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Device-assisted strategies for drug delivery across the blood-brain barrier to treat glioblastoma

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The blood-brain barrier, essential for protecting the central nervous system, also restricts drug delivery to this region. Thus, delivering drugs across the blood-brain barrier is an active research area in immunology, oncology, and neurology; moreover, novel methods are urgently needed to expand therapeutic options for central nervous system pathologies. While previous strategies have focused on small molecules that modulate blood-brain barrier permeability or penetrate the barrier, there is an increased focus on biomedical devices—external or implanted—for improving drug delivery. Here, we review device-assisted drug delivery across the blood–brain barrier, emphasizing its application in glioblastoma, an aggressively malignant primary brain cancer in which the blood-brain barrier plays a central role. We examine the blood-brain barrier and its features in glioblastoma, emerging models for studying the blood-brain barrier, and device-assisted methods for crossing the blood-brain barrier. We conclude by presenting methods to monitor the blood-brain barrier and paradigms for combined cross-BBB drug delivery.

The blood-brain barrier (BBB) is essential for the protection and unimpaired function of the central nervous system (CNS). However, the barrier's limited permeability and highly active efflux transporters make drug delivery to the CNS challenging[1]. With the shift from small molecule drugs to larger biomolecular therapeutics such as monoclonal antibodies, the ability to cross the BBB has grown increasingly vital for effective drug delivery[2]. Invasive and non-invasive drug delivery techniques have been devised to overcome the impermeability of the BBB. Non-invasive techniques include intranasal delivery, codelivery with chemicals that affect influx and efflux transporters, nanoparticle-mediated delivery, transcytosis-mediated delivery, viral-assisted delivery, and cell-mediated delivery. Invasive techniques include direct injection, intrathecal administration, intracerebral grafting, deep brain stimulation, and most of the device-assisted delivery techniques described in this review[2].

The BBB plays a central role in glioblastoma (GBM), an aggressively malignant primary brain cancer. Disruption of the spatially heterogeneous permeability of the BBB is a hallmark of this disease. Due to the invasive nature of GBM, tumor cell migration into the perivascular space and increased angiogenesis disrupt the BBB's neurovascular unit, leading to a leaky BBB known as the blood-tumor barrier (BTB) which accelerates GBM's destruction of the CNS[3,4]. Paradoxically, the loss of BBB integrity does not necessarily lead to improved treatment outcomes. Although the BTB is often disrupted near the tumor core, intact BBB away from the tumor mass can protect infiltrative cancer cells resulting in cancer recurrence. This heterogeneity of the BTB and the inaccessibility of intercortical tumors in GBM represent a challenge for effective chemotherapeutic delivery[5].

Cellular and molecular strategies for modulating BBB permeability and penetrating the barrier have been reviewed previously[2]. Here, we focus on recently developed device-assisted strategies for drug delivery across the BBB. These strategies include laser interstitial thermal therapy, magnetic resonance-guided focused ultrasound, electrical fields and electroporation, convection-enhanced delivery, and sustained delivery using implanted wafers, hydrogels, and composite meshes. Methods used to monitor BBB permeability provide vital information for assessing the efficacy of device-assisted drug delivery across the BBB. Therefore, we will also discuss standard clinical and experimental BBB monitoring.

This review provides (1) an overview of BBB anatomy and function and the challenges the BBB poses for drug development and delivery with a discussion on emerging in vitro modeling techniques alongside traditional in vivo models of BBB; (2) a detailed description of device-assisted strategies for crossing this barrier; and (3) BBB monitoring techniques with a focus on

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clinical utility. These topics will be presented in the context of GBM but are also applicable to other brain diseases.

