frontiers | Frontiers in Physics

TYPE Review
PUBLISHED 18 December 2025
DOI 10.3389/fphy.2025.1723329

OPEN ACCESS

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RECEIVED 12 October 2025
REVISED 11 November 2025
ACCEPTED 18 November 2025
PUBLISHED 18 December 2025

CITATION
Abikenari M, Sjoholm MA, Liu J, Nageeb G, Ha JH, Wu J, Ren A, Sayadi J, Lim J, Cho KB, Verma R, Medikonda R, Banu M and Lim M (2025) Molecular and biophysical remodeling of the blood–brain barrier in glioblastoma: mechanistic drivers of tumor–neurovascular crosstalk.
Front. Phys. 13:1723329.
doi: 10.3389/fphy.2025.1723329

Molecular and biophysical remodeling of the blood–brain barrier in glioblastoma: mechanistic drivers of tumor–neurovascular crosstalk

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Glioblastoma (GBM) resists conventional treatment in large part because the blood–brain barrier (BBB) and its tumor-modified counterpart, the blood–tumor barrier (BTB), form a spatially heterogeneous, actively regulated interface that governs transport. In this setting, permeability, perfusion, and efflux are decoupled so radiographic contrast enhancement is an imperfect surrogate for true therapeutic exposure. Based on breakthroughs in vascular biology, imaging, and transport modeling, single-cell and spatial profiling, and translational delivery studies, we demonstrate how vascular co-option, hypoxia-induced remodeling, and barrier dysregulation generate gradients from relatively intact margins to leaky but sparsely perfused cores. In addition to their function in regulating molecular traffic, perivascular cells and astrocyte programs affect local immune niches that enable myeloid suppression and exclusion of T-cells and suppress systemic immunotherapies. New tools, from novel MRI/PET methods to intravital microscopy and microphysiologic "BBB-on-chip" platforms, facilitate quantitative measurement of regional transport and drug levels. These observations indicate three interrelated paths to enhanced therapy: temporarily normalizing or reversibly opening the barrier, avoiding it by targeted regional delivery, and rationally designing drugs that account for transport and efflux limitations. The integration of barrier modulation with immunotherapies in preclinical models enhances intratumoral exposure and efficacy. Lessons from other neurologic illnesses highlight both the dangers of uncontrolled opening and the potential of localized, reversible modulation. We support a "BBB-first" paradigm that treats the barrier as a quantifiable, targetable organ and demands trials stratified by barrier phenotype and correlating clinical outcome with regional exposure and immune access.

KEYWORDS

glioblastoma, blood–brain barrier, blood–tumor barrier, drug delivery, focused ultrasound, immunotherapy, receptor-mediated transcytosis, convection enhanced delivery

1 Introduction

Glioblastoma (GBM) is the most common malignant primary brain tumor in adults and, under the 2021 WHO criteria, is defined as an IDH-wildtype, grade 4 diffuse astrocytic glioma that typically shows microvascular proliferation or necrosis on pathology [1–4]. While global data on GBM incidence and disability-adjusted life years (DALYs) remain limited, the incidence in the United States is estimated at approximately 3 cases per 100,000 individuals [4, 5]. Despite maximal therapy involving surgery when feasible, radiation, and temozolomide, outcomes remain poor, with population-level datasets highlighting that GBM accounts for roughly 51% of malignant CNS tumors in the U.S., and effects and carries the lowest median observed survival among malignant brain tumors (Even with modern care, most series still report median overall survival on the order of ~14–16 months in trial cohorts [4–6]. Tumor Treating Fields modestly extend survival for selected patients, but durable cures remain rare, underscoring the need to rethink how we deliver drugs and immune effectors to the brain [6].

A defining reason GBM is so hard to treat is the blood–brain barrier (BBB) and its tumor-altered counterpart, the blood–tumor barrier (BTB). The BBB normally protects neural circuits by tight endothelial junctions, low vesicular transport, and active efflux, but GBM converts this into a mosaic of barrier states, from relatively intact, drug-resistant margins to disrupted, edematous cores, with non-uniform permeability and persistent efflux that make "leak" on MRI a poor proxy for effective drug exposure [7, 8]. This variability also shapes immune entry and edema, so the same tumor can be "open" to water and contrast yet "closed" to antibodies or T cells. Clinically, that means delivery strategies must be matched to local BBB state (normalize, open, bypass, or exploit receptor-mediated transport) rather than assuming a single barrier phenotype throughout the lesion.

This spatial heterogeneity forms the basis of the challenges in drug delivery, dictates the behavior of immune cells, and substantially elevates the complexity of neurosurgical planning. Hence, GBM goes beyond the description of a brain tumor with an attendant barrier presentation but, instead, defines a pathological state with BBB remodeling across molecular, cellular, and biophysical axes [9, 10].

In health, BBB function emerges from specialized endothelium with tight and adherens junctions (including claudin-5, occludin, zonula occludens (ZO) scaffolds, and vascular endothelial cadherin (VE-cadherin)), low vesicular transport, polarized efflux and receptor systems (such as ATP-binding cassette (ABC) transporters and receptor-mediated transcytosis), and the neurovascular unit (NVU), which consists of pericytes within a dual basement membrane and astrocytic endfeet that align aquaporin-4 (AQP4) channels and ion/water flux to neuronal demand. Junctional integrity and transporter polarity enforce steep permeability gradients and high transendothelial electrical resistance (TEER), preserving neuronal signaling fidelity [11–13]. Astrocytes and pericytes impose bidirectional control: astrocytic sonic hedgehog (SHH), Wnt/β-catenin, angiopoietin-1 (ANG-1)/Tie2, insulin-like growth factor-1 (IGF-1), glial cell line-derived neurotrophic factor (GDNF), and retinoic acid strengthen junctions; vascular endothelial growth factor (VEGF)–endothelial nitric oxide synthase (eNOS)–nitric oxide (NO), endothelins, matrix metalloproteinases-2 and -9 (MMP-2/9), and glutamate–N-methyl-D-aspartate (NMDA) signaling loosen them. Pericyte platelet-derived growth factor receptor beta (PDGFRβ)–transforming growth factor beta (TGF-β) signaling stabilizes endothelial identity and suppresses permeability phenotypes; Notch3 and metabolic stress tune coverage and contractility [7, 10, 14, 15]. These latent programs are the levers GBM pulls.

GBM corrupts the vasculature in stages. Vascular co-option permits tumor cells to parasitize native microvessels, physically displacing astrocytic endfeet from the endothelial basement membrane and uncoupling perivascular signaling, TEER drops before frank angiogenesis [16]. Hypoxia and acidosis then select for VEGF, IL-8, SDF-1, bFGF, generating tortuous neovessels with high interstitial pressure and chaotic perfusion. At the junctional level, PKC-dependent occludin phosphorylation and MMP-mediated claudin-5 degradation dismantle the paracellular fence, while ZO-1 mislocalization/loss fractures continuity; islands of residual junctions persist, yielding BBB mosaics [17–19]. The extracellular matrix (ECM) modulates barrier tone: laminins and collagen IV support endothelial polarity and AQP4 organization, whereas hyaluronan–TLR signaling propagates inflammation and matrix remodeling that further increases permeability [19–21]. Pericytes are a second control point: PDGFRβ/TGF-β and Notch3 sustain coverage; chronic hypoxia/inflammation erodes these safeguards, and glioma-associated mesenchymal cells can transdifferentiate into pericyte-like cells that are not equivalently barrier-protective [22, 23]. Notably, BBB remodeling is lineage- and model-dependent: patient-derived glioma stem–like cells can preserve perivascular integrity that classic U87 lines do not, emphasizing tumor-intrinsic control of barrier state [23, 24].

Immunologically, the remodeled BBB is not a passive leak but an active gatekeeper that sculpts the tumor ecosystem. VEGF signaling induces Tregs and MDSCs and biases microglia/macrophages toward M2-like programs, while anti-VEGF "normalization" can re-route biology toward SRC-driven invasion and metabolic rewiring rather than durable immune competence [24, 25]. Endothelial ICAM-1/VCAM-1 and perivascular chemokines shape leukocyte adhesion and diapedesis; astrocytic SHH suppresses endothelial ICAM-1, limiting immune entry, whereas NF-κB/STAT3-driven reactive astrogliosis elevates cytokines (IL-6, TNF-α), MMPs, and NO, loosening junctions yet not necessarily improving effector T-cell trafficking [7, 25, 26]. Pericyte dysfunction expands perivascular myeloid niches that buffer cytotoxic lymphocytes; APOE4–CypA–NF-κB–MMP-9 signaling in pericytes exemplifies a barrier–myeloid axis that promotes leak and immunosuppression [7]. Layered on top are non-coding and epigenetic controls such as NEAT1to miR-181d-5p/SOX5, miR-34c/miR-18a networks that repress claudin-5/occludin/ZO-1; NF-κB/STAT3/NFAT programs and histone remodeling that toggle astrocyte secretomes between barrier-stabilizing (SHH, ANG-1) and barrier-loosening (VEGF, MMPs) states [7, 26, 27]. The net effect is spatially adjacent zones that are drug-refractory yet immunologically sealed and edematous, leaky regions that still fail to deliver adequate concentrations to infiltrative margins.

These realities have first-order clinical consequences. Enhancing cores typically exhibit greater leak, whereas invasive peripheries often retain an intact BBB ('imaging-dark' on permeability maps);

importantly, local perfusion–permeability decoupling can produce exceptions, so we stratify by measured barrier state rather than location. In addition, intact-BBB peripheries exert discordant pharmacokinetics; anti-angiogenic therapy obtains short-term radiographic benefit with modest survival; and BBB state dictates fluorescence-guided resection fidelity, convection enhanced delivery distribution, and the benefit-risk balance of osmotic or targeted-ultrasound dilation [7, 28, 29]. For immunotherapy, endothelial state of activation, pericyte coverage, and perivascular composition of myeloids jointly dictate T-cell influx, CAR-T viability, and antibody diffusion [29, 30]. Hence, a BBB-first framework, viewing the barrier as a measurable, druggable organ, naturally follows.

