbated vascular bed inflammation, and edema [29]. In line with this finding, tumor-derived ECs maintain their identity while gaining mesenchymal characteristics, which promotes tumor proliferation, migration, and permeability [30]. Furthermore, tumor-induced angiogenesis might rely on the recruitment of bone marrow-derived endothelial progenitor cells, or other stem cells [31]. Hypoxia also increases local DNA methylation disorder and shifted cell states, which reflects an epigenetically facilitated adaptive stress response [32].

Vasculature is also rerouted for the benefit of the tumor through co-option of pre-existing vessels. This migration of tumor cells toward and along pre-existing vessels provides cancer cells with privileged access to nutrients and oxygen, as well as a trail for invasion [33]. More atypical features, such as vascular mimicry (i.e., the propensity of tumor cells to simulate an endothelial phenotype), might provide additional complexity to the brain TME [34]. This interdependency between cancer cells and vasculature might be even more plastic and dynamic than initially thought, following the discovery of new modalities involved in building a dedicated tumor vasculature, including processes such as *trans*-differentiation by the commitment of GSCs to endothelial and pericyte lineages [35]. This process might be associated with the so-called ‘perivascular niche’, where GSCs and ECs are in close contact [36]. Single cell sequencing of CD31-expressing ECs led identified five clusters when comparing the tumor core to the tumor periphery. Three clusters are present in the tumor core and two in the periphery. Expression profiles of blood–brain barrier (BBB)-associated transporters indicate that the BBB is partially preserved [37]. The variety of

ECs in GBM is further enhanced by the finding that tumor-derived ECs (TDECs), which arise from GBM cell *trans*-differentiation, and tumor-associated vessel ECs (TAVs) are molecularly distinct, although heterogeneity is observed within each group [38].

Future work should better define the different processes involved in vascularizing and perfusing tumors and determine how they dynamically coexist during tumor evolution and in response to treatment. The molecular circuitry driving the adaptability of ECs in hostile conditions remains an open question.

Therapeutic implications

Several clinical studies involving VEGF/VEGFR inhibition have been reported, with mixed outcomes (75 clinical trials, of which 53 involved bevacizumab). Most anti-angiogenic therapies (e.g., bevacizumab, cilengitide, or cediranib) failed to improve the overall survival (OS) and quality of life of patients [39]. There are several possible reasons for this limited efficacy, including VEGF-independent angiogenesis, vessel co-option, and inefficient antiangiogenic factor delivery to the tumor [40]. Alternatively, VEGF is secreted and transported within extracellular vesicles, where it is inefficiently targeted by bevacizumab [41–44].

Efforts have been made to develop novel therapies based on anti-angiogenic factors that target angiogenesis through different mechanisms of action. Recently, regorafenib, an inhibitor of several kinases involved in neoangiogenesis regulation, showed promising results when used as monotherapy for recurrent GBM [45]. Alternative anti-angiogenic therapeutic approaches rely on the targeting of vasculogenesis and vessel co-option through alteration of the CXCR4/CXCL12 axis [46]. As such, the CXCR4 inhibitor plerixafor was evaluated in a Phase 1/2 trial upon continuous infusion during chemoradiation for patients with newly diagnosed GBM. This treatment was well tolerated by patients and was associated with decreasing blood volume in the irradiation field. Other factors involved in vessel co-option included angiopoietin-2, IL8, EGFRvIII, MDGP/FABP3, IRE1 α , CDC42, and Ephrin-B2. The inhibition of angiopoietin-1 and -2 by trebananib for patients with recurrent GBM was well tolerated but did not improve patient survival alone or in combination with bevacizumab [47]. Regarding the targeting of EGFRvIII, the first results of a specific vaccine called rindopepimut were disappointing [48] but new drugs are under investigation [49]. Finally, the MET pathway was explored to disrupt the angiogenic process in GBM. This pathway was suspected to be involved in angiogenesis independent of VEGF and represented an interesting approach alone or in combination with a VEGF inhibitor. However, onartuzumab, a MET inhibitor, used in combination with bevacizumab, yielded inconclusive results [50]. The tyrosine kinase inhibitor cabozantinib was also tested as monotherapy for patients with recurrent GBM who had either benefited or not from previous anti-angiogenic therapy [51,52]. Although it failed in patients who already received bevacizumab, it was associated with a response rate ranging from 14% to 17% in patients naïve to anti-angiogenic therapy, thus opening future opportunities for clinical development.

More recently, combinations with immunotherapy have been proposed. These therapies generally fit into the following categories: targeted molecular inhibitors, vaccine-based therapies, viral therapies, and adoptive T cell therapies. Bevacizumab is the only anti-angiogenic agent that has so far been trialed with immunotherapy in GBM. Bevacizumab has the theoretical advantage of indirectly promoting an immune response by reducing the use of corticosteroids. However, there have not yet been any successful Phase 3 trials in immunotherapy and anti-VEGF combinatorial treatments for GBM. The two best-studied combinations are immune checkpoint inhibitors (ICIs) with bevacizumab and vaccine-based therapies with bevacizumab, such as the Durvalumab (NCT02336165) and Gliovac studies (NCT01903330). Yet, targeting the GBM

TME using a combinatorial strategy (anti-angiogenics and immunotherapy) remains an appealing therapeutic approach.

GBM and the blood–brain barrier

Cellular and molecular aspects

The BBB is an anatomic and biochemical barrier with multiple proteins controlling the transport of various molecules across its cellular and acellular structures. Several mechanisms of transport are described based on molecular weight, liposolubility, electric charge of cargo molecules, or interactions with proteins in circulation or the BBB. Blood pressure is also involved in modulating the traffic of molecules across the BBB. Small lipophilic molecules use passive transcellular and paracellular pathways between and across vascular ECs. Larger or hydrophilic molecules are transported by transcytosis or specific receptor-mediated transport. The efflux pump system is involved in expelling harmful molecules out to the bloodstream [53,54]. In GBM, the BBB is partly disrupted, and the term ‘blood–tumor barrier’ is preferred. However, the BBB remains intact in the bed around the tumor, where invasive cells involved in drug resistance and recurrence are located [55,56].

Therapeutic implications

Overall, antitumor drugs cross the BBB inadequately. The most effective drugs used in neuro-oncology are TMZ and nitrosoureas, with an efficacy for crossing the BBB of 15–30%. Although the dose reaching the tumor cells is limited, increasing the delivered dose can expose the patient to systemic toxicity. Multiple strategies are under development to disrupt, bypass, or overcome the BBB obstacle, such as increasing local or systemic delivery and increasing drug penetration by drug or BBB modulation. Increasing drug delivery relies mainly on high-dose intravenous doses or intra-arterial chemotherapies with modest results in GBM. Another approach to overcome the BBB relies either on the use of carrier agents to promote drug penetration or BBB modulation using drugs or physical interventions [57]. A Phase 2 clinical trial testing TMZ and liposomal doxorubicin showed promising results, with four out of 22 patients with recurrent GBM showing a response [58]. Finally, transient BBB disruption using chemical/physical agents (e.g., hyperosmotic agents or ultrasound) appears a promising approach and is being investigated in GBM clinical trials [55]. Nevertheless, the BBB remains a major factor involved in the chemoresistance of GBM cells.

The GBM neuronal compartment

Cellular and molecular aspects

The effects of neuronal activity and synaptic signaling on GBM dynamics have been studied by grafting the tumor in the visual cortex and controlling light exposure or by optogenetically increasing neuronal activity, resulting in modulation of the number of dividing tumor cells [59]. Neuronal activity-driven GBM growth is first mediated by secreted factors, such as brain-derived neurotrophic factor (BDNF) or the synaptic adhesion molecule Neuroligin-3 [60]. Neuroligin-3 acts on GBM cells, triggers its own secretion (autocrine effect), and favors cell division through the PI3K-Akt-mTOR pathway. The expression of functional receptors for several neurotransmitters in GBM cells constitutes another level of exposure to the neuronal environment. For instance, GBM cells express fully functional dopamine receptors, including the D2 and D4 subtypes [61], the blockade of which affects GBM growth. Glutamatergic excitatory synaptic signaling is a major microenvironmental driver of GBM growth. Extracellular glutamate concentration increases up to 100-fold both in gliomas and their vicinity in humans [62] and mouse models (Box 1). A single dose of sulfasalazine reduces brain glutamate concentration by 20–50%, in correlation with the expression of the cysteine-glutamate antiporter (SXC; SLC7A11) [63]. Direct bona fide synapses from neurons to glioma cells have been documented in animal models and human tissue,

especially in astrocytomas and GBM [11,64]. It was reported that 10–16% of glioma cells contained these synapses, linking axons of pyramidal cells with microtubules of glioma cells, and mainly comprised postsynaptic glutamatergic AMPA receptors, with high rates of edited GluR2 subunits, which allowed for Ca^{2+} permeability. Glutamatergic signaling led to the division and migration of tumor cells [11,64]. GABA also regulates GBM cell proliferation [65]. Glioma cells express ionotropic GABA receptors with a different status according to their state of malignancy, with functional receptors identified on low-grade glioma cells, whereas nonfunctional receptors mostly formed by Theta subunits are expressed by GBM cells [66]. GABA signaling also modulates GBM growth by regulating chloride ion fluxes [67]. However, pharmacological approaches targeting those pathways have not yet translated into efficient clinical strategies.

Therapeutic implications

The fact that glioma stem-like cells can form oncogenic synapses with glutaminergic neurons supports the development of therapies targeting this interaction. Blocking AMPA receptors with the approved antiepileptic drug perampanel resulted in the reduction of glioma proliferation [64] and invasion [11] in animal models, whereas the AMPA antagonist talampanel prolonged the survival of patients [68]. GBM cells also express glutamate metabotropic receptor Grm3. Targeting these receptors by specific inhibitors could constitute an interesting venue for the development of novel therapies [69]. Other potential strategies might include acting directly on glutamine via GLS-mediated glutaminolysis, as reported for SNAP25 [70], repressing glutamate outflow by the Xc system [63], or enhancing glutamate uptake from astrocytes via the upregulation of EAAT2 by the peroxisome proliferator-activated receptor gamma (PPAR γ) [71].