BBB anatomy and function

The BBB is a specialized network of cells that forms a physical barrier between the CNS and circulating blood, selectively permitting the transmission of essential biomolecules while preventing the passage of pathogens and toxins. The BBB maintains CNS homeostasis by regulating ion concentrations, fluid volume balance, pH, and immune cell activity[6]. Disruption of this protective and regulatory structure has been implicated in various diseases. Nonetheless, even in diseases that cause BBB disruption, this barrier can hinder the delivery of therapeutics to the CNS[7]. Here, we provide a brief overview of BBB structure and function; a more detailed description can be found in ref. 6.

The BBB was formerly thought to be comprised primarily of endothelial cells and its permeability is determined by the tight junctions (TJ) between these cells. The BBB is now known to be composed of a mixture of endothelial cells, astrocytes, and pericytes that together form the neurovascular unit (NVU) which forms a dynamic barrier and regulates the transit of molecules through this firewall of the body's central processing unit (Fig. 1)[8]. In the NVU, endothelial cells are primarily responsible for the barrier functionality, with TJs between adjacent cells forming capillary walls in the CNS[9]. TJ proteins such as occludins and claudins play a key role in the impermeability of the BBB with claudins, especially claudin-5, composing the backbone of the BBB, and occludins regulating TJ function and BBB permeability[10,11]. Due to TJ and adhesive junction proteins' strict regulation of the paracellular paths between endothelial cells, larger and polar molecules cannot pass through the BBB; hence, restricting the passage of most drugs to the CNS[6]. Some of the smaller lipophilic drugs can diffuse through the BBB, while there are other small molecules that modulate the TJ proteins to penetrate the BBB[12,13]. For larger molecular and biomolecular drugs, though, other methods of transport across the BBB (e.g., receptor or carrier-mediated transport, or physical disruption of the BBB) are required[13]. Pericytes in the NVU regulate blood flow and maintain barrier integrity[6,14] while also acting as essential regulators of gene expression in adjacent endothelial cells and modulating vessel-associated astrocyte polarization[15]; and astrocyte projections (astrocytic end-feet) provide metabolic and structural support to endothelial cells[16]. Astrocytes also play a role in the localized innate immune system and their disruption has detrimental autoimmune and neurological consequences[8,16,17]. Microglia and neurons are not part of the NVU but indirectly affect BBB permeability. Microglia are part of the localized innate immune system and help maintain BBB integrity through clearing cellular debris; neurons impact the BBB by communicating with pericytes and astrocytes[1,18]. Overall, the NVU and its neighboring cells are crucial in maintaining the BBB's barrier functionality and CNS homeostasis.

The BBB maintains a narrow homeostatic window within the CNS to preserve normal brain activity and protect the brain from large fluctuations in ion and nutrient concentrations that occur elsewhere in the body. Ions and molecules with tightly regulated access to the CNS include potassium, amino acids, and plasma proteins[6]. This narrow homeostasis and tight regulation is accomplished by selective molecular crossing and active transport systems at the capillary's interior (luminal) surface over which blood flows. Nutrient influx is allowed via specialized transporters and carriers[6,19]. An example of a BBB influx transporter is the glucose transporter membrane-spanning glycoprotein (GLUT1) which is expressed by