The emergent literature presented collectively support the notion of a BBB-first paradigm within the scope of GBM: the blood-brain barrier should not be viewed merely as a barrier but as a modifiable organ that plays a crucial role in the disease, with its immune and transport characteristics being amenable to assessment, modeling, and therapeutic intervention. In the following sections, we aim to (i) outline the biophysical characteristics of a healthy blood-brain barrier, (ii) explore the lineage- and niche-specific modifications of GBM that result in quantifiable permeability and immune-gating phenotypes, and (iii) evaluate strategies that either utilize endogenous transport mechanisms (such as RMT-aware carriers, prodrugs, and efflux modulation) or temporarily modify the physical attributes of the barrier (through convection, osmotic effects, and focused-ultrasound opening). Our goal is to convert BBB heterogeneity from a source of therapeutic challenges into a design criterion for precision neuro-oncology.

What this Review instantiates is operationalizing a BBB-first paradigm: Beyond summarizing BBB/BTB biology, we provide a decision framework that links barrier phenotype to imaging biomarkers and delivery strategy (open, bypass, normalize, exploit transport) to PK/PD and clinical endpoints as predictable failure modes/mitigations. We harmonize micro-to macro-scale readouts (junctional/perivascular programs, single-cell/spatial maps, DCE/DSC-MRI and PET) into actionable patient stratification, and specify trial design primitives (timing windows, exposure assays, immune ingress, safety liabilities) to prospectively test delivery-efficacy hypotheses in GBM. BBB-first means treating barrier state as the primary stratifier that determines route, schedule, and endpoints: classify regions (intact rim vs. heterogeneous core vs. normalized beds), match modality accordingly, predefine PK/PD and safety readouts, and embed mitigations for known failure modes. We also incorporate very recent advances (e.g., refinements in osmotic BBB opening and image-guided territory control) within this risk-benefit framework.

2 The healthy BBB: biophysical architecture

CNS homeostasis and protection are maintained through a specialized set of tissues and barriers. Although the brain is richly vascularized, the BBB provides a critical separation between peripheral circulation and the CNS. This barrier function is achieved through the coordinated actions of three major cell types: (1) endothelial cells (ECs), (2) mural cells (MCs), including smooth muscle cells (SMCs) and pericytes (PCs), and (3) astrocytic endfoot projections [31–33].

ECs of the BBB differ from those of the peripheral vasculature in that they lack fenestrations and form a high density of specialized tight junctions (TJs) that restrict paracellular diffusion of large or polar molecules into the CNS interstitium and preserve apical–basolateral EC polarity [33–35]. While oxygen and carbon dioxide can readily diffuse across, large and charged molecules like glucose, amino acids, insulin, and iron cannot passively diffuse. TJs are primarily composed of claudins and occludins, which establish intercellular connections and are anchored to the actin cytoskeleton via scaffolding proteins such as ZO-1, ZO-2, ZO-3, and cingulin [36, 37]. Among the claudin family, multiple isoforms have been identified, with claudin-1, claudin-3, and claudin-5 being of particular importance to the BBB [18, 38, 39]. In addition, TJs depend on adherens junctions (AJs) for proper assembly, stability, and intercellular tension. AJs mediate cell–cell adhesion through occludins, claudins, and junctional adhesion molecules, while alpha, beta, and gammma-catenins provide cytoskeletal anchoring [34–36]. Without AJ support, TJs fail to form functional barrier structures.

The second key component of the BBB is MCs. In cerebral arteries, MCs are predominantly SMCs, but as the vasculature narrows to arterioles, they transition to PCs [31, 35]. Fibroblast-like cells may also reside in the perivascular space between the endothelial basement membrane and the astrocytic basement membrane of venules and some arteries. PCs are undifferentiated contractile cells housed within the endothelial basement membrane that contribute to the regulation of vessel diameter, cerebral blood flow, and provide critical microvascular support [33, 35, 40, 41]. At the capillary level, the EC and astrocytic basement membranes become directly apposed. The EC basement membrane is composed primarily of laminin α4 and α5 isoforms, which anchor ECs via α and β integrins, while the astrocytic basement membrane contains laminin α1 and α2 isoforms and connects to astrocytic endfoot projections through dystroglycans and integrins [35]. Together, these interactions create a tightly integrated structural scaffold, providing a foundation for astrocytic regulation of BBB function.

The third key component of the BBB is the astrocytic endfoot projection. These extensions of astrocytes envelop the cerebral vasculature through tight junctions to form the glia limitans, the final barrier between the systemic circulation and the brain parenchyma [35]. Astrocytic endfeet regulate and support BBB function through the expression of ion and water channels, such as aquaporin-4 (AQP4), and by secreting signaling molecules including vascular endothelial growth factor (VEGF), nitric oxide (NO), apolipoprotein E, and insulin-like growth factor-1 [42, 43]. In addition, astrocytic expression of laminin has also been show to be critical proper PC function and BBB integrity [43]. The close crosstalk among ECs, astrocytes, and neurons underscores that the BBB is not a static wall, but rather a highly dynamic and adaptive interface [44]. Taken together, ECs, MCs, and astrocytic endfoot projections form a multilayered and mutually supportive barrier that restricts the diffusion of most molecules into the brain and sets the stage for the highly regulated transport needed for CNS homeostasis.

The despite the layered boundaries, molecules can pass the BBB through passive diffusion, carrier-mediated transport, receptor-mediated transport, and active efflux. Lipid-soluble molecules can

passively diffuse across the BBB, with diffusion rate across the BBB generally increases with lipid solubility up to 400 Da in size, likely due to steric limits within membrane lipid pores. Additional factors, including hydrogen-bonding capacity, molecular shape, and the number of rotatable bonds, also influence permeability [44]. Carrier-mediated transporters are a diverse family of proteins responsible for shuttling polar molecules including glucose, amino acids, ions, nucleosides, and peptides across the BBB. These molecules can be transported through various mechanisms, including passive or active transport, and in either a bidirectional or unidirectional manner [36, 45, 46]. The distribution of carrier-mediated transporters differs between the apical and basolateral membranes, reflecting the established polarity of endothelial cells. Similarly, receptor-mediated transport and transcytotic pathways facilitate the selective uptake of larger proteins and hormones [33]. Finally, active efflux is mediated by ATP-binding cassette (ABC) transporters, which expel exogenous xenobiotics and metabolic byproducts back into the circulation [37, 47]. Together, these complementary mechanisms establish a finely tuned balance that allow the CNS access to essential nutrients and signaling molecules while simultaneously protecting it from toxins and pharmacologic agents.

Another critical function of the BBB is the regulation of the interface between the CNS and the peripheral immune system. Cerebral interstitial fluid drains through narrow pathways between basement membranes into the perivascular space and ultimately toward lymph nodes [48]. This environment presents challenges for immune surveillance, as immune cells cannot readily migrate from the parenchyma to lymphatic vessels. By contrast, soluble antigens, but not larger particulate matter such as viruses, can drain into perivascular regions [35, 49, 50]. Antigen-presenting cells (APCs), such as dendritic cells and macrophages, localize within these perivascular spaces. T cells gain access by crossing endothelial cells at venules and entering the perivascular compartment, but they may only proceed into the brain parenchyma if they recognize antigens presented by perivascular APCs. As a result, only a limited number of $CD4^+$ and $CD8^+$ T cells are permitted entry into the CNS parenchyma [35, 51]. Together, these mechanisms reinforce the concept of the CNS as an immune-privileged site, allowing selective immune surveillance while limiting widespread inflammation.

3 BBB dysregulation in glioblastoma

GBM reorganizes the brain's microvasculature into something more complex than an "open" or "leaky barrier." Concomitant reorganization of junctional composition, endothelial transcytosis, basement membrane structure, perivascular cell identity, and astrocyte polarity induced by tumors is regionally heterogeneous and dynamically regulated by hypoxia, inflammatory signals, and mechanical pressure. Below we integrate the major axes of dysfunction that, taken together, form a mosaic of intact and disrupted barrier states in GBM, with particular emphasis on immunologic crosstalk at the neurovascular interface.

Host factors intersect with GBM genomics in ways that shape disease risk and course: epidemiologically, GBM occurs more often in males, and recent multi-omic and methylome studies report sex-associated molecular differences that may influence outcomes [52]. With aging, GBM is predominantly IDH-wildtype; IDH1/2 mutations are enriched in younger adults, whereas TERT-promoter mutations, common in IDH-wildtype GBM, track with adverse features and poorer prognosis [53]. Race and/or ethnicity are associated with both epidemiology and tumor markers. Population-based cohorts show incidence and survival differences across racial/ethnic groups and these data highlight that age, sex, and ancestry contribute to the GBM genomic landscape (e.g., IDH, TERT, EGFR, MGMT) and, by extension, therapeutic response context that complements vascular–immune heterogeneity at the GBM neurovascular interface [53, 54].

3.1 Tumor angiogenesis and leaky vasculature

GBM remodels the neurovascular unit from a high-resistance, low-permeability interface into a blood-tumor barrier (BTB) with non-uniform permeability, aberrant flow, and persistent efflux due to abnormal junctions and increased transcytosis. Dynamic contrast-enhanced MRI (DCE-MRI) captures this as elevated $K^{\wedge}trans$ in enhancing regions, yet drug exposure remains patchy because perfusion and permeability are spatially uncoupled [7]. At the endothelial layer, angiogenic signaling mislocalizes tight-junction proteins (claudin-5, occludin, ZO-1) and up-shifts transcytosis pathways (caveolin-1/PLVAP), a combination that yields focal paracellular gaps plus heightened vesicular transport [7, 55]. Single-cell and ultrastructural studies from human GBM further show PLVAP-high, caveolae-rich endothelium within enhancing core vasculature, consistent with leak and increased transcytosis [55].