The GBM immune compartment

Cellular and molecular aspects

Immune cell infiltration into GBM is controlled by the release of factors from tumor cells (Box 2), which is regulated by specific pathways in tumors cells, such as metabolic pathways and the unfolded protein response (UPR) (Box 3). Furthermore, autophagy (Box 4) might impact directly on the TME by modifying the immune response. The immune response can be divided into adaptive and innate immunity.

Box 2. The GBM secretome

The secretome corresponds to the material released by cells (proteins and metabolites) into the extracellular space, either using conventional (e.g., the secretory pathway) or unconventional mechanisms. Secreted proteins account for 9–15% of the total human proteome. The GBM microenvironment is impacted by tumor and stromal cell secretion, which are major factors in tumor aggressiveness. Secretome analysis in the human GBM cell line U87 showed high levels of proteins, such as ADAM9, ADAM10, Cathepsin B, Cathepsin L1, Semaphorin7A, Semaphorin3A, and Neuropilin1 [153]. Some of these proteins are proteases, which might help cell detachment and migration. GBMs also secrete cytokines, such as IL1 β , IL6, IL8, or IL18, which are associated with poor prognosis, either through conventional or unconventional routes. Cytokines, such as IL1 β and IL18, are secreted via autophagy-mediated secretion [154]. A secretory reticulophagy route was recently shown to mediate the release of reticulons in a VAMP7-dependent manner and this pathway was increased in cells defective in degradative reticulophagy [155,156]. Therefore, it will be important to characterize unconventional secretion, such as that of endoplasmic reticulum elements, in GBM, its potential effect on tumor development and communication with neurons. For a long time, the functional consequences of the close proximity between GBM cells and neurons were not well understood, but there is now ample evidence to suggest that they participate in a mutually beneficial relationship. Secreted molecules from neurons or the neuronal secretome modulate the GBM microenvironment and impact glioma growth and survival prognosis. Reciprocally, GBM cells produce metabolites that can be secreted into the extracellular space, such as lactate, glutamine, or glutamate. A lactate shuttle has been identified between glycolytic astrocytes and oxidative neurons. The release of glutamate has dual consequences. First, it results in enhanced neuronal activity or hyperexcitability, which sometimes manifests as seizures and can even cause neuronal death to create more space for GB expansion. Second, it has prosurvival effects on GBM cells [157]. This indicates the existence of positive feedback loops because GBMs can enhance neuronal activity by secreting glutamate, thereby fueling their own growth in turn. The characterization of molecules secreted as part of the neuronal secretome likely to impact GB progression opens new avenues for disease treatment and management.

Box 3. The unfolded protein response in GBM

The UPR is an adaptive mechanism triggered in response to endoplasmic reticulum (ER) stress. It is transduced by three sensors: protein kinase R-like endoplasmic reticulum kinase (PERK), inositol-requiring enzyme 1 alpha (IRE1alpha, referred to as IRE1 hereafter), and activating transcription factor 6 (ATF6), and is a key factor in cancer development. GBM cells are exposed to cellular stresses that trigger the UPR. Exploring the role of the UPR showed the involvement of ATF6, IRE1, and PERK in different aspects of GBM pathophysiology [158]. The ATF6 arm of the UPR is involved in GBM resistance to radiotherapy [159]. The UPR also regulates GBM stemness [160] and pharmacological inhibition of PERK limits ER stress-induced GSC death, thus highlighting PERK as an important regulator of GSC growth and differentiation.

IRE1 has been identified as a key regulator of GBM aggressiveness associated with tumor growth, neo-angiogenesis, tumor migration/invasion properties, and proinflammatory processes [158]. Moreover, IRE1 signaling has an active role in tumor immune reshaping, in particular of monocytes/macrophages and neutrophils [161]. IRE1 signaling also contributes to the organization of the tumor architecture, favoring tumor growth, infiltration, and mesenchymal cell phenotypes *in vivo* [162,163]. Antagonistic effects of IRE1 signaling towards XBP1 mRNA non-conventional splicing or RNA degradation were described in tumor invasion, neo-angiogenesis, and the inflammatory response, influencing GBM aggressiveness [161]. Patients with GBM characterized by high IRE1 activity had a poorer prognosis and displayed increased immune, angiogenic, and migration markers. Overall, IRE1 represents an appealing therapeutic target in GBM, and several IRE1 inhibitors are currently available, targeting either its kinase or RNase activities [164]. A recent preclinical study using a mouse model recapitulating the SOC for patients with GBM confirmed the efficacy of such IRE1 inhibitors [165]. Thus, the UPR controls major features related to GB aggressiveness and represents an actionable pathway to improve disease management.

Adaptive immunity

Early studies report the presence of rare infiltrating lymphocytes within tumors, mainly CD4 and CD8 T cells. These cells represent 4–40% of infiltrating immune cells [72]. Such variability was associated with biological heterogeneity, the methods used [e.g., immunohistochemistry (IHC) or fluorescence-activated cell sorting (FACS)], or the characterization performed after *in vitro* expansion [73]. Reduced immune T cell infiltration is attributed to the high immunosuppressive properties displayed by GBM cells, including TGF β secretion [74], and, thus, GBM is defined as an immunologically cold tumor [75]. These initial observations are further explained by the increased

Box 4. Autophagy in GBM

Autophagy is a multistep process of protein/organelle quality control that maintains cell homeostasis under different cellular stress inducers. Autophagy can be associated with either a protumorigenic or tumor-suppressive response depending on various parameters, including the cellular context, type of stimulus, and phase of tumor development. Current data describing a role of macroautophagy (referred to as autophagy hereafter) and chaperone-mediated autophagy (CMA) in TME regulation in GBM are summarized here.

Several studies have demonstrated the link between key players in gliomagenesis and autophagy regulation. Notably, it has been shown that p53, a key tumor suppressor involved in GBM etiology, controls the transcription of two master regulators of mitophagy: PRKN and PINK1 [166]. Interestingly, p53 and PRKN protein levels are inversely correlated according to the grade of gliomas [167] and decreased PRKN microenvironment levels lead to increased tumor progression [168]. The reciprocal relationships between autophagy and the immune system point toward autophagy as an important GB TME modulator. Autophagy promotes tumor progression by increasing the phagocytotic activity of macrophages and by inhibiting inflammasome-mediated responses of microglial cells. It might also foster tumor evolution by either stimulating the differentiation of monocytes into anti-inflammatory M2 macrophages or by delaying monocyte polarization into proinflammatory M1 macrophages [169]. Autophagy has also been shown to decrease tumor activity by inducing an inflammatory response in neutrophils [170].

CMA is a type of autophagy responsible for the lysosomal degradation of cytosolic proteins harboring the KFERQ consensus motif. CMA is constitutively activated in cancer cells [171] and tightly linked to TME regulatory stresses [172]. CMA is important in maintaining aerobic glycolysis in cancer cells and in the catabolism of cell cycle regulatory proteins [173]. Analysis of patient biopsies indicated an increase in the CMA makers LAMP1 and LAMP2A after treatment with TMZ [174]. In addition, CMA has been linked to microglial cell functions in neurodegenerative diseases, providing a potential link to the innate immune response, which might be relevant in GBM [175]. Cellular interactions between perivascular and GB cells are important for tumor progression. CMA-defective pericytes led to a reduction in GB development compared with CMA-competent pericytes [176]. In brief, studies to analyze the role of the various types of autophagy in TME impact on GB development are still needed and could lead to the identification of new therapeutic pipelines.

naïve T cell sequestration in the bone marrow compartment in patients with GBM, resulting in a reduced number of T cells at the periphery and in the tumor site, a phenomenon linked to tumor-induced Sphingosine-1-phosphate receptor 1 (S1P1) loss on the T cell surface [76]. Recently, single-cell analysis of GBM-infiltrating T cells from human glioma unveiled four major T cell clusters, including CD4 (conventional and regulatory), CD8, and cycling T cells [77]. Among the CD8 T cell cluster, one predominant subtype comprised cytotoxic T cells that express not only a cytotoxicity related-gene signature (including perforin-1 and granzymes), but also natural killer (NK) cell-related genes that negatively modulate cytotoxic functions, such as that encoding the inhibitory receptor CD161. This finding opens avenues to target new molecular pathways to transform GBMs from immunologically 'cold' to 'hot' tumors using novel immunotherapy approaches.

Innate immunity

NK cells recognize target cells lacking major histocompatibility complex (MHC) expression. They represent crucial antitumor response agents when cytotoxic T lymphocytes are compromised. NK cells can target GBM cells because these cells express low levels of MHC. GBM cells, including GSCs, are recognized and killed by NK cells *in vitro* [78,79], and high CD56⁺ CD16⁺ activated NK cells are associated with favorable patient outcomes [80]. However, limitations remain to challenging NK cell efficiency in GBM. First, the number of GBM-infiltrating NK cells remains low, representing <1% of all infiltrated immune cells [12]. Second, GBM-infiltrating NK cells harbor a reduced expression of tumor-sensing NK cell-activating receptors, such as NKp30, NKG2D, DNAX accessory molecule-1 (DNAM1), or surface molecules, including CD317 and CD210. Moreover, GBM tumor cells, including GSCs, are directly responsible for NK cell inactivation by expressing immunosuppressive CD7312 and overexpressing MHC class I [81,82].

The most abundant cells in GBM are tumor-associated macrophages (TAMs). These constitute up to 40% of the tumor mass and correspond to brain-resident microglia and bone marrow-derived myeloid cells. TAMs contribute to tumor heterogeneity and tumor progression. As innate immune cells, TAMs express Toll-like receptor-2, the activation of which leads to the expression of matrix metalloproteinase (MMP)-14, which is necessary for MMP2 release and GBM invasion. TAM infiltration has been associated with poor outcomes in patients with GBM, and in preclinical mouse models of GBM. TAMs also promote GSC proliferation, a mesenchymal phenotype, and resistance to radiotherapy and anti-VEGF therapy [83].