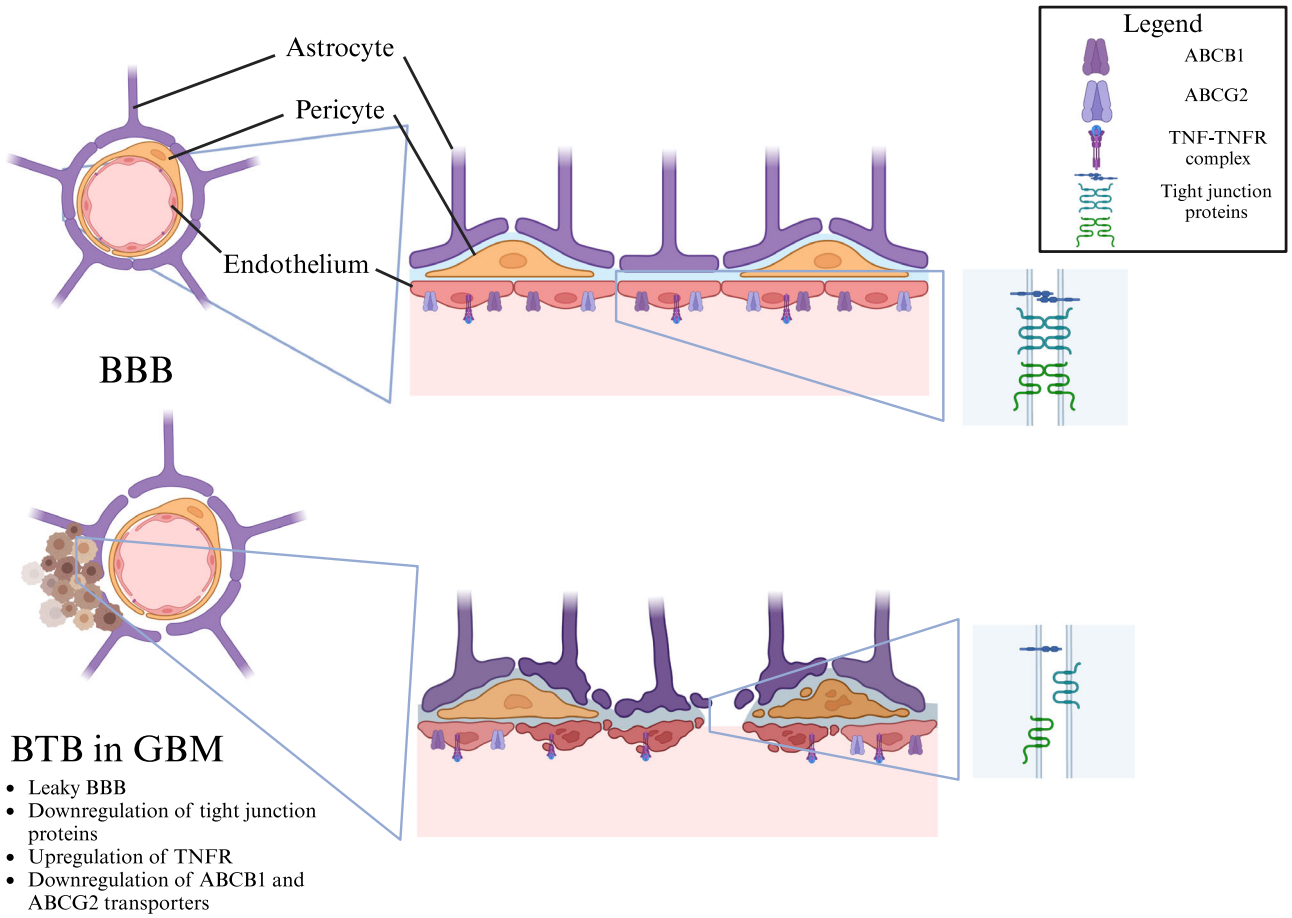

Fig. 1 | Comparison of the BBB and BTB. The BBB is composed of endothelial cells, astrocytes, and pericytes, with tight junctions forming between endothelial cells. The BTB in GBM is leakier than normal BBB in part due to lower expression of tight junction proteins and active efflux transporters (ABCB1 and ABCG2). BBB, blood-brain barrier; BTB, blood-tumor barrier; GBM, glioblastoma. Figure created in BioRender. Lab, B. (2024) https://BioRender.com/b97c299.

endothelial cells and facilitates glucose uptake into the CNS. In addition, numerous efflux transporters line the luminal surface of the BBB, moving molecules from the CNS into the blood. Many efflux transporters hydrolyze ATP to move molecules counter to the concentration gradient[6]. For example, ABCB1 (P-glycoprotein) is responsible for the efflux of neurotoxins and drugs[20]. Another example is ABCG2 (BCRP) which expels drugs and various endogenous metabolites such as steroids and folates[21]. Efflux transporters are important for rapid adjustments of the CNS microenvironment and waste removal; accordingly, they pose a challenge for drug delivery and dosing. Other transport mechanisms between the BBB cells are limited. For example, transcytosis activity is lower in BBB endothelial cells than in other endothelial cells, and immune cell infiltration into the CNS is limited by the downregulation of leukocyte adhesion molecules[22]. The numerous selection mechanisms employed by the BBB allow only a small number of specific molecules to cross the membrane resulting in concentration gradients between the capillary lumen and CNS, confounding systemic drug delivery to the CNS.