Abnormal tumor vessels are tortuous, dilated, and flow-heterogeneous, elevating interstitial fluid pressure and creating diffusion–perfusion mismatches that hinder delivery even where contrast enhancement suggests "leak." These hallmarks motivate time-boxed vascular normalization to transiently improve perfusion/oxygenation and reduce edema [56, 57]. Clinically, $K^{\wedge}trans$ and related DCE metrics correlate with angiogenic phenotype and prognosis in GBM and help separate progressive disease from treatment effects, though repeatability and model choice matter [58, 59]. Together, these data support a BTB continuum, from vesicle rich, junctionally abnormal endothelium in enhancing cores to relatively intact vessels at infiltrative margins [7].

3.2 Hypoxia-driven VEGF signaling and pericyte detachment

Hypoxia in pseudopalisading/necrotic GBM regions stabilizes HIF programs that upregulate VEGFA, loosening junctions, increasing endothelial vesicular transport, and driving immature sprouting [60, 61]. In GBM, hypoxia and VEGF intersect with the Angiopoietin/TIE axis: ANG2 (upregulated in GBM endothelium and after antiVEGF therapy) destabilizes the vessel wall and promotes pericyte detachment/regression, priming leaky, immature angiogenesis in the presence of VEGF [62, 63]. Mechanistically, pericyte expressed Tie2 helps stabilize sprouting vessels, and perturbing pericyteTie2 signaling renders pericytes promigratory and barrier ineffective, contributing to

leak despite apparent coverage [64]. Human GBM single cell datasets corroborate endothelial BBBtoBTB state shifts (junctional programs partly retained, PLVAP/caveolae increased) and show mural cell remodeling consistent with pericyte dysfunction [31, 55]. Therapeutically, judicious VEGF/VEGFR2 blockade can normalize vessels, tightening junctions, reducing transcytosis, restoring pericyte–endothelial coupling, and lowering IFP, but prolonged or mistimed inhibition risks evasive invasion and re-hypoxia, arguing for time-boxed combinations (e.g., with radiotherapy or immunotherapy) [54, 56, 57]. Emerging GBM data suggest Tie2 agonism can also promote normalization across core and periphery by reducing transcytosis and stabilizing junctions, offering an Ang/TIE directed complement to VEGF blockade.

3.3 Heterogeneity: intact vs. disrupted BBB regions

The GBM BBB is mosaic involving an angiogenic, contrast enhancing core with junctional discontinuities and high transcytosis, abutting an infiltrative rim that coopts native vessels and retains BBBlike features (tight junction transcripts, active efflux), producing pharmacologically "dark" disease beyond enhancement [65]. Vessel cooption at the invasive front is a GBM hallmark and a mechanism of resistance to antiangiogenic therapy, reinforcing the persistence of intact BBB territories despite radiographic response. Spatial omics and imaging show that permeability and flow markers diverge across microdomains. For example, PLVAP-high but poorly perfused patches versus intact-BBB, efflux-rich margins, explaining why contrast enhancement does not equate uniform drug delivery. Recent human/mouse work explicitly delineates core versus margin BTB states and demonstrates that state matched modulation (optoBBTB) can enhance delivery in both compartments, underscoring the translational value of BBB phenotyping [66].

3.4 Interactions with tumor associated macrophages and microglia

The GBM-remodeled BBB is an active immunologic gate shaped by perivascular myeloid niches. Spatial atlases of high-grade glioma reveal perivascular enrichment of macrophage/microglia states that correlate with immune exclusion and patient outcome, distinguishing GBM from brain metastases. CyTOF single-cell mapping confirms GBM's predominance of tissue-resident microglia with distinct activation states from infiltrating monocytes, reinforcing niche-specific crosstalk at vessels [55, 67]. Angiogenic cues are immunomodulatory: VEGF can suppress dendritic maturation and T-cell function, while vascular normalization partially re-tunes the perivascular milieu to permit better lymphocyte trafficking, principles now tested in GBM combination strategies [57, 67]. Moreover, GBM stroma contains perivascular fibroblasts linked to immune-checkpoint non-response and poor survival, adding another vascular-adjacent suppressive element [68]. Collectively, these data argue for BBB-aware immunotherapy: myeloid reprogramming in leaky, myeloid-rich cores and spatially targeted opening/normalization at intact-BBB rims to facilitate antibody/CAR-T entry while preserving safety.

4 Biophysical and imaging insights into BBB dynamics

4.1 Advances in imaging BBB permeability

Imaging advances have greatly enhanced our understanding of BBB permeability in GBM. Magnetic resonance imaging (MRI) techniques, especially dynamic contrast-enhanced MRI (DCE-MRI) and dynamic susceptibility contrast (DSC) perfusion MRI, allow quantitative mapping of tumor vascular permeability. Early studies showed that higher-grade gliomas exhibit increased contrast leakage and cerebral blood volume on perfusion MRI, correlating with their more disrupted BBB Recent refinements in DCE-MRI provide parametric maps of BBB leakiness, helping identify heterogeneous areas of permeability within a tumor [69]. For example, *in vivo* MRI of GBM models has demonstrated regions of leaky vasculature adjacent to relatively intact areas, reflecting the spatial variability of the blood–tumor barrier (BTB) [66, 70, 71]. Such imaging is not only diagnostic but can be used to correlate increases in post-treatment DCE-MRI permeability with disease severity (i.e., prognosticate) [72]. These imaging modalities, DCE-MRI and DSC, also allow for the gathering of robust information in bulk, a significant advantage over the aforementioned *in vivo* methods.

Positron emission tomography (PET) offers complementary insights by using radiotracers to quantify BBB function. Unlike MRI (which mostly detects structural leakage), PET can measure molecular transport across the BBB. New PET tracers such as radiolabeled amino acids and metabolites cross via specific transport mechanisms and can map regional BBB permeability with kinetic modeling [73]. For instance, [^{11}C]aminoisobutyric acid (AIB) and ^{68}Ga-EDTA are used to identify areas of compromised barrier in brain tumors [74]. PET can even assess efflux transporter activity at the BBB by employing substrates like [^{11}C]verapamil for P-glycoprotein function. Multimodal imaging that combines PET and MRI is now being explored to improve spatial resolution of BBB imaging via MRI and using the quantitative aspect of PET imaging [69]. Together, the dual imaging modalities of MRI and PET enable noninvasive "permeability mapping" of GBM, guiding both diagnosis and the evaluation of therapies aimed at modulating the BBB.

At the microscopic scale, intravital optical imaging has provided real-time views of BBB disruption in GBM models. Two-photon microscopy in orthotopic gliomas reveals how invading tumor cells physically perturb the neurovascular unit. Watkins et al. observed that glioma infiltration disrupted astrocyte–vascular coupling, leading to focal loss of endothelial tight junction integrity and increased leakage of fluorescent tracers [9]. Similarly, longitudinal multiphoton imaging has visualized macromolecular dye extravasation from tumor micro-vessels, confirming that BBB permeability is highest in regions of dense tumor and neovasculature [75]. Three-photon microscopy now permits imaging deeper into brain tissue, such as the invasive tumor margins in white matter [76]. These optical approaches have been demonstrated in pre-clinical models and enrich our understanding of BBB dynamics by

delineating where and when the barrier fails. For example, intravital imaging of nanoparticle delivery to GBM has demonstrated that functionalizing nanoparticles with targeting ligands (e.g., transferrin) enables them to transcytose across an otherwise intact BBB, whereas untargeted particles do not cross [77]. Furthermore, alternative nanoscale techniques addressing the nanomechanics in glioblastoma may serve as a useful biomarker to distinguish between health and GBM samples [78, 79]. In particular, AFM/ECIS data show that GBM lines harbor distinct nanomechanical phenotypes, e.g., stiffer, more viscous T98G versus more elastic U87 MG, that track with migration behavior and temozolomide sensitivity, underscoring actionable heterogeneity. Complementing intravital optical imaging of BBB failure, advanced AFM modalities quantify morphological, mechanical, and chemical features at nanoscale resolution, positioning AFM-derived mechanics as biomarkers to stratify therapy and interpret delivery across an otherwise intact or variably compromised BBB [78, 79].

Overall, advances in MRI, PET, and optical imaging provide a multiscale picture: from whole-tumor permeability down to cellular-level barrier breaches. This convergence of imaging modalities is illuminating the heterogeneous landscape of BBB integrity in and around GBM.

4.2 Modeling of solute and therapeutic flux

Beyond imaging, researchers are using biophysical modeling and experimental paradigms to quantify how solutes and drugs traverse the GBM-altered BBB. A consistent and major finding from both models and *in vivo* studies is that BTB permeability is highly heterogeneous, which profoundly affects drug delivery. Lockman et al. demonstrated in a breast cancer brain metastasis model that drug efficacy correlated with local BTB permeability regions with "tight" vasculature resisted therapy, whereas leaky regions saw better drug penetration [80]. By extension, GBM's patchy BBB disruption means some tumor niches receive sub-therapeutic drug concentrations. In human studies, Fine et al. directly measured chemotherapy deposition in resected brain tumors, finding that paclitaxel levels were <2% of plasma levels in many GBM samples due to poor penetration [81]. Such data underscore the need for quantitative models of drug transport in GBM.