The TME modulates the response to radiotherapy because hypoxia is a major factor for resistance in several tumors, including GBM. Preclinical studies showed that radiation-induced hypoxia prompts an increase in stromal cell-derived factor-1 (SDF1/CXCL12) secretion [84]. This signaling pathway has been targeted in GBM animal models, leading to macrophage exclusion and improvement of local control and survival after radiotherapy [85]. In addition, radiotherapy promotes the expression of tumor antigens along with the translocation of calreticulin to the tumor cell surface, leading to their phagocytosis [86]. This results in cGAS/STING activation and the subsequent induction of interferon β (IFN β) [87]. Interestingly, whereas hypo-fractionated radiotherapy (3 \times 8 Gy) induced an immune cell death through cGAS/STING, 12–18 Gy induced the DNA exonuclease Trex1, thus clearing DNA fragments from the cytosol and inhibiting tumor immunogenicity [88].

Neutrophils, the first line of innate immune defense, are present in the TME as tumor-associated neutrophils (TANs) and can be polarized toward a proinflammatory N1 phenotype in the presence of IFN β or to a protumoral N2 phenotype in the presence of TGF β . Whereas the N1 phenotype inhibits tumor growth and induces the immune response, the N2 phenotype, which is prominent

in GBM, promotes tumor growth, stemness, invasion, and angiogenesis, and suppresses immune responses [89]. Moreover, myeloid cell content changes during tumor evolution and standard-of-care (SOC) therapy. In early-stage tumors, proinflammatory microglia are predominant, whereas in end-stage tumors, anti-inflammatory macrophages and protumorigenic myeloid-derived suppressor cells are present [90]. TMZ decreased myeloid-derived suppressor cells and, when combined with irradiation, intratumoral Granzyme B⁺ CD8⁺ T cells influx into the tumor. Brain meningeal lymphatic vasculature can also be remodeled using ectopic expression of VEGF-C, thus enhancing immune surveillance and T cell-mediated immunity against GBM cells [91].

Therapeutic implications

Four main immunotherapeutic approaches are considered in GBM: (i) immune check point inhibitors (ICIs), (ii) cell, vaccine and dendritic cell (DC) therapies; (iii) oncolytic viruses; and (iv) immune cytokine inhibitors. The main immunotherapy approaches, stimulating the antitumor activities of cytotoxic T cells through inhibition of immune checkpoints, failed to improve the survival of patients with GBM [92]. The current perspectives for ICIs in brain tumors are to refine their administration schedule, to combine ICIs or to test new ICIs (e.g., LAG3, TIM3 or TIGIT inhibitors). ICI efficacy is based on the ability of T cells to infiltrate the tumor and on the development of an environment that is favorable to cytotoxic responses. These conditions are rarely met in GBM because of the abundance of immunosuppressive TAMs. Resistance to ICIs can be circumvented by depleting tumor macrophages with a CSF1R1 receptor inhibitor [93].

One of the most promising cell therapy approaches are chimeric antigen receptor (CAR)-T cells, comprising a modified T cell receptor containing a single-chain variable antibody fragment (scFv) against a tumor-associated antigen. In neuro-oncology, several CAR-T cells are under investigation, targeting EGFRvIII, HER2, and IL13Rα2. First results obtained from Phase 1/2 trials suggested an acceptable safety profile with promising results [94,95]. Recently, a CAR-engineered NK cell that targets both wild-type EGFR and EGFRvIII mutant has been developed, with promising results in mice.

Vaccination and DCs also present an attractive method for actively targeting GBM. First results using EGFRvIII vaccination reported disappointing results in a Phase 3 trial [48] but personalized approaches, based on patient tumor antigen expression profiles, appear promising despite the complexity of their management [96]. DCs pulsed by tumor-specific peptides, lysates, or antigens can also enhance antitumor T cell responses. Different DC-based vaccinations have been evaluated, showing interesting response rates and excellent safety profiles [97]. However, this approach remains challenging and expensive.

Another immunogenic active approach is the use of oncolytic viruses, which can selectively infect and kill tumor cells. The aim of using these viruses is to turn an immunosuppressive microenvironment into an immune-supporting environment. Various oncolytic viruses are under investigation, including herpes virus, parvovirus, adenovirus, and cytomegalovirus. In a Phase 1/2 trial, an oncolytic H-1 parvovirus was injected before and during surgical resection of recurrent GBM [98]. This virus was able to cross the BBB, spread through the tumor, and trigger an immunogenic stimulus, opening promising perspectives. Similar results were observed in another early-development clinical trial using an oncolytic reovirus, the results of which support the combination of oncolytic viruses and PD1 blockade [99].

Finally, among the different cytokines implicated in the immunosuppressive GBM TME, tumor cell-secreted CSF1 might be involved in the switch of tumor-resident macrophages/microglia

to an immunoinhibitory phenotype. Conversely, TAM depletion by LyG6 antibodies prolonged survival in a GBM mouse model [100], whereas the combination of the arginine-depleting agent ADI-PEG20 with irradiation led to almost-complete tumor eradication in an immunocompetent mouse model by promoting microglial recruitment and M2 to M1 polarization [101]. The first results of phase II trial evaluating the CSF-1 receptor inhibitor alone showed no efficacy [102] but additional studies are ongoing.

Few clinical trials have aimed to optimize irradiation with immunotherapy. In one trial, immunotherapy was added to irradiation in combination with bevacizumab [103]. In another trial, stereotactic 3 × 8Gy re-irradiation in the presence or absence of the anti-PDL1 durvalumab (NCT02866747) was compared. The use of tumor-treating fields (TTFields) as adjuvant treatment after standard radiochemotherapy in patients with *de novo* GBM also increased progression-free survival and OS [104], and presented, beside disrupting mitosis, the capacity to induce immunogenic cell death. In models other than GBM, TTFields increase peritumoral lymphocyte infiltration compared with nontreated animals [105]. The combination of such a device with standard radiochemotherapy is being investigated for *de novo* GBM (Trident EF-32).

GBM-associated stromal cells

Cellular and molecular aspects

The GBM TME also harbors glioma-associated stromal cells (GASCs). These cells have phenotypic and functional properties similar to those of mesenchymal stem cells (MSCs) and cancer-associated fibroblasts (CAFs) [106]. GASCs are present in human low-grade gliomas (LGGs) and GBMs, mostly around blood vessels, but also in tumor tissue. Cultured GASCs have properties similar to MSCs, such as adhesion to plastic, expression of surface antigens characteristic of MSCs (CD73, CD90, and CD105), mesenchymal differentiation potential, and lack of tumorigenesis potential. They also have phenotypic and functional properties in common with CAFs, such as expressing markers FSP1/S100A4 and promoting angiogenesis, invasion, and tumor growth by secreting soluble factors and extracellular vesicles [106]. The origin of GASCs has yet to be determined. They might arise from the *trans*-differentiation of local brain cells, such as astrocytes, pericytes, vascular smooth muscle cells, or ECs. GASCs can also originate from bone marrow-derived MSCs. They are not a homogeneous stromal population, given that GASCs isolated from LGGs and GBMs present different proteomic profiles and cancer cell adhesion capacities [107]. Furthermore, GASCs might also differ between gliomas with the same histological classification. Two peritumoral microenvironments can be found in patients with GBM: a peripheral microenvironment containing GASCs with tumor-promoting and angiogenic properties, and another containing GASCs without such properties [108]. The genetic background of tumor cells could account for these different GASC statuses. GASCs might also have prognostic value in gliomas because the percentage of GASCs in GBMs [109] inversely correlates with overall survival. These cells, which complicate our understanding of the GBM TME, should not be ignored. Thus, additional studies on the impact of GASCs on the response to treatment are urgently required.

Therapeutic implications

Given their tumor-supporting functions, GASCs are promising new targets for glioma treatment. However, additional studies are required to identify candidate target molecules. One way to circumvent this problem is by using the properties of these cells to trap, rather than target, tumor cells [110]. The concept of tumor cell traps, which emerged from the ecological trap strategy, is designed to attract residual brain cancer cells surrounding the surgical cavity into the cavity, where they are trapped within a biomaterial support that can be targeted by treatment, such as stereotactic radiosurgery [111–113]. Molecules or particles with radio-sensitizing and/or chemotherapy characteristics could also be embedded within the biomaterial to improve tumor cell

destruction. Given that GASCs increase the invasiveness of GBM cells, a bacterial cellulose scaffold that was loaded with GASC-conditioned medium was evaluated for use as a trap matrix. The bacterial cellulose membranes released and attracted tumor cells *in vitro* [110]. Once attached to the surface of the membrane, tumor cells were unable to move on, go through, or escape the membrane, even when an attractive medium was present in close proximity [110]. However, further studies are needed to improve the chemotaxis properties of bacterial cellulose membranes to ensure the diffusion of chemoattractants over large distances and at high enough concentrations to trap GBM cells that infiltrate tissues several centimeters away from the resection cavity.

Extracellular matrix in GBM

Cellular and molecular aspects

ECM remodeling and stiffening is associated with a poorer prognosis [114]; however, analysis of ECM components and their spatial distribution in samples from patients with GBM is limited. Given that the brain is a soft tissue, its ECM differs from that of other solid organs. Elasticity of normal brain and brain tumors, measured using shear wave elastography, showed a stiffness increase from 7.3 kPa for normal brain to 11.4–33.1 kPa in GBM of different grades [115]. This observation was confirmed in preclinical models, with heterogeneous GBM tissues becoming softer as hypoxic/necrotic areas develop over time or, conversely, stiffer in zones of dense cell-containing abnormal vascular components [116]. At the molecular level, these differences in tissue stiffness result from modifications in the ECM composition in GBM. Accordingly, increased amounts of hyaluronic acid (HA) are detected in the ECM surrounding the tumor, as a result of locally increased HA synthase 1–3 activity, which promotes ECM remodeling and invasion of GBM cells [117]. Furthermore, tenascins, glycosaminoglycans, laminins, fibronectins, and collagens secreted by tumor cells or by stromal cells also contribute to ECM softening [118].