The BBB and Glioblastoma

The heterogeneity of the BBB is an important consideration when treating intracranial tumors such as GBM. The BBB exhibits large-scale regional heterogeneity and smaller-scale variations in its microvasculature network; capillaries form at higher densities in gray matter and the subfornical organ compared to white matter and the corpus callosum, venules possess looser BBB junctions than capillaries, and arterioles have lower ABCB1 transporter expression and thicker astrocytic perivascular sheaths[23,24]. The cells that form the NVU also vary regionally; astrocytes express higher levels of intermediate filament proteins in gray versus white matter which affects BBB permeability[25,26]. Cerebral microvascular endothelial cells (CECs) exhibit greater expression of transferrin receptors and barrier function proteins such as ABCB1 transporters in capillary CECs than in microvessels or other tributary subtypes[27,28]. CECs also exhibit regional heterogeneity in the structure of TJs and their astrocytic connectivity, although the expression level of TJ proteins appears to be consistent throughout the brain[29]. This cellular-anatomical heterogeneity leads to heterogeneous permeability and variable expression of therapeutically relevant inflammatory or drug-inhibitory proteins which is exacerbated in intracranial tumors.

BBB cell composition and gene expression vary depending on the molecular profile and location of GBM tumors[30]. For example, in the BTB, capillaries in the tumor periphery are modified, and transcellular transport is dysregulated heterogeneously. Modifications include a decrease in active efflux transporters (e.g., ABCB1 and ABCG2), adherens, and TJ proteins (Fig. 1)[31]. Clinical and preclinical drug distribution and imaging studies have revealed heterogeneity within a primary tumor and among the various invading and metastatic lesions within the same brain[31–36]. When Paclitaxel and Doxorubicin were injected into a mouse model of breast-to-brain metastases, the BTB displayed greater overall permeability than intact BBB, and there was considerable variability in compound distribution between and within the various metastases, with some lesions showing compound concentration differences as high as 200 fold[35]. A clinical study revealed a noteworthy variance in Paclitaxel concentration in CNS tissue, with the highest levels observed in the tumor periphery of metastatic lesions, followed by the tumor core of the primary tumor, and the lowest concentrations detected in tumor-free brain tissue[36]. The variations in intra- and intertumoral drug accumulation and permeability are attributed to structural changes in the BTB which manifest as the tumor progresses. These structural changes are induced by neuronal death and displacement of astrocytes[32–34]. Furthermore, cancer treatments such as radiation introduce variations to the BTB; radiation causes pericyte cell death, a decrease in TJs, and an increase in the ABCB1 efflux transporter expression[37]. All of the mentioned structural changes contribute to heterogeneity in permeability and perfusion, impacting drug distribution[32–34] which highlights the need for improved drug delivery methods to address these issues.

Modeling the BBB to Study Permeability

In vitro models

Despite in vivo models being the gold standard for studying BBB permeability and its therapeutic implications, in vitro models provide a more accessible, higher-throughput platform for BBB modeling and are becoming increasingly accurate in their recapitulation of in vivo 3D BBB properties (Fig. 2)[22,38–40]. With advances in stem cell technology and the growing emphasis on personalized medicine, in vitro platforms have become substantial in preclinical research[39,41–43]. Patient-derived and 3D in vitro tissue modeling systems have been developed with varying degrees of physiological relevance, utility, and cost[44,45]. However, due to the complexities in BBB anatomy and physiology, such as the adaptability of TJ characteristics and the various efflux transporters, developing an in vitro BBB model that represents the in vivo conditions of the BBB with high fidelity is challenging.