Mathematical modeling of solute flux often treats the BBB/BTB as a semipermeable barrier with parameters like permeability (P) and surface area (S). DCE-MRI data are commonly fit to biophysical models (e.g., the Patlak or Tofts models) to estimate the permeability–surface area product (PS) in different tumor regions. These models show that GBM's PS for gadolinium demonstrates very low leakage [82–84]. However, when active transport is considered, PS can be an order of magnitude higher for substrates of facilitated transporters [73]. These computational models highlight that the GBM BBB is not simply open or closed, but rather that transport is compound-specific, depending on size, lipophilicity, and transporter affinity.

To better recapitulate human BBB dynamics, microfluidic and organ-on-chip models of the GBM microvasculature have been developed. A 2022 study by Straehla et al. created a microfluidic GBM model with perfused human endothelial cells, tumor spheroids, and astrocytes to simulate the BTB. This platform accurately predicted the trafficking of nanoparticles across the BBB and into tumor regions [85]. This model was tested using various nanoparticle designs and their ability to penetrate the barrier, providing a tool for modeling drug delivery before moving to animal or human trials [85]. Similarly, other 3D *in vitro* models using patient-derived induced pluripotent stem cells (iPSC) have been used to quantify flux and have demonstrated that a mildly intact BBB can drastically reduce chemotherapy uptake, consistent with observations in humans [86–88]. Furthermore, Seano et al. reported that alleviating solid stress with lithium in mice restored perfusion and improved neurological function [16]. While that study focused on neurons, it implies that mechanical forces contribute to BBB dysfunction and could be modeled in drug delivery simulations. In summary, biophysical modeling, whether through computational equations or physical microsystems, is shedding light on the complex kinetics of drug delivery in GBM. These approaches consistently indicate that without intervention, therapeutic molecules have uneven and often inadequate distribution in GBM due to a variably intact BBB. This understanding motivates the design of strategies to improve drug flux into all parts of the tumor. Table 1 recapitulates the mechanisms of transfer and support across the BBB.

4.3 Single-cell and spatial transcriptomic insights into BBB heterogeneity

Recent single-cell and spatial transcriptomic approaches are delineating the cellular and molecular heterogeneity of the blood–brain barrier within and adjacent to glioblastoma. Traditional bulk analyses showed that GBM endothelium expresses lower levels of tight junction proteins and higher levels of inflammatory signals than normal brain vasculature [22, 89]. Single-cell RNA sequencing provides identification of single-cell subpopulations. A recent single-cell study of human GBM vascular and perivascular cells identified distinct clusters of tumor-associated endothelial cells, pericytes, and astrocytes that collectively form an abnormal barrier niche [31]. Endothelial cells in GBM showed downregulation of BBB junction genes and upregulation of pathways related to antigen presentation and angiogenesis, indicating an inflamed, leaky phenotype [12, 15]. Notably, many tumor endothelial cells co-expressed genes for efflux pumps, such as ABCB1 and ABCG2, as well as angiogenic factors, reflecting a state that is both drug-exclusionary and pro-permeability (vessel tortuosity and leak) [69].

Pericytes serve as the support cells that wrap capillaries and have garnered special interest in single-cell analyses [31]. In normal brain, pericytes help regulate the function of the BBB; however, in GBM, they appear reprogrammed. Li and colleagues performed single-cell RNA sequencing on GBM tissues and found a subset of blood-brain-tumor-barrier-associated pericytes marked by high PTH1R expression [90]. These pericytes showed stark upregulation of extracellular matrix genes like collagen IV (COL4A1/A2) and fibronectin (FN1) compared to normal pericytes. Interestingly, patients whose tumors had higher expression of these pericyte ECM genes had worse survival, underscoring the clinical relevance of this BBB-modulating subpopulation. Functional tests confirmed that knocking down PTH1R in pericytes *in vitro* drove up collagen

TABLE 1 The healthy BBB: an engineering blueprint for transport and measurement.

Layer/unit	Principal molecular features	Core functions (biophysics)	Transport routes and gating	Electrophys/ barrier metrics	Measurement (bench/bedside)
Brain microvascular endothelium	Claudin-5, occludin, ZO-1/2/3; VE-cadherin; caveolin-1; GLUT1, LAT1/2; P-gp/ABCB1, BCRP/ABCG2	Tight paracellular seal; polarity; low transcytosis	Paracellular (TJ pores, size/charge-selective); Transcellular (RMT, AMT, caveolae); Efflux (ABC)	High TEER; low hydraulic conductivity; minimal vesicle density	TEER (chips), FITC-dextran flux, tracer EM; DSC-MRI, CBF/CBV baselines
Pericytes (PDGFRβ+)	TGF-β, Ang/Tie2 crosstalk; Notch3; integrins	Junction stabilization; suppression of transcytosis; tone	Indirect gating via endothelial signaling and BM composition	High TEER; Low transcytosis when coverage high	Pericyte coverage index (IMC/IF PDGFRβ); BM ultrastructure; permeability-coverage correlation
Basement membranes (endothelial/parenchymal)	Laminins (α4/5, β1/2), collagen IV, nidogens, agrin	Mechanical scaffold; charge selectivity; AQP4 anchoring	Electro-osmotic effects; diffusion tortuosity	Low effective diffusivity for charged solutes	Laminin/collagen IV mapping; ECM charge density; diffusion phantoms
Astrocytic endfeet (AQP4, Kir4.1)	SHH, Wnt, RA, ANG-1; AQP4 polarity	Junction "hardening"; water/ion homeostasis; NVC	Indirect RMT tuning; cytokine gating of adhesion molecules	Stable AQP4 polarity supports high TEER	AQP4 polarity index; endothelial ICAM-1/VCAM-1 at baseline
NVU coupling	Neurovascular signals (PGE2, EETs, NO)	Match flow to metabolism; preserve gradients	Controls shear, pressure	Maintains low IFP	ASL perfusion; pressure surrogates

This table reframes the healthy BBB, as a quantifiable biophysical system: all of the layers of the neurovasculatures have corresponding transport processes and electrophysiology with combined bench-to-bedside metrics, themselves then utilized throughout the manuscript as a reference with which to compare glioblastoma (GBM) conditions.
Abbreviations: ABC, ATP-binding cassette; AQP4, aquaporin-4; ANG-1, angiopoietin-1; AMT, adsorptive-mediated transcytosis; ASL, arterial spin labeling; BBB, blood–brain barrier; BCRP/ABCG2, breast cancer resistance protein; BM, basement membrane; BMECs, brain microvascular endothelial cells; CBF/CBV, cerebral blood flow/volume; DCE/DSC-MRI, dynamic contrast-enhanced/dynamic susceptibility contrast MRI; ECM, extracellular matrix; EM, electron microscopy; EETs, epoxyeicosatrienoic acids; ICAM-1/VCAM-1, intercellular/vascular cell adhesion molecule-1; IF, immunofluorescence; IFP, interstitial fluid pressure; IMC, imaging mass cytometry; LAT1/2, large amino-acid transporters; NVU, neurovascular unit; P-gp/ABCB1, P-glycoprotein; PGE2, prostaglandin E2; RA, retinoic acid; RMT, receptor-mediated transcytosis; SHH, sonic hedgehog; TEER, transendothelial electrical resistance; TJ, tight junction; VE-cadherin, vascular endothelial cadherin; Wnt, Wingless/Int; ZO-1/2/3, zonula occludens-1/2/3.

IV and FN1 production, and *in vivo* modeling showed an inverse correlation between PTH1R levels and BBB leakiness, suggesting $PTH1R^+$ pericytes regulate the permeability of the BBB [90].

In parallel, spatial transcriptomics has illuminated how BBB-related gene expression varies across different tumor regions [91]. GBM exhibits well-defined histologic niches, for example, microvascular proliferation (MVP) zones and hypoxic pseudopalisading necrosis zones, which were known to have different vascular phenotypes [91, 92]. Spatial transcriptomic analysis of 16 GBM patients identified region-specific gene signatures for MVP regions versus perinecrotic (PAN) regions. MVP regions (dense abnormal vessels) showed enrichment of endothelial and stromal genes like COL4A1, COL4A2, and FN1, as well as SPARC and IGFBP3, which are associated with angiogenesis and matrix remodeling. In contrast, PAN regions (around necrosis) had upregulation of genes like *CHI3L1* and *VEGFA* in their vasculature and associated myeloid cells [93]. These findings support the idea that different parts of the same tumor harbor blood vessels with distinct molecular profiles with their own respective phenotypes. In summary, single-cell and spatial omics are revealing BBB heterogeneity at the cellular and molecular level. These studies highlight potential targets for therapy, such as modulating pericyte-ECM interactions or targeting region-specific vessel phenotypes. Moreover, these approaches reinforce the concept that the BBB in GBM is not a monolith; but rather a heterogenous population of capillaries in the invasive margin to profoundly abnormal vessels in the tumor core. Table 2 recapitulates the drivers of immune evasion by the tumor in the context of utilizing the BBB's structural and functional vulnerability.

5 Translational strategies for overcoming the GBM BBB

5.1 Pharmacological approaches

One strategy to surmount the BBB in GBM is through pharmacological therapy to permeabilize or modulate the BBB. A straightforward approach is to use small-molecule therapeutics that inherently cross the BBB. The success of the oral alkylator temozolomide (TMZ) in GBM is partly due to its low molecular weight and lipophilicity, allowing therapeutic fractions to reach the brain [94]. Small-molecule PI3K/mTOR inhibitors have been evaluated for brain penetrance [95]. Additionally, medicinal chemistry efforts often produce prodrug compounds which have

TABLE 2 GBM reprograms the BBB: drivers, consequences, readouts, levers.