Mechanical stress has been previously shown to specifically control Rho GTPase [119]. Myosin II appears essential for migration properties by squeezing nuclei upon stiffness [120], suggesting an ECM-induced promotion of glioma invasiveness. *In vitro* biochemical investigations established that the morphology and the migratory behavior of GBM cells were inversely correlated with HA density [121], suggesting that a major requirement for glioma invasion is linked, at least in part, to the interaction between HA and its cognate receptor CD44. This finding also indicates that reduced HA leads to more flexibility [122] and, in turn, could favor the degradation of the surrounding ECM by MMPs, such as MMP2, MMP9, and MMP13, allowing tumor cells to invade surrounding brain tissue [123].

A pro-invasive network of matrix collagens, collagen-interacting proteins, and collagen-processing enzymes is present in mesenchymal tumors and transcriptionally regulated by IRF3. The expression levels of these collagens is significantly correlated with GBM progression and OS [124]. In addition to its fibrous composition, the ECM dynamically controls fluid concentrations and gradients of chemokines within the tumor bulk and in the periphery, via GBM-induced secretion of different enzymes, including hyaluronidases, MMPs, disintegrin, metalloproteinases with thrombospondin sequence (ADAMTs), cathepsins, urokinases (uPAs), and tissue plasminogen activators (TPAs). This leads to chemokine and growth factor accumulation, either by increased production or by the release of pre-adsorbed ligands, such as the CXC family of chemokines or TGF β [125] following ECM degradation, which control GBM invasion and progression. ECM components or ECM-entrapped secreted proteins also have the ability to modify the immune landscape of GBM. For example, M2 TAMs (known to exhibit protumoral functions) are recruited through Integrin $\alpha\beta 3$ in the tumor by GSC-secreted periostin to promote malignant growth [126]. Tenascin-C impairs T cell infiltration in tumor nodules [127]. Osteopontin overexpressed in GBM microenvironment by immune cells is required for M2 macrophage gene signature maintenance and recruitment to the tumor [128]. However, dynamic changes in brain ECM during

GBM development and progression are not yet extensively characterized or understood. Recently, comprehensive work demonstrated how the compression exerted by GBM on the surrounding brain tissue causes vascular perfusion defects and death of adjacent neurons, likely contributing to extra-tumoral ECM remodeling [129].

Therapeutic implications

Targeting ECM in GBM appears an attractive strategy based on the altered expression of components responsible for many aspects of tumor progression. Hyaluronan is the most studied component [130], with HA oligomers, hyaluronidases, or HA synthesis inhibitors yielding promising results in preclinical studies, despite not yet reaching clinical applications. Among the HA synthesis inhibitors, 4MU induced senescence and decreased CD44 and RHAMM in glioma cells independently of its effect on HA and, thus, is an alternative therapy [131] requiring more investigation. HA was also identified as a therapeutic target in oncolytic adenovirus immunotherapy. ICOVIR17, a conditionally replicating adenovirus expressing soluble hyaluronidase, mediates degradation of HA when directly injected in GBM in mice, resulting in virus spread and antitumor efficacy [132]. Moreover, ICOVIR17 dramatically modified the immune landscape in immunocompetent GBM mice by increasing tumor-infiltrating CD8⁺ T cells and glioma-associated macrophages, and upregulating PDL1 [133]. The combination of ICOVIR17 with anti-PD1 therapy led to increased survival in mice, suggesting strong preclinical evidence in support of a clinical trial.

Other ECM molecules have also been targeted. A brevicin deglycosylated form (dg-Bcan), which accumulates specifically in GBM, could be targeted by a dg-Bcan-targeting peptide (BTP-7) specifically homing to GBM [134], enabling drug delivery with therapeutic effects [135]. Similarly, a tumor-penetrating Tenascin-C binding peptide (PL3 peptide) was used to coat nanoparticles, which then accumulated at Tenascin-C- and NRP1 receptor-positive areas in clinical GBM samples. When loaded with pro-apoptotic factors, these PL3-guided nanoparticles provoked GBM caspase-3-dependent apoptosis and decreased vascularization associated with mouse survival [136]. This finding led to the creation of a bispecific Tenascin-C and fibronectin-targeted peptide (PL1 peptide), which also shows antiglioma activity [137]. Recent development of an antibody–cytokine fusion or immunocytokines, based on an L19 antibody specific for the tumor-associated extra epitope B of fibronectin in fusion with IL12 or TNF α , showed promising results in mouse models. The fusion promoted tumor-infiltrating lymphocytes and increased proinflammatory cytokines within the TME [138], which resulted in L19/TNF α -induced GBM necrosis. This compound is now being tested in a pilot trial in patients with GBM (NCT03779230). Targeting the ECM brings new hope to being able to provide therapeutic alternatives that not only remodel the GBM microenvironment (in particular to enhance anti-tumoral immunity), but also, when used in combination, guide therapeutics inside tumors or promote immune attack.

Concluding remarks

Although no significant improvements in the survival of patients with GBM have been seen in the general population since 2005, two important Phase 3 randomized clinical trials reported positive results in two subgroups of patients with newly diagnosed GBM. First, the EF-14 clinical (NCT00916409) showed that TTFIELDS plus SOC improved progression-free survival and OS in patients with newly diagnosed GBM in good clinical condition (i.e., Karnofsky performance score ≥ 70) after the concurrent radiochemotherapy part of the SOC [104]. Second, the CeTeG/NOA-09 (NCT01149109) clinical trial reported that lomustine added to SOC increased the OS of patients with MGMT-methylated newly diagnosed GBM [139] (a more complete list of current clinical trials is provided in Table S1 in the supplemental information online). Nevertheless, novel therapeutic targets, such as those derived from the study of the TME, must be identified to increase the therapeutic efficacy.

Outstanding questions

What are the molecular events and their dynamics that occur between tumor cells and the nervous, vascular, and immune compartments?

What contributes to tumor regional heterogeneity?

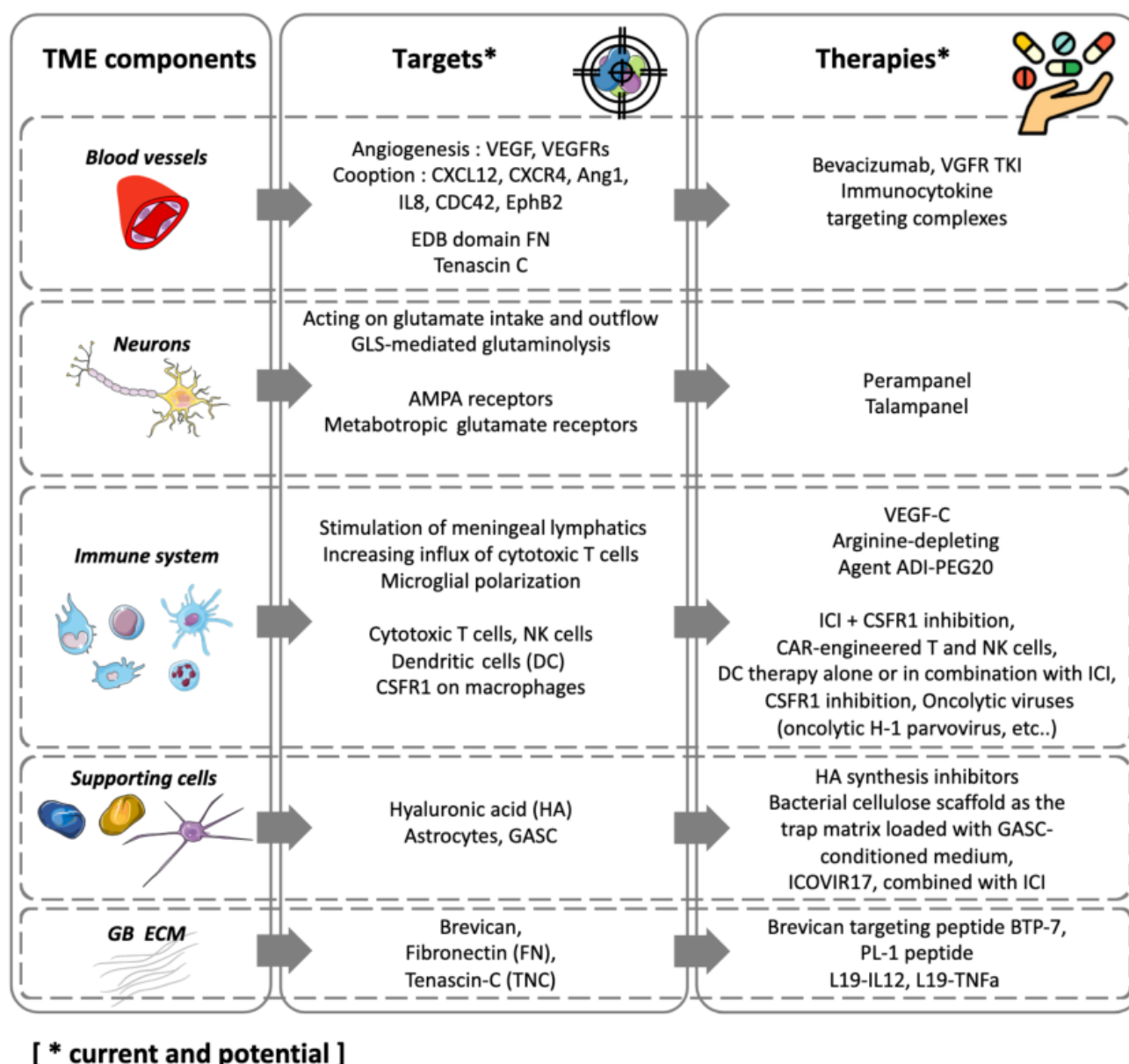
How can 3D GBM models be improved to account for the complexity of the TME?

What are the external constraints and cues regulating the TME and what is their impact on tumor development?

What is the impact of therapeutic intervention on a complex brain tumor environment?

Which therapeutic strategies that associate with TME/tumor cell-targeting combinations contribute to an improved outcome of the disease?