Conventional 2D in vitro BBB models typically utilize either patient-derived primary brain endothelial cells (BECs) or commercially available immortalized BEC cell lines[44–46]. BECs are conventionally cultured in a homogenous 2D monolayer, with contemporary cultures typically paired with parallel-plate fluid flow chambers to recreate the shear stress experienced by BECs in vivo[47–49]. 2D monolayers have been used to study BBB disruption and permeability, immune interactions and regulation, transport mechanisms, and secretome composition[22]. These 2D models are less physiologically relevant than in vivo models due to not accurately recapitulating the structures and mechanical stresses that occur in vivo, and the reduction of cell-cell contacts limits paracrine signaling and affects BEC proliferation and phenotype[39,50]. The convenience and lower cost of these models make them an attractive option for high-throughput permeability assays, but the results obtained from these models should be tested against more sophisticated in vitro and in vivo models.

To address the limitations of 2D models, 3D monolayer systems in which cells are cultured in multiple layers or plated in a 3D geometry, such as in the circular lumen of a collagen gel, were developed. These techniques yield increased cell-cell contact and better recreation of in vivo physiological geometry, but remain limited by poor simulation of in vivo mechanical stress[22,51,52]. In addition to these structural artifacts, the cell-type homogeneity in BEC monolayers does not accurately recreate the BBB and BTB microenvironment and molecular characteristics. A BEC-only culture lacks key transporters, immune processes, and metabolic regulators. To address this issue, co-cultures of astrocytes and pericytes (autologous, allogenic, and xenografts) cultured in proximity to BECs have been used to recapitulate the heterogeneous cell-cell signaling and differentiation processes that occur in vivo[53–55]. Although these co-cultures of multiple cells provide a better representation of the BBB than BEC monolayers, they do not present an accurate representation of the BTB and tumor microenvironment due to the lack of tumor. For example, the further increase in heterogeneity of the BTB due to progression of the disease and treatment cannot be studied accurately in these in vitro models.

Organoid models provide the most physiologically relevant in vitro models of the BBB and BTB[56–60]. An organoid is a micro- or mini-organ-like structure that can be derived from stem cells, among other methods[61]. One of the most researched and popular sources of organoid stem cells is induced pluripotent stem cells (iPSC). iPSC systems offer the dual benefit of being patient-specific and allowing low-risk, minimally-invasive sampling[41,62,63]. Patient fibroblasts from a skin punch biopsy can be used to create iPSC lines[48], which are then differentiated into BBB-related cell types, including neurons, microglia, endothelial cells, vascular smooth muscle cells, and vascular pericytes[62–64]. Various culture compositions and ratios are used depending on which aspect of the BBB is being studied; for example, endothelial cells and pericytes at a 1:1 to 5:1 ratio are required for TJ formation, an essential characteristic for permeability analysis[62,63]. Microglial processes such as immune cell recruitment and metabolic support have been recapitulated in co-cultures, enabling drug efficacy and immune regulation investigations[65,66]. iPSC-derived organoid systems have the potential to mimic the in vivo development of the BBB[62,63], allowing longitudinal investigation of developmental pathologies and identification of transient,