Driver/axis	Predominant cellular source(s)	Primary BBB effect	Immediate junctional/ transport consequence	Immune-ecology consequence	Biophysical/ imaging readout	Therapeutic lever(s)
VEGF–VEGFR2 with eNOS/NO and SRC–PAK signaling	Tumor cells, tumor-associated macrophages, and endothelial cells	Increased permeability ("leaky" microdomains)	PKC/SRC-dependent occludin phosphorylation; decreased claudin-5; increased vesicular trafficking	Induction of Tregs and MDSCs; M2 macrophage bias	Increased Ktrans on DCE-MRI; vasogenic edema; junction discontinuities on STED	Anti-VEGF during normalization window; pair with anti-invasion or efflux-aware delivery
MMP-2 and MMP-9	Reactive astrocytes, tumor cells, myeloid cells	Basement-membrane and tight-junction degradation	Claudin-5/occludin cleavage; extracellular-matrix loosening	Increased myeloid influx; serum antigen leakage	ECM fragmentation; albumin extravasation	MMP inhibitors; ANG-1 co-therapy; localized delivery
Endothelin-1 engaging ETB with reactive oxygen/nitric oxide bursts	Astrocytes, endothelial cells	Vasoconstriction with transient permeability spikes	Induction of MMP-9; NO and ROS burst	Edema; innate-immune skewing	Perfusion variability; transient pressure spikes	Endothelin-receptor modulation; edema-mitigation protocols
SHH, ANG-1, retinoic acid, and canonical Wnt signaling	Astrocytes communicating with endothelium and pericytes	Barrier stabilization ("hardening")	Increased ZO-1/occludin/claudin-5; decreased caveolae; PTPN2-dependent dephosphorylation of occludin	Reduced ICAM-1/VCAM-1 expression; decreased leukocyte adhesion	Low Ktrans at tumor rim; high AQP4 polarity; high TEER	SHH/ANG-1 augmentation; PTPN2 stabilization; sequence with a controlled opening if immunotherapy is planned
PDGFRβ–TGF-β and Notch3 pathways	Pericytes	Increased pericyte coverage and reduced transcytosis	Decreased vesicle density; stabilized VE-cadherin	Restricts T-cell ingress; perivascular myeloid hubs	Pericyte-coverage index inversely related to Ktrans	Spare PDGFRβ signaling when planning immunotherapy
Hyaluronan–TLR–driven ECM remodeling	ECM compartment, glioma cells	Shift in matrix charge and tortuosity	Increased diffusion hindrance despite vascular leak	Chemokine trapping; inflammatory retention	ADC–perfusion mismatch patterns	ECM-targeted timing coordinated with delivery
Non-coding regulators (NEAT1; miR-34c/18a/181d-5p)	Astrocytes, endothelial cells	Tight-junction program rewiring	Post-transcriptional downregulation of claudin-5/occludin/ZO-1	Barrier loosening without effective T-cell entry	EV-miRNA assays; TJ mRNA/protein mapping	Antisense/miRNA therapies; biomarker-guided opening
APOE4–Cyclophilin A–NF-κB–MMP-9 cascade	Pericytes	Pericyte-mediated vascular leak	Basement-membrane degradation; junction loss	Myeloid-rich perivascular niches	Genotype-stratified leak patterns	Cyclophilin A or MMP-9 inhibition; genotype-aware treatment planning

GBM, creates a BBB, mosaic via alternation of individual axes of signaling, either to relax or tighten junctions. Correlating each driver with immune fates and quantitative readouts (microscopy: MRI) offers decision hooks for targeted modulation substantiated thereafter in the manuscript.

Abbreviations: ADC, apparent diffusion coefficient; ANG-1, angiopoietin-1; APOE4, apolipoprotein E4; ASO, antisense oligonucleotide; AQP4, aquaporin-4; BM, basement membrane; CypA, cyclophilin A; DCE-MRI, dynamic contrast-enhanced MRI; EC, endothelial cell; ECM, extracellular matrix; eNOS, endothelial nitric oxide synthase; ET_B, endothelin-B, receptor; EV-miRNA, extracellular-vesicle microRNA; ICAM-1/VCAM-1, intercellular/vascular cell adhesion molecule-1; MRI, volume transfer constant; MDSC, myeloid-derived suppressor cell; MMP-2/9, matrix metalloproteinase-2/9; NF-κB, nuclear factor-κB; PDGFRβ, platelet-derived growth factor receptor-β; PKC, protein kinase C; PTPN2, protein tyrosine phosphatase non-receptor type 2; RA, retinoic acid; RMT, receptor-mediated transcytosis; ROS, reactive oxygen species; SHH, sonic hedgehog; Src–PAK, SRC, kinase–p21-activated kinase; STED, stimulated emission depletion microscopy; TAM, tumor-associated macrophage; TEER, transendothelial electrical resistance; TJ, tight junction; TLR, Toll-like receptor; Wnt, Wingless/Int; ZO-1, zonula occludens-1.

enhanced BBB-permeability by chemically modifying polar groups, which later convert into the active drug inside the CNS [96, 97].

Interestingly, some anti-angiogenic drugs might indirectly normalize or tighten the BBB. Low doses of VEGF inhibitors can prune leaky vessels and restore BBB integrity, potentially improving drug penetration into the remaining vessels by normalizing flow [98]. However, anti-VEGF therapy in GBM (e.g., bevacizumab) also rapidly reduces contrast enhancement and edema by *re-establishing* an intact barrier, which may actually impede drug delivery to the tumor [99]. Thus, pharmacological approaches require a delicate balance: one might open the BBB with one agent while delivering a second agent or design a drug that subverts BBB transport

mechanisms altogether. Thus, pharmacological strategies aim either to bypass BBB defenses or temporarily disable those defenses. While purely pharmacologic BBB modulation has yet to yield a breakthrough in GBM therapy, it remains an area of active research, especially in combination with other methods.

5.2 Physical and biophysical disruption approaches

Given the insights gained from pharmacologic methods, a number of physical techniques have been developed to breach the BBB/BTB in GBM patients. These approaches directly disrupt the barrier or circumvent it, often transiently and in a targeted fashion. Focused ultrasound (FUS) with microbubbles uses low-intensity ultrasound beams, targeted to the tumor region, in combination with circulating microbubble contrast agents [100]. The ultrasound causes the microbubbles to oscillate and produce mechanical opening of tight junctions in the local vasculature [101]. Magnetic resonance-guided FUS (MRgFUS) can localize this effect precisely. Idbaih et al. showed that pulsed ultrasound via an implanted device safely opened the BBB in recurrent GBM, evidenced by gadolinium uptake on MRI, and early data hinted at improved chemotherapy concentrations in tumor tissue [102]. Indeed, FUS produces a 4–6 h window of enhanced permeability, allowing intravenous chemotherapy, like doxorubicin or TMZ, to penetrate more effectively [103]. Excitingly, FUS is also being combined with immunotherapy: an *in vivo* study demonstrated that FUS BBB opening can potentiate anti-PD-1 checkpoint therapy by increasing lymphocyte infiltration into GBM [104].

As an alternative strategy to opening the BBB from within, convection enhanced delivery (CED) bypasses it by surgically placing one or more catheters directly into the tumor or resection cavity and infusing therapy under positive pressure [105, 106]. This creates a bulk flow that carries drug molecules into the brain tissue, allowing for greater concentration delivery than IV administration of a therapy [107]. Furthermore, infusion of MRI-contrast enhancing agents, like gadolinium, can map the volume of distribution during CED, allowing clinicians to tailor infusion rates or catheter positions in real time. While still an invasive approach, CED effectively circumvents the BBB and continues to be a viable approach for treatment delivery in the perioperative period of recurrent GBM. Furthurmore, a recent study showed that osmotic opening of the BBB with 25% mannitol +4% NaCl (doubling osmotic power) in mice resulted in wider, hemisphere-scale BBB permeabilization compared with mannitol alone, with significantly higher brain uptake of ^{89}Zr-labeled antibodies on PET; IA delivery outperformed IV, and serial MRI/histology showed no edema or injury up to 7 days. This refines OBBBO by boosting efficacy without increasing infusion rate and suggests a safer, more effective SIACI protocol for the delivery of large molecules [108].

5.3 Immunotherapeutic strategies and BBB modulation

Immunotherapy for GBM faces unique hurdles, one being the BBB, which limits immune cell entry and immuno-modulatory drug delivery. Innovative strategies are therefore focusing on modulating the BBB or exploiting immune pathways to penetrate it. Chimeric antigen receptor (CAR) T-cells targeting GBM-associated antigens have shown some dramatic responses in early trials, but getting these T-cells to the tumor is challenging [109]. The brain's relative immune privilege and the BBB impede T-cell trafficking from blood to tumor parenchyma [110]. To address this, most GBM CAR-T trials have delivered the cells locally, either directly into the resection cavity, or intraventricularly via an Ommaya reservoir [111–113]. For example, IL13Rα2-specific CAR-T cells were infused into the post-surgical cavity and ventricular system; several patients had transient tumor regressions and one patient survived over 5 years [114]. This suggests CAR-T cells can exert potent effects if they can be delivered past the BBB. There is evidence that CAR-T cells administered systemically can infiltrate GBM to some degree as O'Rourke and colleagues demonstrated that EGFRvIII CAR-T cells given intravenously were later detectable in GBM tissue, indicating they crossed into the tumor [115]. However, the efficiency of this trafficking is low as evidenced by the numerous clinical trials administering CAR T cells through more direct methods, such as intrathecal or CED. One method of overcoming the lack of CAR T cell infiltration to the tumor is through the functionalization of CAR-T cells with chemokine receptors, like CXCR4 or CCR2, to attract them to chemokines emitted by the tumor, thereby enhancing migration across the endothelium [116, 117]. Preclinical results show that such modifications increase CAR-T accumulation in brain tumors and improve survival in mice [116]. Overall, while CAR-T therapy holds promise, its efficacy in GBM requires working around the trafficking obstacles posed by the BBB. Figure 1 recapitulates how GBM remaps the BBB's neurovasculature.