Many components of the GBM environment have been reported in the literature, such as vascular and immune components, neurons, and ECM. They interact closely with GSCs or differentiated GBM cells through a network of growth factors, cytokines, and chemokines. Figure 2 summarizes



Trends in Cancer

Figure 2. Cellular and molecular actors and potential targets and therapeutic interventions in glioblastoma (GBM). Abbreviations: ADI-PEG20, pegylated arginine deiminase; AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; Ang, angiotensin; CAR, chimeric antigen receptor; CDC42, cell division control protein 42 homolog; CSFR1, colony-stimulating factor receptor-1; CXC, CXC chemokine; CXCR, CXC chemokine receptor; DC, dendritic cell; ECM, extracellular matrix; EDB, extra domain-B; EphB2, ephrin B2; FN, fibronectin; GASC, glioma-associated stromal cell; GB-ECM, glioblastoma-extracellular matrix; GLS, glutaminase; HA, hyaluronic acid; ICI, immune checkpoint inhibitor; IL8, interleukin-8; L19-IL12, L19 antibody-bound interleukin-12; L19-TNFa, L19 antibody-bound TNF α ; NK, natural killer; PL1 peptide, 12-mer targeting peptide selectively interacts with both TNC-C and FN-EDB; TKI, tyrosine kinase inhibitor; TKI, tyrosine kinase inhibitor; VEGF(R), vascular endothelial growth factor (receptor); VEGF, vascular endothelial growth factor.

the cellular and molecular actors and potential targets and therapeutic interventions in GBM. This variety of cell-cell and molecular interactions reflects the heterogeneous state of GBMs, of which tumor cell plasticity is a main feature. New technologies [e.g., *in vivo* imaging, artificial intelligence, single cell (sc)RNA sequencing or single nucleus (sn)RNA sequencing, or spatial gene expression] combined with more sophisticated and controlled experimental models are likely to provide relevant novel information to improve our understanding of the GBM TME (Box 5). These might, in turn, contribute to the identification of actionable targets for the development of effective therapeutic strategies (e.g., TME-based tumor traps). Finally, current therapeutic approaches impact the components of the TME through different means, which modulate and modify the therapeutic response. For example, neurosurgical actions alone or in combination with the frequent use of steroid therapy can impact the TME and surrounding brain tissue [140]. Although tumor surgical resection has shown modest effects when used as a stand-alone approach, it can be coupled with local therapy in GBM. Approaches to local drug delivery have included the use of implantable, controlled-release polymer systems [141], delivery into the cerebrospinal fluid (CSF) or a cyst cavity (often using an implanted reservoir [142]), and catheter-based convection-enhanced delivery (CED) [143]. Other therapeutic agents have been investigated (reviewed in [144]). For instance, there are studies using intra-arterial cerebral infusion of anti-angiogenics in relapsed GBM (NCT01269853), opening of the BBB by using a sonocloud-9 device (NCT04614493), infusion of B7-H3 CAR-T in recurrent GBM (NCT04077866), or administration of autologous DCs in primary GBM (NCT04115761).

Although results are limited, these methodologies are promising for targeted local drug diffusion with innovative and effective delivery tools (scaffold or fluidic) for delivery to the tumor and surrounding central nervous system of novel therapeutic agents (CAR-T cells, ICIs, chemo- or targeted therapies). For instance, medical devices, including Ommaya reservoirs, convection-enhanced delivery reservoirs, and *in situ* biodegradable devices, are interesting strategies to increase local drug delivery. A Phase 3 clinical trial reported the efficacy of carmustine wafers in high-grade gliomas, although statistical significance was not reached for GBM [141]. Thus, understanding how to either enhance or minimize these effects for treatment improvement represents a major challenge (see Outstanding questions).

Box 5. Experimental and preclinical GBM models

Improved models of GBM are needed to faithfully recapitulate the landscape of GBM states and TMEs and to contribute to a thorough understanding of the developmental cues yielding GBM heterogeneity. Current models include standard *in vitro* models, rodent implant, or genetically engineered models (GEMMs), and chick or quail, microfluidic or assembloid engineered models.

Rodent implant models for GBM have provided valuable information about various molecular regulations, but are limited. Indeed, syngeneic systems do not recapitulate the genetics of human tumors and xenotransplanted cell lines or patient-derived xenograft (PDX) models lack a fully competent immune system. GEMMs do not recapitulate the genetics of human tumors or their invasive behavior. As an alternative with fewer ethical issues, avian-embryo models have been developed for short-term studies [177]. Microfluidic or bioprinted models are generated with optically clear material-based microchannels networks containing cells and/or matrix to recreate *in vitro* tissue or organ structure and function [178]. Different types of microfluidic device exist, such as the 3D organotypic microfluidic platform [179], the 3D bioprinted tumor vascular model [180] or a microchannel set-up mimicking the GBM microenvironment [181]. These microfluidic-derived models provide some improvement over existing models but have still limitations, such as lacking developmental dynamics, plasticity, and a relevant immune cell component. This complex tissue system maintains the cytoarchitecture of the brain with functional astrocytes and neurons for 2 weeks but lacks functional vessels, a problem also seen in brain/GBM organoids. Micro- and macrovessel engineering has been performed to provide artificial vascularized tissue for functional studies and tissue vascularization. The emergence of organ-on-a-chip technologies has attracted much attention [182]. These technologies aim to create bioactive artificial tissues from cultured cells, often using microfluidic techniques.

Acknowledgments

We apologize deeply to colleagues whose work could not be cited in this review because of space constraints. This work was funded by grants from: EU Transcan ERA Net Consortium grant 'GliomaPRD' (2018-2021); ARC research grant 'Fondation ARC' 2018-2019 Role of PRL phosphatases in angiogenesis; Labellisation ARC 'GliomaGuide' (2018-2020); 'Conseil Régional d'Aquitaine' (2018-2020), Ligue Contre le Cancer (2017-2021) to A.B.; INCa PLBIO17-220 to M.G.; INCa PLBIO17-20 to E.C.; Cancéropôle Grand-Ouest to E.C., F.V., J.G., and L.B.; INCa PLBIO20-23 to T.D., E.C., and G.S.; INCa PLBIO21-24, Ligue Contre le Cancer, Plan Cancer, Fondation ARC, ARTC, Cancéropôle Grand Sud Ouest to T.D.; la Ligue Contre le Cancer (Comité Charente Maritime) to B.C. and V.C.; INCa PLBIO17-145 to M.D.; and ARTC-sud to E.T. All authors belong to the French Preclinical Brain Tumor Study Group 'GrOupe Préclinique de l'Anocéf' (GOPA).

Declaration of interests

None declared by authors.

Supplemental information

Supplemental information to this article can be found online at <https://doi.org/10.1016/j.trecan.2022.09.005>.

References

- Ostrom, Q.T. *et al.* (2021) CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the united states in 2014-2018. *Neuro-Oncol.* 23, iii1-iii105.
- Fabbro-Peray, P. *et al.* (2019) Association of patterns of care, prognostic factors, and use of radiotherapy-temozolomide therapy with survival in patients with newly diagnosed glioblastoma: a French national population-based study. *J. Neuro-Oncol.* 142, 91-101.
- Louis, D.N. *et al.* (2021) The 2021 WHO Classification of Tumors of the Central Nervous System: a summary. *Neuro-Oncol.* 23, 1231-1251.
- Stupp, R. *et al.* (2005) Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N. Engl. J. Med.* 352, 987-996.
- DeWeerd, S. (2018) The genomics of brain cancer. *Nature* 561, S54-S55.
- Gonzalez Castro, L.N. *et al.* (2021) Decoding cancer biology one cell at a time. *Cancer Discov.* 11, 960-970.
- Aldape, K. *et al.* (2019) Challenges to curing primary brain tumours. *Nat. Rev. Clin. Oncol.* 16, 509-520.
- Kowal, J. *et al.* (2019) Re-education of macrophages as a therapeutic strategy in cancer. *Immunotherapy* 11, 677-689.
- Perus, L.J.M. and Walsh, L.A. (2019) Microenvironmental heterogeneity in brain malignancies. *Front. Immunol.* 10, 2294.
- Bikfalvi, A. (2018) *A Brief History of Blood and Lymphatic Vessels*, Springer.
- Venkatarani, V. *et al.* (2019) Glutamatergic synaptic input to glioma cells drives brain tumour progression. *Nature* 573, 532-538.
- Klemm, F. *et al.* (2020) Interrogation of the microenvironmental landscape in brain tumors reveals disease-specific alterations of immune cells. *Cell* 181, 1643-1660.
- Almairac, F. *et al.* (2020) ERK-mediated loss of miR-199a-3p and induction of EGR1 act as a "toggle switch" of GBM cell dedifferentiation into NANOG- and OCT4-positive cells. *Cancer Res.* 80, 3236-3250.
- El-Habr, E.A. *et al.* (2017) A driver role for GABA metabolism in controlling stem and proliferative cell state through GHB production in glioma. *Acta Neuropathol. (Berl)* 133, 645-660.
- Jung, J. *et al.* (2017) Nicotinamide metabolism regulates glioblastoma stem cell maintenance. *JCI Insight* 2, 90019.
- Natsume, A. *et al.* (2013) Chromatin regulator PRC2 is a key regulator of epigenetic plasticity in glioblastoma. *Cancer Res.* 73, 4559-4570.
- Tchoghandjian, A. *et al.* (2012) Cortical and subventricular zone glioblastoma-derived stem-like cells display different molecular profiles and differential in vitro and in vivo properties. *Ann. Surg. Oncol.* 19, S608-S619.
- Khalifa, J. *et al.* (2017) Subventricular zones: new key targets for glioblastoma treatment. *Radiat. Oncol. Lond. Engl.* 12, 67.
- Seano, G. (2018) Targeting the perivascular niche in brain tumors. *Curr. Opin. Oncol.* 30, 54-60.
- Guelli, S. *et al.* (2016) Vascular transdifferentiation in the CNS: a focus on neural and glioblastoma stem-like cells. *Stem Cells Int.* 2016, 2759403.
- Deshors, P. *et al.* (2019) Ionizing radiation induces endothelial transdifferentiation of glioblastoma stem-like cells through the Tie2 signaling pathway. *Cell Death Dis.* 10, 816.
- Wu, A. *et al.* (2010) Glioma cancer stem cells induce immunosuppressive macrophages/microglia. *Neuro-Oncol.* 12, 1113-1125.
- Laprie, A. *et al.* (2008) Proton magnetic resonance spectroscopic imaging in newly diagnosed glioblastoma: predictive value for the site of postradiotherapy relapse in a prospective longitudinal study. *Int. J. Radiat. Oncol. Biol. Phys.* 70, 773-781.
- Pinto, G. *et al.* (2021) Patient-derived glioblastoma stem cells transfer mitochondria through tunneling nanotubes in tumor organoids. *Biochem. J.* 478, 21-39.
- Assad Kehn, S. *et al.* (2016) The anti-hypertensive drug prazosin inhibits glioblastoma growth via the PKC δ -dependent inhibition of the AKT pathway. *EMBO Mol. Med.* 8, 511-526.
- Piccirilli, S.G.M. *et al.* (2006) Bone morphogenetic proteins inhibit the tumorigenic potential of human brain tumour-initiating cells. *Nature* 444, 761-765.
- Fareh, M. *et al.* (2012) The miR-302-367 cluster drastically affects self-renewal and infiltration properties of glioma-initiating cells through CXCR4 repression and consequent disruption of the SHH-GLI-NANOG network. *Cell Death Differ.* 19, 232-244.
- Red-Horse, K. *et al.* (2007) Endothelium-microenvironment interactions in the developing embryo and in the adult. *Dev. Cell* 12, 181-194.
- Le Guellec, A. *et al.* (2011) Jumping the barrier: VE-cadherin, VEGF and other angiogenic modifiers in cancer. *Biol. Cell* 103, 593-605.
- Huang, M. *et al.* (2016) c-Met-mediated endothelial plasticity drives aberrant vascularization and chemoresistance in glioblastoma. *J. Clin. Invest.* 126, 1801-1814.
- Das, S. and Marsden, P.A. (2013) Angiogenesis in glioblastoma. *N. Engl. J. Med.* 369, 1561-1563.
- Johnson, K.C. *et al.* (2021) Single-cell multimodal glioma analyses identify epigenetic regulators of cellular plasticity and environmental stress response. *Nat. Genet.* 53, 1456-1468.
- Griveau, A. *et al.* (2018) A glial signature and Wnt7 signaling regulate glioma-vascular interactions and tumor microenvironment. *Cancer Cell* 33, 874-889.
- El-Hallani, S. *et al.* (2010) A new alternative mechanism in glioblastoma vascularization: tubular vasculogenic mimicry. *Brain J. Neurol.* 133, 973-982.
- Cheng, L. *et al.* (2013) Glioblastoma stem cells generate vascular pericytes to support vessel function and tumor growth. *Cell* 153, 139-152.
- Calabrese, C. *et al.* (2007) A perivascular niche for brain tumor stem cells. *Cancer Cell* 11, 69-82.