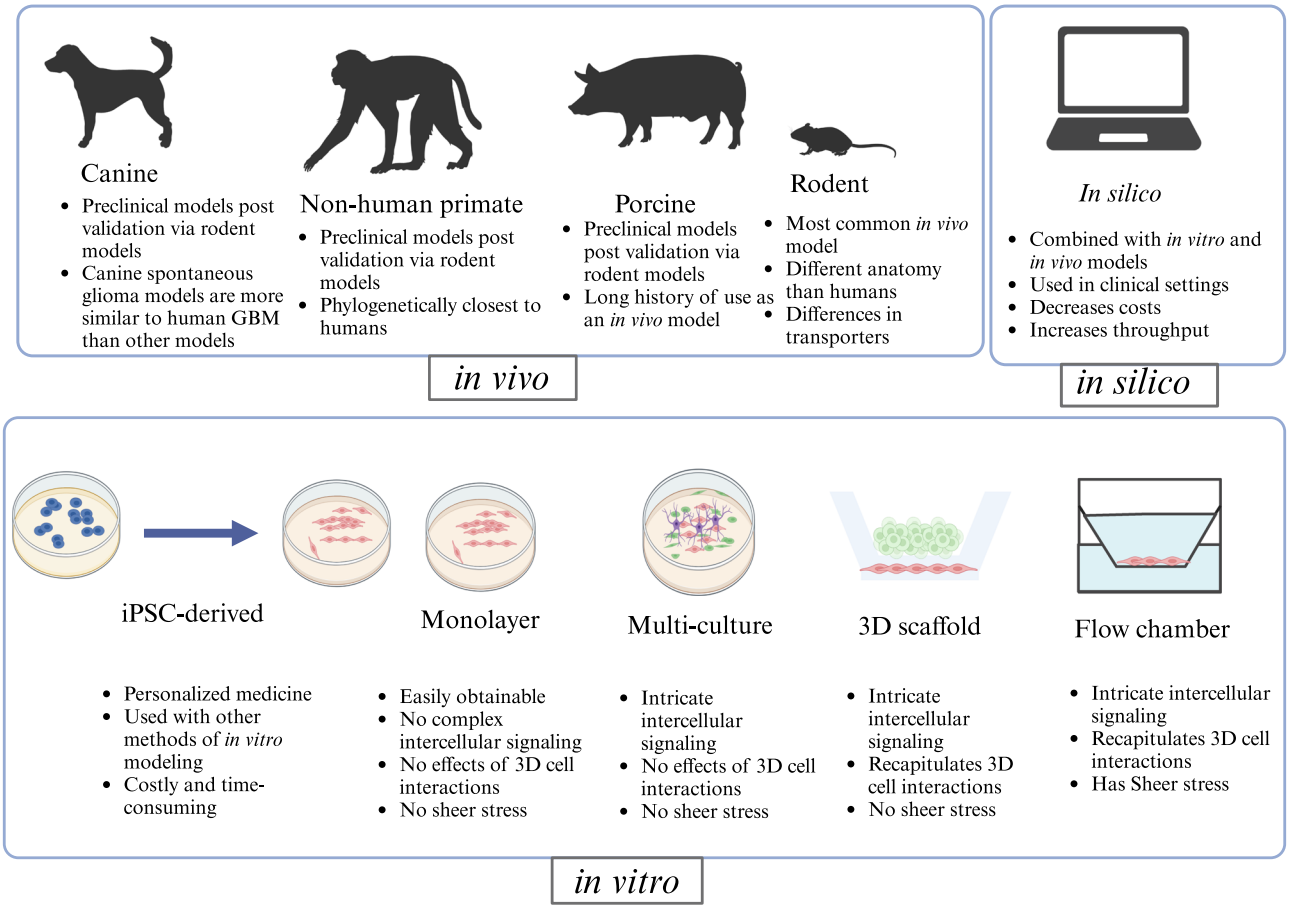

Fig. 2 | BBB and BTB Models. BBB and BTB can be modeled in vivo, in vitro, and in silico. Each model has limitations, and no model can completely recapitulate human conditions. in vitro models vary in their ability to replicate key aspects of the BBB, such as cell-cell interactions, 3D characteristics, and mechanical forces like sheer stress. Some of the greatest challenges of in vitro models include their inability to accurately mimic mechanical forces and the variations observed in the different areas of the BBB and BTB. The most used in vivo models are rodents, but rodents have significantly different BBB structures and functions than humans, including with the efflux transporters; hence, the application of other preclinical validation models such as canines, non-human primates, and porcine is necessary. Nevertheless, all in vivo models have the burden of ethical concerns, high cost, and maintenance, rendering them unsuitable for high-throughput assays. BBB, blood-brain barrier; BTB, blood-tumor barrier. Figure created in BioRender. Lab, B. (2024) https://BioRender.com/y94i838.