Interestingly, some immunotherapies can directly alter BBB properties. Cytokine therapies like IL-2 or IFN-γ can make the BBB more permeable by inducing endothelial inflammation [118–120]. While the increased permeability can help immune cells traffic to the site of the tumor, this carries the inherent risk of fluid overload and subsequent cerebral edema [121]. Even checkpoint blockade itself, if effective, may trigger an immune response that secondarily opens the BBB [52, 122, 123]. Moreover, there is interest in targeting myeloid cells (like tie2-expressing macrophages) that regulate vessel permeability; depleting or reprogramming these cells could harden the BBB against tumor-favoring leaks while enabling leukocyte transmigration [124, 125]. Notably, bevacizumab does not need to cross into tumor cells; it binds VEGF in the extracellular space. When given to GBM patients, it often causes a rapid "normalization" of tumor vasculature, within days, MRI shows reduced contrast enhancement and vasogenic edema, reflecting a restoration of BBB tightness. This can greatly improve symptoms through decreased cerebral edema, thus resulting in decreased ICP. However, by decreasing the vascular permeability of a leaky BTB, bevacizumab might impair delivery of concurrently given chemotherapies to the tumor [99]. There is evidence that bevacizumab's effects include increased pericyte coverage and deposition of basement membrane around vessels (i.e., a more intact BBB) [126]. From a translational standpoint, bevacizumab failed to extend overall survival in newly diagnosed GBM trials, despite prolonging progression-free survival [28]. Furthermore, relapse

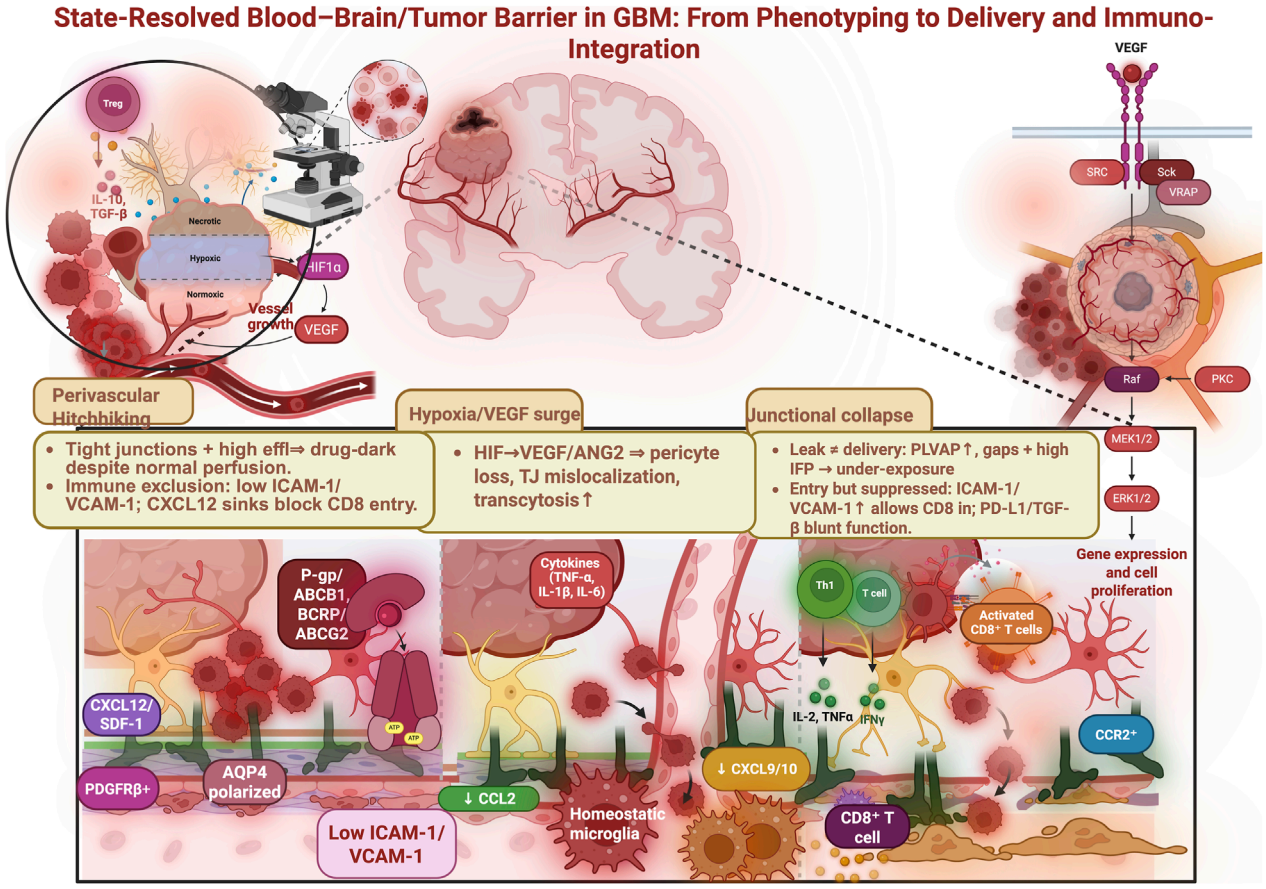

FIGURE 1
State-resolved BBB/BTB in GBM: Barrier phenotypes orchestrate delivery and immunity. This figure recapitulates how glioblastoma re-maps the neurovascular unit from efflux-protected, co-opted edge (tight junction-intact, high P-gp/BCRP, low ICAM-1/VCAM-1 and CXCL12) to a hypoxia/VEGF-driven dominated transition (ANG2-mediated pericyte loss, ZO-1 mislocalization, transcytosis/PLVAP upshift, patchy flow) to junctional collapse in the core (paracellular gaps, PLVAP-high endothelium, edema/high IFP). Under states, permeability, perfusion, and efflux get uncoupled, so enhancement does not equate to exposure. These conversions are made by the immune layer: from rim exclusion, to partial arrest in the transition zone, to diapedesis within a myeloid-suppressive core (PD-L1, TGF-β/IL-10). The schematic provokes state-matched immune evasion tactics: normalization windows or CED for leaky, under-perfused cores; and suggests trials stratified by BBB/BTB phenotype and matched with delivery endpoints (tissue drug, efflux/ICAM readouts, CD8 ingress).

following bevacizumab therapy results in tumors that recur in non-enhancing ways, infiltrating far from the original core, presumably in regions where an intact BBB shielded cells from therapy [127]. Therefore, immunotherapeutic strategies for GBM may ultimately benefit from further understanding of the tumor BBB. As our understanding deepens, we expect to see immunotherapy protocols that deliberately include a BBB modulation component, making the CNS tumor microenvironment more permissive for an immune attack.

5.4 Safety considerations

BBB-targeting strategies enhance delivery yet bring unique risks that should be managed prospectively. Vascular normalization (anti-VEGF) may alleviate edema but induces hypertension, thromboembolic/hemorrhagic events, and defective wound healing, and can re-tighten the barrier and decrease co-therapy penetration; careful timing and BP control mitigate this. FUS may elicit transient edema, headache, and petechial microbleeds; cavitation-based feedback, staged sonications, and PK-aligned dosing reduce this risk. Osmotic opening is effective yet nonselective, with seizure or focal-deficit risk; tight vascular-territory control and detailed hemodynamic/neurologic monitoring are necessary. CED avoids the barrier but has procedural complications such as catheter tract hemorrhage/infection and reflux/ventricular leakage; reflux-resistant cannulas, real-time distribution imaging, and stepwise flow rates reduce these. RMT/efflux strategies and nanocarriers bear the risks of off-tumor uptake, transporter pathway effects, RES accumulation, or complement activation; affinity/valency tuning, degradable cores, and complement-sparing surface chemistries help to contain this risk. Immunotherapies such as checkpoint blockade, cytokines, and CAR-T may trigger inflammatory edema, CRS/ICANS, or peritumoral swelling; route and dose tailoring along with standardized neurologic monitoring and steroid-sparing edema management are recommended. The mechanism, risks, and mitigants identified for each modality herein are cross-referenced to the failure modes/mitigations listed.

6 Clinical impact and future neurological interventions

6.1 Implications for neurosurgical planning

Maximizing safe resection in glioblastoma is critical, and BBB properties directly influence surgical strategies and planning. Fluorescence-guided resection with 5-aminolevulinic acid (5-ALA) is now an established approach to improve tumor visualization during surgery. In a landmark phase III clinical trial, 5-ALA guidance nearly doubled the rate of complete tumor resection (65% vs. 36% in white light) and significantly prolonged 6-month progression free survival (41.0% vs. 21.1% in white light) [128]. More recent clinical series have confirmed these benefits as well. In a 343-patient cohort, 5-ALA-guided surgery achieved greater gross-total resection rates (47.4% vs. 22.9%) and improved median overall survival (17.47 vs. 10.63 months) compared to conventional surgery [129]. Furthermore, the same study found that 5-ALA-guided surgery significantly reduced postoperative focal neurological deficits (23.3% vs. 44.9%) when compared to conventional surgery, most likely by helping delineate tumor margins. However, a known limitation of 5-ALA fluorescence-guided resection is that infiltrative tumor cells beyond regions of contrast enhancement or with intact BBB may not sufficiently accumulate protoporphyrin IX, the fluorescent metabolite produced after 5-ALA administration which accumulates selectively in glioma cells. This may lead to false negatives at the invasive margin, where fluorescence is absent in histologically malignant glioma tissue [130]. Thus, while fluorescence-guided resection significantly improves complete tumor resection and survival in GBM patients, the heterogeneity of BBB permeability means that neurosurgeons must remain cautious about tumor cells in non-fluorescing, BBB-intact tissue.