37. Xie, Y. *et al.* (2021) Key molecular alterations in endothelial cells in human glioblastoma uncovered through single-cell RNA sequencing. *JCI Insight* 6, e150861
38. Carlson, J.C. *et al.* (2021) Identification of diverse tumor endothelial cell populations in malignant glioma. *Neuro-Oncol.* 23, 932–944
39. Clavreul, A. *et al.* (2019) Nanocarriers and nonviral methods for delivering antiangiogenic factors for glioblastoma therapy: the story so far. *Int. J. Nanomedicine* 14, 2497–2513
40. Martin, J.D. *et al.* (2019) Normalizing function of tumor vessels: progress, opportunities, and challenges. *Annu. Rev. Physiol.* 81, 505–534
41. Treps, L. *et al.* (2017) Glioblastoma stem-like cells secrete the pro-angiogenic VEGF-A factor in extracellular vesicles. *J. Extracell. Vesicles* 6, 1359479
42. Skog, J. *et al.* (2008) Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat. Cell Biol.* 10, 1470–1476
43. Ma, S. *et al.* (2021) CD63-mediated cloaking of VEGF in small extracellular vesicles contributes to anti-VEGF therapy resistance. *Cell Rep.* 36, 109549
44. Simon, T. *et al.* (2018) Shedding of bevacizumab in tumour cells-derived extracellular vesicles as a new therapeutic escape mechanism in glioblastoma. *Mol. Cancer* 17, 132
45. Lombardi, G. *et al.* (2019) Regorafenib compared with lomustine in patients with relapsed glioblastoma (REGOMA): a multicentre, open-label, randomised, controlled, phase 2 trial. *Lancet Oncol.* 20, 110–119
46. Belotti, D. *et al.* (2021) Alternative vascularization mechanisms in tumor resistance to therapy. *Cancers* 13, 1912
47. Lee, E.C. *et al.* (2020) NRG/RTOG 1122: a phase 2, double-blinded, placebo-controlled study of bevacizumab with and without trebananib in patients with recurrent glioblastoma or gliosarcoma. *Cancer* 126, 2821–2828
48. Weller, M. *et al.* (2017) Rindopepimut with temozolomide for patients with newly diagnosed, EGFRvIII-expressing glioblastoma (ACT IV): a randomised, double-blind, international phase 3 trial. *Lancet Oncol.* 18, 1373–1385
49. Rosenthal, M. *et al.* (2019) Safety, tolerability, and pharmacokinetics of anti-EGFRvIII antibody-drug conjugate AMG 595 in patients with recurrent malignant glioma expressing EGFRvIII. *Cancer Chemother. Pharmacol.* 84, 327–336
50. Cloughesy, T. *et al.* (2017) Randomized, double-blind, placebo-controlled, multicenter Phase II Study of Onartuzumab Plus Bevacizumab Versus placebo plus bevacizumab in patients with recurrent glioblastoma: efficacy, safety, and hepatocyte growth factor and O6-methylguanine-DNA methyltransferase biomarker analyses. *J. Clin. Oncol.* 35, 343–351
51. Cloughesy, T.F. *et al.* (2018) Phase II study of cabozantinib in patients with progressive glioblastoma: subset analysis of patients with prior antiangiogenic therapy. *Neuro-Oncol.* 20, 259–267
52. Wen, P.Y. *et al.* (2018) Phase II study of cabozantinib in patients with progressive glioblastoma: subset analysis of patients naive to antiangiogenic therapy. *Neuro-Oncol.* 20, 249–258
53. Castro Dias, M. *et al.* (2019) Structure and junctional complexes of endothelial, epithelial and glial brain barriers. *Int. J. Mol. Sci.* 20, E5372
54. Engelhardt, B. *et al.* (2017) The movers and shapers in immune privilege of the CNS. *Nat. Immunol.* 18, 123–131
55. Dréan, A. *et al.* (2016) Blood-brain barrier, cytotoxic chemotherapies and glioblastoma. *Expert. Rev. Neurother.* 16, 1285–12300
56. Sarkaria, J.N. *et al.* (2018) Is the blood-brain barrier really disrupted in all glioblastomas? A critical assessment of existing clinical data. *Neuro-Oncol.* 20, 184–191
57. Hersh, D.S. *et al.* (2016) Evolving drug delivery strategies to overcome the blood brain barrier. *Curr. Pharm. Des.* 22, 1177–1193
58. Chua, S.L. *et al.* (2004) Phase 2 study of temozolomide and Caelyx in patients with recurrent glioblastoma multiforme. *Neuro-Oncol.* 6, 38–43
59. Tantillo, E. *et al.* (2020) Differential roles of pyramidal and fast-spiking, GABAergic neurons in the control of glioma cell proliferation. *Neurobiol. Dis.* 141, 104942
60. Venkatesh, H.S. *et al.* (2015) Neuronal activity promotes glioma growth through neuropilin-3 secretion. *Cell* 161, 803–816
61. Doima, S. *et al.* (2016) Inhibition of dopamine receptor D4 impedes autophagic flux, proliferation, and survival of glioblastoma stem cells. *Cancer Cell* 29, 859–873
62. Marcus, H.J. *et al.* (2010) In vivo assessment of high-grade glioma biochemistry using microdialysis: a study of energy-related molecules, growth factors and cytokines. *J. Neuro-Oncol.* 97, 11–23
63. Robert, S.M. *et al.* (2015) SLC7A11 expression is associated with seizures and predicts poor survival in patients with malignant glioma. *Sci. Transl. Med.* 7, 289ra86
64. Venkatesh, H.S. *et al.* (2019) Electrical and synaptic integration of glioma into neural circuits. *Nature* 573, 539–545
65. Young, S.Z. and Bordey, A. (2009) GABA's control of stem and cancer cell proliferation in adult neural and peripheral niches. *Physiology* 24, 171–185
66. Smits, A. *et al.* (2012) GABA-A channel subunit expression in human glioma correlates with tumor histology and clinical outcome. *PLoS ONE* 7, e37041
67. Habela, C.W. *et al.* (2009) Chloride accumulation drives volume dynamics underlying cell proliferation and migration. *J. Neurophysiol.* 101, 750–757
68. Grossman, S.A. *et al.* (2010) Survival of patients with newly diagnosed glioblastoma treated with radiation and temozolomide in research studies in the United States. *Clin. Cancer Res.* 16, 2443–2449
69. Wirsching, H.G. *et al.* (2021) Negative allosteric modulators of metabotropic glutamate receptor 3 target the stem-like phenotype of glioblastoma. *Mol. Ther. Oncolytics* 20, 166–174
70. Huang, Q. *et al.* (2021) SNAP25 inhibits glioma progression by regulating synapse plasticity via GLS-mediated glutaminolysis. *Front. Oncol.* 11, 698835
71. de Groot, J.F. *et al.* (2005) The excitatory amino acid transporter-2 induces apoptosis and decreases glioma growth in vitro and in vivo. *Cancer Res.* 65, 1934–1940
72. Dunn, G.P. *et al.* (2012) Cancer immunosediting in malignant glioma. *Neurosurgery* 71, 201–222 discussion 222–223
73. Yang, I. *et al.* (2010) CD8+ T-cell infiltrate in newly diagnosed glioblastoma is associated with long-term survival. *J. Clin. Neurosci.* 17, 1381–1385
74. Avril, T. *et al.* (2011) Mechanisms of immunomodulation in human glioblastoma. *Immunotherapy* 3, 42–44
75. Quail, D.F. and Joyce, J.A. (2017) The microenvironmental landscape of brain tumors. *Cancer Cell* 31, 326–341
76. Chongsathidkiet, P. *et al.* (2018) Sequestration of T cells in bone marrow in the setting of glioblastoma and other intracranial tumors. *Nat. Med.* 24, 1459–1468
77. Mathewson, N.D. *et al.* (2021) Inhibitory CD161 receptor identified in glioma-infiltrating T cells by single-cell analysis. *Cell* 184, 1281–1298
78. Castriconi, R. *et al.* (2009) NK cells recognize and kill human glioblastoma cells with stem cell-like properties. *J. Immunol. Baltim. Md. 1950* 182, 3530–3539
79. Gras Navarro, A. *et al.* (2014) NK cells with KIR2DS2 immunogenotype have a functional activation advantage to efficiently kill glioblastoma and prolong animal survival. *J. Immunol.* 193, 6192–6206
80. Mostafa, H. *et al.* (2016) Immune phenotypes predict survival in patients with glioblastoma multiforme. *J. Hematol. Oncol.* 9, 77
81. Friese, M.A. *et al.* (2003) MICA/NG2D-mediated immunogenic therapy of experimental gliomas. *Cancer Res.* 63, 8996–9006
82. Kepp, O. *et al.* (2017) Extracellular nucleosides and nucleotides as immunomodulators. *Immunol. Rev.* 280, 83–92
83. Zhang, C. *et al.* (2017) Tumor purity as an underlying key factor in glioma. *Clin. Cancer Res.* 23, 6279–6291
84. Goffart, N. *et al.* (2017) CXCL12 mediates glioblastoma resistance to radiotherapy in the subventricular zone. *Neuro-Oncol.* 19, 66–77
85. Thomas, R.P. *et al.* (2019) Macrophage exclusion after radiation therapy (MERT): a first in human Phase I/II trial using a CXCR4 inhibitor in glioblastoma. *Clin. Cancer Res.* 25, 6948–6957
86. Gameiro, S.R. *et al.* (2014) Radiation-induced immunogenic modulation of tumor enhances antigen processing and calreticulin exposure, resulting in enhanced T-cell killing. *Oncotarget* 5, 403–416
87. Deng, L. *et al.* (2014) Irradiation and anti-PD-L1 treatment synergistically promote antitumor immunity in mice. *J. Clin. Invest.* 124, 687–695