Beyond resection, intra-arterial therapies have re-emerged as an approach to bypass the BBB during drug delivery. Intra-arterial infusion of chemotherapy, often combined with osmotic BBB disruption using hyperosmolar mannitol, can transiently open tight junctions and flood the tumor region with high drug concentrations. Early iterations of the intra-arterial delivery mechanism were hindered by deleterious side effects, namely, decreased visual acuity, encephalopathy, and myelosuppression, to chemotherapies such as BCNU, ACNU, and cisplatin [131–134]. However, modern superselective catheter techniques target tumor-feeding arteries, limiting the systemic toxicities which plagued earlier intra-arterial chemotherapy trials. For example, a 2021 phase I/II single-arm study repeated superselective intra-arterial bevacizumab after mannitol BBB disruption in newly diagnosed GBM and achieved a median overall survival of 23.1 months, with 32% of patients alive at 3 years [135]. Reported toxicities were primarily grade 1–3, including seizures, aphasia, and thromboembolic events, while no grade 4–5 toxicities were observed among evaluable patients. However, this trial lacked a control group, and larger randomized studies are needed to determine whether intra-arterial bevacizumab plus chemoradiation is superior to standard therapy alone. While these approaches are still experimental, they illustrate the principle that aggressive regional therapy can exploit areas of BBB leak or actively induce BBB permeability to improve drug uptake.

From a surgical planning perspective, knowing a tumor's vascular supply and BBB integrity may guide the use of intra-arterial versus intravenous routes or the application of adjuncts like osmotic opening during surgery, such as intra-arterial mannitol infusion prior to resection. As an extension of neurosurgical care, combining meticulous resection, aided by 5-ALA fluorescence, with targeted intra-arterial therapies represent several forthcoming strategies to overcome the BBB barrier and treat both the core and infiltrative margins of GBM.

6.2 BBB-targeting as a therapeutic endpoint: integrating drug delivery with Immunotherapy

Given the BBB's role in limiting both drug and immune cell entry into the brain, a growing paradigm treats the BBB itself as a modifiable therapeutic target in GBM. Rather than viewing the BBB as a static obstacle, firstly approaching the BBB proposes manipulating barrier function as a part of therapy. One specific application is in immunotherapies, where the efficacy of treatments such as immune checkpoint inhibitors (ICIs) or chimeric antigen receptor (CAR)-T cells may be blunted by poor trafficking into the tumor bed. The abnormal tumor vasculature and associated BBB dysfunction in GBM create an immune-privileged microenvironment, often excluding effector T-cells and fostering immunosuppressive myeloid cells. Consequently, systemic immunotherapies may fail to achieve adequate effector cell trafficking throughout the tumor. Approaches to integrate BBB modulation in conjunction with immunotherapy are being actively explored. Preclinical studies demonstrate that deliberately opening the BBB can synergistically enhance immunotherapeutic efficacy. In murine glioma models, low-intensity focused ultrasound (FUS) disruption of the BBB was shown to improve anti-PD-1 checkpoint blockade, evidenced by an increased median survival from 39 days with anti-PD-1 alone to 58 days with the addition of FUS. Furthermore, a subset of mice rejected contralateral hemisphere tumor rechallenge, implying a more robust immune memory [136]. The same study demonstrated that FUS-mediated BBB opening significantly increased CAR-T cell homing to intracranial tumors, approximately doubling CNS CAR-T counts, and extended survival by 129% compared with CAR-T therapy alone. Table 3 recapitulates the emerging therapeutic landscape that utilized the blood-brain-barrier as it's advantage.

6.3 Cross-disease lessons: Alzheimer's, stroke, and multiple sclerosis insights for GBM

The importance of the BBB in GBM is further underscored by parallels in other neurological diseases. Insights from Alzheimer's disease (AD), stroke, and multiple sclerosis (MS) illustrate how BBB dynamics can drive pathology and inform therapeutic strategies. Firstly, AD exemplifies how chronic barrier dysfunction can contribute to neurodegeneration. Recent research has shown that individuals carrying the APOE4 allele, a major AD risk gene, exhibited accelerated breakdown of the BBB in the hippocampus and cortex, even prior to amyloid plaque accumulation [137]. This BBB leakage correlates with cognitive decline in APOE4 carriers,

TABLE 3 Matching intervention to BBB state, payload, and endpoints.

Approach	Core mechanism	Best-fit BBB context	Works best for (payload class)	Key biomarkers to stratify	Primary success endpoints	Common failure modes/mitigations
Vascular normalization (anti-VEGF)	Prunes abnormal vessels, tightens vascular leak, decreases interstitial fluid pressure, increases flow homogeneity	Leaky tumor cores with edema and poor perfusion	Small molecules; radiotherapy synergy	High K trans with low CBF; FLAIR edema	Decreased edema, increased CBF; improved progression-free survival in some cohorts	Invasion or metabolic switch (SRC or GLUT programs); mitigation: Combine with anti-invasion and metabolic agents
Focused ultrasound (MRgFUS with microbubbles)	Transient tight-junction opening via stable cavitation	Intact-BBB rims and invasive margins	Antibodies and antibody–drug conjugates; large small-molecule drugs; facilitation of immune-cell ingress	Low rim K trans; high pericyte coverage; low ICAM-1	Increased intratumoral drug by LC-MS/MS; increased $CD8^+$ T cells at re-operation	Edema or intracranial hemorrhage risk; mitigation: Acoustic feedback control, steroid protocol, pharmacokinetic-aligned timing
Osmotic opening (SIACI mannitol)	Endothelial cell shrinkage produces territorial tight-junction opening	Arterial territories with otherwise intact BBB	Chemotherapy cocktails; nanoparticles	Vascular territory maps	Increased tissue drug exposure; expansion of contrast enhancement	Seizure or stroke risk; mitigation: Anesthesia and hemodynamic monitoring; avoid fragile vasculature
Convection enhanced delivery (CED)	Pressure-driven interstitial infusion that bypasses BBB	Poorly perfused cores with dense extracellular matrix	Toxins, RNA or antisense oligos, large biologics	Tractography; ECM density; reflux modeling	Distribution-to-infusion (V_d_/V_i_) ratio; on-target coverage	Reflux or ventricular leak; mitigation: step-design cannulas, co-infused visible tracers
Efflux modulation (P-gp/BCRP)	Reduces active drug export at endothelium	Intact-BBB regions with high efflux activity	Efflux-substrate tyrosine-kinase inhibitors	[11C]-verapamil PET; transporter SNPs	Increased tissue-to-plasma ratio	Systemic toxicity; mitigation: local or temporally restricted inhibition
Receptor-mediated transcytosis carriers (transferrin, insulin, LDLR)	Uses endothelial receptors for transcytosis	Intact or normalized BBB	Biologics and enzymes	Receptor density and affinity; ligand competition	Target engagement with adequate exposure	Off-target pathway effects; mitigation: tune affinity and valency
Angiopoietin-1/Sonic hedgehog augmentation	Junction stabilization to reduce washout after local delivery	Used in combination with CED or targeted deposition	Any payload needing precise localization	Low ZO-1 continuity; edema on imaging	Reduced backflow; improved on-target retention	Excessive hardening may limit immune entry; mitigation: sequence before or after a controlled opening

(Continued on the following page)

10.3389/fphy.2025.1723329

TABLE 3 (*Continued*) Matching intervention to BBB state, payload, and endpoints.

Approach	Core mechanism	Best-fit BBB context	Works best for (payload class)	Key biomarkers to stratify	Primary success endpoints	Common failure modes/mitigations
Nanocarriers and enhanced permeability/retention	Size and stealth to exploit leak and retention	Heterogeneously leaky cores	NP-encapsulated chemotherapy or siRNA	Ktrans heterogeneity; interstitial fluid pressure map	Nanoparticle deposition with verified payload release	Intact rims resist deposition; mitigation: Combine with focused ultrasound or CED
Immunotherapy (checkpoint blockade, CAR-T)	Restores or delivers antitumor immunity	BBB-tight, immune-cold tumors that need opening; or myeloid-rich tumors that need reprogramming	Monoclonal antibodies; cellular therapies	ICAM-1/VCAM-1; perivascular M2 markers; AQP4 polarity	Increased $CD8^+$infiltration; progression-free and overall survival	Trafficking failure or myeloid "sinks"; mitigation: pair with focused ultrasound plus CSF-1R or TGF-β modulators

A pragmatic map from BBB, phenotype to strategy: which barrier state benefits from normalization, opening, bypass, or receptor-mediated delivery; what payloads suit each; what biomarkers stratify patients; and how to recognize and mitigate predictable failure modes.

Abbreviations: ADC, antibody–drug conjugate; AQP4, aquaporin-4; BBB, blood–brain barrier; BCRP, breast cancer resistance protein; CBF, cerebral blood flow; CED, convection enhanced delivery; CSF-1R, colony-stimulating factor-1, receptor; DCE-/DSC-MRI, dynamic contrast-/dynamic susceptibility-contrast MRI; ECM, extracellular matrix; EPR, enhanced permeability and retention; FLAIR, fluid-attenuated inversion recovery; FUS, focused ultrasound; ICAM-1/VCAM-1, intercellular/vascular cell adhesion molecule-1; IFP, interstitial fluid pressure; K-trans, MRI, volume transfer constant; LC-MS/MS, liquid chromatography–tandem mass spectrometry; LDLR, low-density lipoprotein receptor; MRgFUS, MRI-guided focused ultrasound; NP, nanoparticle; OS/PFS, overall/progression-free survival; PET, positron emission tomography; PK, pharmacokinetics; P-gp, P-glycoprotein; RMT, receptor-mediated transcytosis; RT, radiotherapy; SIAMI, superselective intra-arterial mannitol infusion; SNP, single-nucleotide polymorphism; SRC, SRC, family kinase; TEER, transendothelial electrical resistance; TJ, tight junction.

independent of classic AD pathology, suggesting that a leaky BBB is itself neurotoxic and a potential therapeutic target in AD. For GBM, these observations imply that sustained BBB disruption can impair normal neural function, necessitating precise control and timing of therapeutic opening strategies. Ongoing AD trials using FUS to transiently open the BBB for enhanced drug delivery or amyloid clearance illustrate how barrier modulation itself can be leveraged therapeutically, a concept translatable to GBM where transient BBB opening may enable delivery of large biologics, cellular therapies, or nanoparticles otherwise excluded from the CNS [138, 139].

In stroke, ischemia causes tight junctions to open within hours, allowing plasma proteins and fluid to flood the brain, manifesting as vasogenic edema. This BBB breakdown is a pathological hallmark that contributes to hemorrhagic transformation and worsened outcomes if left untreated [140]. Clinically, stroke management must carefully time reperfusion therapies, such as tPA or thrombectomy, because a severely compromised BBB increases the risk of intracerebral hemorrhage upon reperfusion. In GBM, peritumoral edema frequently arises from BBB leakage and is typically managed with corticosteroids. Interventions that further perturb the BBB, including radiation or focused ultrasound, many exacerbate edema or precipitate microhemorrhages, paralleling complications observed in stroke. Stroke research emphasizes neurovascular unit protection, suggesting that adjunct therapies such as ROS scavengers or MMP inhibitors can be used to stabilize the BBB during or after aggressive interventions [141, 142]. Additionally, stroke models indicate that BBB opening may aid in clearing waste from the brain, implying that periods of BBB permeability in GBM may facilitate immune cell entry or clearance of tumor lysis products if managed and timed properly. Moving forward, lessons from stroke encourage GBM clinicians to monitor BBB status, for example, via MRI permeability imaging, after treatments such as surgery, radiation, or BBB-opening procedures, and to deploy supportive measures to mitigate harmful barrier disruption when needed.

Multiple sclerosis (MS), an autoimmune demyelinating disease, is essentially a disorder of immune cells breaching the BBB. In early MS lesions, inflammatory cytokines and activated leukocytes disrupt the endothelial junctions, enabling T-cells and macrophages to infiltrate the CNS and target myelin. Pathology and imaging studies demonstrate that BBB disruption occurs not only in active MS lesions but also in chronic lesions and normal-appearing white matter, indicating that BBB dysfunction precedes and accompanies demyelination [143]. In fact, the presence of gadolinium-enhancing lesions on MRI, indicative of focal BBB breakdown, is a diagnostic hallmark of active MS. A direct insight from MS is the success of therapies that target the BBB to modulate disease, which can be translated to GBM. Natalizumab, a monoclonal antibody targeting the α4-integrin adhesion molecule, prevents leukocytes from adhering to and crossing the BBB. By blocking immune cell transmigration at the vascular endothelium, natalizumab dramatically reduces CNS inflammation in MS [144]. This establishes that blocking the BBB can be therapeutic in an immune-driven condition, the converse of the challenge seen in GBM. However, GBM actively suppresses immune entry in part via the BBB and associated cells, such as reactive astrocytes which release factors that tighten the barrier and limit T-cell trafficking. The insights gained from BBB-derived treatments in MS suggest that specific molecular targets at the BBB could be manipulated

to either enhance or reduce immune cell passage. In the case of GBM, one potential therapy could be selectively increasing effector T-cell entry while excluding immunosuppressive cells by blocking certain endothelial checkpoints that admit regulatory T-cells but not cytotoxic T-cells.

Parallels from Alzheimer's disease, stroke, traumatic brain injuries and multiple sclerosis highlight that BBB dysfunction can drive pathology but also serve as a therapeutic target [145–148]. These conditions illustrate both the risks of uncontrolled barrier disruption and the potential of targeted modulation to improve outcomes. For GBM, such lessons reinforce that precise, context-specific BBB modulation, whether to enhance drug and immune cell delivery or to limit edema and neurotoxicity, will be central to future therapeutic strategies.

7 Conclusion

Glioblastoma's blood–brain barrier is not merely a static wall to drug delivery, but an active and heterogeneous organ that profoundly shapes therapy outcomes. As reviewed, the BBB in GBM exists on a spectrum from intact, impermeable regions at the invasive margins to leaky, abnormal vessels in the tumor core. This patchwork limits therapeutic penetration and creates sanctuary sites for tumor cells, contributing to treatment failure and relapse. In fact, even standard chemotherapies like temozolomide achieve only a fraction of their systemic concentrations in the brain due to efflux pumps and tight junctions at the BBB. The BBB thus stands as both a physical barrier to current treatments and a biological driver of GBM's resistance and immune evasion. Recognizing this, a "BBB-first" paradigm has emerged treating the barrier itself as a therapeutic target rather than an afterthought. By viewing the BBB as a measurable, druggable interface, we can aim to modulate its properties (tight junction integrity, transporter activity, and permeability) to improve drug and immune cell entry into the tumor.

Importantly, overcoming GBM's notorious therapeutic resistance will require interdisciplinary strategies that bridge biophysics, neuroscience, and oncology. A key lesson from recent advances is that no single approach is sufficient. Instead, combining insights from multiple disciplines offers the best promise for breaching this tumor's defenses. Biophysical innovations are allowing us to map and manipulate the BBB like never before. Advanced imaging techniques (e.g., DCE-MRI, PET) now quantify regional BBB permeability *in vivo*, guiding where to target treatments. Microfluidic models and "organ-on-a-chip" systems simulate drug and nanoparticle transport across a human BBB, predicting delivery efficacy before clinical trials. Physical delivery methods such as focused ultrasound (FUS) with microbubbles can reversibly open tight junctions in a targeted manner, creating a 4–6 h window for therapeutics to flood into the tumor bed. Early clinical studies show that FUS-induced BBB opening increases chemotherapy accumulation in GBM and may even enhance immunotherapy by boosting T-cell infiltration. Likewise, convection enhanced delivery bypasses the BBB entirely via catheters, achieving drug concentrations in the tumor that systemic infusion cannot. Each of these tools, imaging, modeling, and focused delivery, arises from fields outside traditional oncology, yet together they are addressing the BBB challenge head-on.

Equally crucial is integrating these biophysical tools with biological and pharmacological innovations. GBM researchers are leveraging molecular biology and immunology to turn the BBB from an obstacle into an ally. For example, single-cell RNA sequencing and spatial transcriptomics have exposed novel molecular targets in the tumor-associated endothelium and pericytes that regulate barrier leakiness and immune cell trafficking. Exploiting such targets can recalibrate the barrier: recent work identified an IL-6/STAT3 signaling axis by which glioma cells induce barrier permeability, and blocking this pathway was shown to tighten the BBB or, conversely, could be timed to loosen it for drug entry. Similarly, nanomedicine and medicinal chemistry are producing therapies optimized for BBB traversal, from receptor-mediated transcytosis shuttles to lipophilic prodrugs, ensuring drugs reach infiltrative tumor cells shielded by an intact BBB. On the immune front, strategies like locally delivered CAR-T cells or immune cell attractants (chemokine receptor engineering) are being employed so that immunotherapies can penetrate the tumor's protective vascular niche. Notably, these approaches underscore the need to finely tune the BBB: opening it enough to let drugs and effector cells in, while avoiding excessive disruption that could harm normal brain function (a lesson reinforced by parallels in stroke and neuroinflammation). In essence, the most promising breakthroughs arise when engineering solutions (e.g., FUS, nanocarriers), biophysical modeling, and oncologic therapies are designed in concert.

In summary, the BBB in glioblastoma should be viewed as both a challenge and an opportunity. It remains a major reason why many conventional and experimental treatments fall short, but it is also a key to unlocking improved outcomes. By treating the BBB as a controllable variable, something that can imaged, modeled, targeted, and transiently modified, next-generation therapies can be tailored to each tumor's barrier phenotype. Interdisciplinary collaboration is driving this shift: neurosurgeons, bioengineers, neuro-oncologists, and immunologists are together devising ways to deliver drugs and immune cells past the BBB safely and more uniformly. The ultimate vision is a precision neuro-oncology approach in which BBB characteristics guide therapy selection and delivery method. Through such a BBB-centric framework, the field can convert the current barrier heterogeneity from a source of therapy resistance into a design criterion for personalized treatment. Harnessing both the biological insights and the biophysical tools at our disposal, we can begin to erode GBM's defenses, not by circumventing the blood–brain barrier, but by actively engaging and remolding it as part of the treatment strategy.

Author contributions

MA: Investigation, Writing – review and editing, Conceptualization, Visualization, Writing – original draft. MS: Methodology, Writing – review and editing, Writing – original draft, Investigation. JuL: Supervision, Writing – original draft, Investigation, Writing – review and editing, Project administration. GN: Validation, Writing – original draft, Writing – review and editing, Investigation. JH: Writing – review and editing, Methodology, Writing – original draft, Conceptualization. JW: Investigation, Writing – review and editing, Writing – original draft. AR: Investigation, Writing – original draft; Software;

Writing – review and editing. JS: Writing – original draft, Investigation, Writing – review and editing. JaL: Writing – review and editing, Resources, Software, Writing – original draft, Investigation. KC: Investigation, Writing – original draft, Writing – review and editing, Methodology. RV: Writing – review and editing; formal analysis; Writing – original draft; RM: Writing – original draft; Investigation; Writing – review and editing. MB: Writing – original draft, Investigation, Writing – review and editing, Methodology. ML: Supervision, Writing – review and editing, Writing – original draft, Conceptualization.

Funding

The authors declare that no financial support was received for the research and/or publication of this article.

Conflict of interest

ML: Funding from Arbor Pharmaceuticals, Accuracy, BMS, Novartis; Consultant: BMS, Merck, SQZ Biotechnologies, Tocagen, VBI; Patents: Combining Focused Radiation and Immunotherapy, Combining Local Chemotherapy and Immunotherapy; Shareholder: Egret Therapeutics. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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