88. Vanpouille-Box, C. *et al.* (2017) DNA exonuclease Trex1 regulates radiotherapy-induced tumour immunogenicity. *Nat. Commun.* 8, 15618
89. Khan, S. *et al.* (2020) Role of neutrophils and myeloid-derived suppressor cells in glioma progression and treatment resistance. *Int. J. Mol. Sci.* 21, 1954
90. Lakshmanachetty, S. and Mitra, S.S. (2022) Mapping the tumor-infiltrating immune cells during glioblastoma progression. *Nat. Immunol.* 23, 826–828
91. Song, E. *et al.* (2020) VEGF-C-driven lymphatic drainage enables immunosurveillance of brain tumours. *Nature* 577, 689–694
92. Reardon, D.A. *et al.* (2020) Effect of nivolumab vs bevacizumab in patients with recurrent glioblastoma: the CheckMate 143 Phase 3 randomized clinical trial. *JAMA Oncol.* 6, 1003
93. Antonios, J.P. *et al.* (2017) Immunosuppressive tumor-infiltrating myeloid cells mediate adaptive immune resistance via a PD-1/PD-L1 mechanism in glioblastoma. *Neuro-Oncol.* 19, 796–807
94. Brown, C.E. *et al.* (2016) Regression of glioblastoma after chimeric antigen receptor T-cell therapy. *N. Engl. J. Med.* 375, 2561–2569
95. O'Rourke, D.M. *et al.* (2017) A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. *Sci. Transl. Med.* 9, eaad9864
96. Hill, N. *et al.* (2019) Actively personalized vaccination trial for newly diagnosed glioblastoma. *Nature* 565, 240–245
97. Liao, L.M. *et al.* (2018) First results on survival from a large Phase 3 clinical trial of an autologous dendritic cell vaccine in newly diagnosed glioblastoma. *J. Transl. Med.* 16, 142
98. Geletneky, K. *et al.* (2017) Oncolytic H-1 parvovirus shows safety and signs of immunogenic activity in a first Phase I/IIa glioblastoma trial. *Mol. Ther.* 25, 2620–2634
99. Samson, A. *et al.* (2018) Intravenous delivery of oncolytic reovirus to brain tumor patients immunologically primes for subsequent checkpoint blockade. *Sci. Transl. Med.* 10, eaam7577
100. Fujita, M. *et al.* (2010) Role of type 1 IFNs in anti-glioma immunosurveillance—using mouse studies to guide examination of novel prognostic markers in humans. *Clin. Cancer Res.* 16, 3409–3419
101. Hajji, N. *et al.* (2022) Arginine deprivation alters microglial polarity and synergizes with radiation to eradicate non-arginine-auxotrophic glioblastoma tumors. *J. Clin. Invest.* 132, e142137
102. Butowski, N. *et al.* (2016) 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.* 18, 557–564
103. Sahetjani, S. *et al.* (2021) Hypofractionated stereotactic re-irradiation with pembrolizumab and bevacizumab in patients with recurrent high-grade gliomas: results from a phase I study. *Neuro-Oncol.* 23, 677–686
104. Stupp, R. *et al.* (2017) Effect of tumor-treating fields plus maintenance temozolomide vs maintenance temozolomide alone on survival in patients with glioblastoma: a randomized clinical trial. *JAMA* 318, 2306–2316
105. Karanam, N.K. and Story, M.D. (2021) An overview of potential novel mechanisms of action underlying Tumor Treating Fields-induced cancer cell death and their clinical implications. *Int. J. Radiat. Biol.* 97, 1044–1054
106. Clavreul, A. and Menai, P. (2020) Mesenchymal stromal-like cells in the glioma microenvironment: what are these cells? *Cancers* 12, E2628
107. Taghipour, M. *et al.* (2017) Comparative proteomic analysis of tumor mesenchymal-like stem cells derived from high grade versus low grade gliomas. *Cell J.* 19, 250–258
108. Clavreul, A. *et al.* (2015) Identification of two glioblastoma-associated stromal cell subtypes with different carcinogenic properties in histologically normal surgical margins. *J. Neuro-Oncol.* 122, 1–10
109. Shahar, T. *et al.* (2017) Percentage of mesenchymal stem cells in high-grade glioma tumor samples correlates with patient survival. *Neuro-Oncol.* 19, 660–668
110. Autier, L. *et al.* (2019) A new glioblastoma cell trap for implantation after surgical resection. *Acta Biomater.* 84, 268–279
111. van der Sanden, B. *et al.* (2013) Translation of the ecological trap concept to glioma therapy: the cancer cell trap concept. *Future Oncol.* 9, 817–824
112. Najberg, M. *et al.* (2019) Reversing the tumor target: establishment of a tumor trap. *Front. Pharmacol.* 10, 887
113. Kasapidou, P.M. *et al.* (2021) Hyaluronic acid-based hydrogels loaded with chemoattractant and anticancer drug - new formulation for attracting and tackling glioma cells. *Soft Matter* 17, 10846–10861
114. Miroshnikova, Y.A. *et al.* (2016) Tissue mechanics promote IDH1-dependent HIF1 α -tenascin C feedback to regulate glioblastoma aggression. *Nat. Cell Biol.* 18, 1336–1345
115. Chauvet, D. *et al.* (2016) In vivo measurement of brain tumor elasticity using intraoperative shear wave elastography. *Ultraschall. Med. Stuttg. Ger.* 1980 37, 584–590
116. Schragel, K. *et al.* (2018) Characterization of glioblastoma in an orthotopic mouse model with magnetic resonance elastography. *NMR Biomed.* 31, e3840
117. Perrin, S.L. *et al.* (2019) Glioblastoma heterogeneity and the tumour microenvironment: implications for preclinical research and development of new treatments. *Biochem. Soc. Trans.* 47, 625–638
118. Mehesparan, R. *et al.* (2003) Expression of extracellular matrix components in a highly infiltrative in vivo glioma model. *Acta Neuropathol. (Berl)* 105, 49–57
119. Torroni, A.S. *et al.* (2005) RhoA/ROCK signaling is critical to FAK activation by cyclic stretch in cardiac myocytes. *Am. J. Physiol. Heart Circ. Physiol.* 289, H1488–H1496
120. Ivkovic, S. *et al.* (2012) Direct inhibition of myosin II effectively blocks glioma invasion in the presence of multiple motogens. *Mol. Biol. Cell* 23, 533–542
121. Rao, S.S. *et al.* (2013) Glioblastoma behaviors in three-dimensional collagen-hyaluronan composite hydrogels. *ACS Appl. Mater. Interfaces* 5, 9276–9284
122. Chen, J.W.E. *et al.* (2017) The combined influence of hydrogel stiffness and matrix-bound hyaluronic acid content on glioblastoma invasion. *Macromol. Biosci.* 17
123. Manini, I. *et al.* (2018) Role of microenvironment in glioma invasion: what we learned from in vitro models. *Int. J. Mol. Sci.* 19, E147
124. Pencheva, N. *et al.* (2017) Identification of a druggable pathway controlling glioblastoma invasiveness. *Cell Rep.* 20, 48–60
125. Ferrer, V.P. *et al.* (2018) Glioma infiltration and extracellular matrix: key players and modulators. *Glia* 66, 1542–1565
126. Zhou, W. *et al.* (2015) Periostin secreted by glioblastoma stem cells recruits M2 tumour-associated macrophages and promotes malignant growth. *Nat. Cell Biol.* 17, 170–182
127. Huang, J.Y. *et al.* (2010) Extracellular matrix of glioblastoma inhibits polarization and transmigration of T cells: the role of Tenascin-C in immune suppression. *J. Immunol.* 185, 1450–1459
128. Wei, J. *et al.* (2018) Osteopontin mediates glioblastoma-associated macrophage infiltration and is a potential therapeutic target. *J. Clin. Invest.* 129, 137–149
129. Seano, G. *et al.* (2019) Solid stress in brain tumours causes neuronal loss and neurological dysfunction and can be reversed by lithium. *Nat. Biomed. Eng.* 3, 230–245
130. Pibuel, M.A. *et al.* (2021) The scrambled story between hyaluronan and glioblastoma. *J. Biol. Chem.* 296, 100549
131. Pibuel, M.A. *et al.* (2021) Antitumor effect of 4MU on glioblastoma cells is mediated by senescence induction and CD44, RHAMM and p-ERK modulation. *Cell Death Discov.* 7, 280
132. Martinez-Quintanilla, J. *et al.* (2015) Encapsulated stem cells loaded with hyaluronidase-expressing oncolytic virus for brain tumor therapy. *Mol. Ther. J. Am. Soc. Gene Ther.* 23, 108–118
133. Kyokawa, J. *et al.* (2021) Modification of extracellular matrix enhances oncolytic adenovirus immunotherapy in glioblastoma. *Clin. Cancer Res.* 27, 889–902
134. von Spreckelsen, N. *et al.* (2021) Targeting glioblastoma using a novel peptide specific to a deglycosylated isoform of brevican. *Adv. Ther.* 4, 2000244

135. Cho, C.F. *et al.* (2022) A tumor-homing peptide platform enhances drug solubility, improves blood-brain barrier permeability and targets glioblastoma. *Cancers* 14, 2207
136. Lingasamy, P. *et al.* (2020) Tumor-penetrating peptide for systemic targeting of Tenascin-C. *Sci. Rep.* 10, 5809
137. Lingasamy, P. *et al.* (2019) Bi-specific tenascin-C and fibronectin targeted peptide for solid tumor delivery. *Biomaterials* 219, 118373
138. Weiss, T. *et al.* (2020) Immunocytokines are a promising immunotherapeutic approach against glioblastoma. *Sci. Transl. Med.* 12, eabb2311
139. Herflinger, U. *et al.* (2019) Lomustine-temozolomide combination therapy versus standard temozolomide therapy in patients with newly diagnosed glioblastoma with methylated MGMT promoter (CeTeG/NOA-09): a randomised, open-label, phase 3 trial. *Lancet Lond. Engl.* 393, 678–688
140. Otvos, B. *et al.* (2021) Preclinical modeling of surgery and steroid therapy for glioblastoma reveals changes in immunophenotype that are associated with tumor growth and outcome. *Clin. Cancer Res.* 27, 2038–2049
141. Westphal, M. *et al.* (2003) A phase 3 trial of local chemotherapy with biodegradable carmustine (BCNU) wafers (GliaGel wafers) in patients with primary malignant glioma. *Neuro-Oncol.* 5, 79–88
142. Bolandi, A. *et al.* (1996) Interstitial chemotherapy with mitoxantrone in recurrent malignant glioma: preliminary data. *J. Neuro-Oncol.* 27, 157–162
143. Laske, D.W. *et al.* (1997) Tumor regression with regional distribution of the targeted toxin TF-CRM107 in patients with malignant brain tumors. *Nat. Med.* 3, 1362–1368
144. D'Amico, R.S. *et al.* (2021) Convection-enhanced drug delivery for glioblastoma: a review. *J. Neuro-Oncol.* 151, 415–427
145. Bi, J. *et al.* (2018) Targeting cancer's metabolic co-dependencies: a landscape shaped by genotype and tissue context. *Biochim. Biophys. Acta BBA – Rev. Cancer* 1870, 76–87
146. Lin, J. *et al.* (2019) The roles of glucose metabolic reprogramming in chemo- and radio-resistance. *J. Exp. Clin. Cancer Res.* 38, 218
147. Oizel, K. *et al.* (2017) Efficient mitochondrial glutamine targeting prevails over glioblastoma metabolic plasticity. *Clin. Cancer Res.* 23, 6292–6304
148. Gouirand, V. *et al.* (2018) Influence of the tumor microenvironment on cancer cells metabolic reprogramming. *Front. Oncol.* 8, 117
149. Vander Heiden, M.G. *et al.* (2009) Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* 324, 1029–1033
150. Lan, X. *et al.* (2017) Fate mapping of human glioblastoma reveals an invariant stem cell hierarchy. *Nature* 549, 227–232
151. Morfouace, M. *et al.* (2014) Control of glioma cell death and differentiation by PKM2-Oct4 interaction. *Cell Death Dis.* 5, e1036
152. Hoang-Minh, L.B. *et al.* (2018) Infiltrative and drug-resistant slow-cycling cells support metabolic heterogeneity in glioblastoma. *EMBO J.* 37, e98772
153. Formolo, C.A. *et al.* (2011) Secretome signature of invasive glioblastoma multiforme. *J. Proteome Res.* 10, 3149–3159
154. Almiron Bonnin, D.A. *et al.* (2018) Glioma cell secretion: a driver of tumor progression and a potential therapeutic target. *Cancer Res.* 78, 6031–6039
155. Vats, S. and Galli, T. (2021) Introducing secretory reticulophagy/ER-phagy (SERP), a VAMP7-dependent pathway involved in neurite growth. *Autophagy* 17, 1037–1039
156. Wojnacki, J. *et al.* (2020) Role of VAMP7-dependent secretion of reticulum 3 in neurite growth. *Cell Rep.* 33, 108536
157. Savaraj, J.P. *et al.* (2019) Early glioma is associated with abnormal electrical events in cortical cultures. *Med. Biol. Eng. Comput.* 57, 1645–1656
158. Obacz, J. *et al.* (2017) Endoplasmic reticulum proteostasis in glioblastoma-From molecular mechanisms to therapeutic perspectives. *Sci. Signal.* 10, eaai2323
159. Dadey, D.Y.A. *et al.* (2016) The ATF6 pathway of the ER stress response contributes to enhanced viability in glioblastoma. *Oncotarget* 7, 2080–2092
160. Peñaranda-Fajardo, N.M. *et al.* (2019) ER stress and UPR activation in glioblastoma: identification of a noncanonical PERK mechanism regulating GBM stem cells through SOX2 modulation. *Cell Death Dis.* 10, 690
161. Lhomond, S. *et al.* (2018) Dual IRE1 RNase functions dictate glioblastoma development. *EMBO Mol. Med.* 10, e7929
162. Dejeans, N. *et al.* (2012) Autocrine control of glioma cells adhesion and migration through IRE1 α -mediated cleavage of SPARC mRNA. *J. Cell Sci.* 125, 4278–4287
163. Drogat, B. *et al.* (2007) IRE1 signaling is essential for ischemia-induced vascular endothelial growth factor-A expression and contributes to angiogenesis and tumor growth in vivo. *Cancer Res.* 67, 6700–6707
164. Langlais, T. *et al.* (2021) Structural and molecular bases to IRE1 activity modulation. *Biochem. J.* 478, 2953–2975
165. Le Reste, P.J. *et al.* (2020) Local intracerebral inhibition of IRE1 by MKC866 sensitizes glioblastoma to irradiation/chemotherapy in vivo. *Cancer Lett.* 494, 73–83
166. Goiran, T. *et al.* (2018) Nuclear p53-mediated repression of autophagy involves PINK1 transcriptional down-regulation. *Cell Death Differ.* 25, 873–884
167. Viotti, J. *et al.* (2014) Glioma tumor grade correlates with parkin depletion in mutant p53-linked tumors and results from loss of function of p53 transcriptional activity. *Oncogene* 33, 1764–1775
168. Rouland, L. *et al.* (2021) Therapeutic potential of parkin as a tumor suppressor via transcriptional control of cyclins in glioblastoma cell and animal models. *Theranostics* 11, 10047–10063
169. Zhang, Y. *et al.* (2012) Induction of autophagy is essential for monocyte-macrophage differentiation. *Blood* 119, 2895–2905
170. Bhattacharya, A. *et al.* (2015) Autophagy is required for neutrophil-mediated inflammation. *Cell Rep.* 12, 1731–1739
171. Kon, M. *et al.* (2011) Chaperone-mediated autophagy is required for tumor growth. *Sci. Transl. Med.* 3, 109ra117
172. Hubbi, M.E. and Hu, H. (2013) Kshitz null, Ahmed I, Levchenko A, Semenza GL. Chaperone-mediated autophagy targets hypoxia-inducible factor-1 α (HIF-1 α) for lysosomal degradation. *J. Biol. Chem.* 288, 10703–10714
173. Hubbi, M.E. *et al.* (2014) Cyclin-dependent kinases regulate lysosomal degradation of hypoxia-inducible factor 1 α to promote cell-cycle progression. *Proc. Natl. Acad. Sci. U. S. A.* 111, E3325–E3334
174. Natsumeda, M. *et al.* (2011) Induction of autophagy in temozolomide treated malignant gliomas. *Neuropathology* 31, 486–493
175. Liu, L. *et al.* (2018) Ac2-26 induces IKK β degradation through chaperone-mediated autophagy via HSPB1 in NCM-treated microglia. *Front. Mol. Neurosci.* 11, 76
176. Vaidor, R. *et al.* (2019) Glioblastoma ablates pericytes antitumor immune function through aberrant up-regulation of chaperone-mediated autophagy. *Proc. Natl. Acad. Sci. U. S. A.* 116, 20655–20665
177. Clarke, K. *et al.* (2015) Inference of low and high-grade glioma gene regulatory networks delineates the role of md3 in establishing multiple hallmarks of cancer. *PLoS Genet.* 11, e1005325
178. Sontheimer-Pheips, A. *et al.* (2019) Modelling cancer in microfluidic human organs-on-chips. *Nat. Rev. Cancer* 19, 65–81
179. Truong, D. *et al.* (2019) A three-dimensional (3D) organotypic microfluidic model for glioma stem cells – vascular interactions. *Biomaterials* 198, 63–77
180. Ozturk, M.S. *et al.* (2020) High-resolution tomographic analysis of in vitro 3D glioblastoma tumor model under long-term drug treatment. *Sci. Adv.* 6, 7513–7519
181. Pahl, L.S. *et al.* (2020) Predicting confined 1D cell migration from parameters calibrated to a 2D motor-clutch model. *Biophys. J.* 118, 1709–1720
182. Pollat, A.M.A.O. and den Toonder, J.M.J. (2020) Recapitulating the vasculature using organ-on-chip technology. *Bioengineering* 18, 17