development-associated drug targets that may have been impossible with a BEC monolayer or more complex co-culture systems[62,67]. Further, the pluripotent nature of these cells allows a degree of self-arrangement and cell heterogeneity that was previously infeasible[41,62]. Although these iPSC-derived systems provide a patient-specific platform for drug discovery and disease investigation, there are multiple limitations in the model that need to be addressed before the iPSC-organoid model can significantly decrease the need for in vivo models. A few of these limitations include inaccurate modeling of sheer stress, the absence of key transporters, and the inability to replicate the BBB and BTB heterogeneity observed in different parts of the brain[62,68]. Moreover, more research on iPSC-derived multi-cell organoid models is needed since most of the research on iPSC models of the BBB has focused on single-cell models.

In silico techniques are frequently combined with in vitro methods to create a configurable, high-throughput, low-cost drug development platform (Fig. 2)[69–71]. New databases characterize the ability of synthetic molecules and biomolecules to cross and interact with the BBB and BTB[72–74]. Novel computational techniques identify and classify motifs in these molecules that facilitate elevated influx into the CNS[69,75]. Artificial intelligence (AI) technologies, such as convolutional neural networks, can be used to predict permeability and luminal transporter targeting[76,77]. Machine learning and large language models have also been applied to predict the permeability of compounds through the BBB[78]. These models can be utilized to identify therapeutics that are more likely to pass through the BBB, streamlining the selection of drug candidates for further in vitro and in vivo testing. Progress in computational modeling, together with improvement in the accuracy of in vitro recapitulation of the BBB and BTB, has the potential to reduce reliance on expensive in vivo models and accelerate the development of drug delivery strategies in GBM.

In vivo models

In vivo models have been used extensively to study GBM and assess the BBB's role in disease, identify drug candidates, and examine their ability to cross the BBB (Fig. 2)[79]. Animal models, usually rodents, are used in BBB-related pharmacokinetic and toxicity analysis and in developing new drug delivery vehicles (Fig. 2)[80,81]. Genetically modified animals (e.g., knockout models) are used to assess transporter functionality, develop efflux enzyme inhibitors, and elucidate metabolic, biochemical, pathophysiologic, and immune regulation within the CNS[82,83].

While these models benefit from a long history of use and characterization, it must be taken into consideration that minor differences between these in vivo models and humans can encumber the translation of in vivo research to clinical research. For example, ABCB1 efflux occurs more readily in rodents than in humans for a number of positron emission tomography (PET) radiotracers[84]. In a study comparing the efflux of 3 radiotracers ([^{11}C]verapamil, [^{11}C]vofopitant, and [^{18}F]altanserin) in rodents versus humans, significant differences between species were observed, with humans having a

higher concentration of all 3 radiotracers in their brain tissue[84]. This implies that a potential drug candidate could be dismissed based on its elevated expulsion rate from the brain in rodent models when, in reality, this observation may not necessarily hold true in clinical settings.

Investigating the constraints of each in vivo model can optimize the selection process for the most suitable model for a given study. Currently, although in vitro and in silico models have improved and are bound to receive further enhancement, in vivo models remain the gold standard. Nevertheless, given the expense, maintenance, and potential inaccuracies stemming from excessive reliance on in vivo models, it is evident that prioritizing the establishment of high-fidelity in silico and in vitro models is imperative.

Device-Assisted Disruption of the BBB

Many previous methods of drug delivery to the CNS focused on pharmaceuticals and exploiting the cellular and molecular biology of the BBB. These include prodrugs, transcytosis, nanoparticle systems, viruses, and cell-mediated drug delivery[2]. The categories associated with these techniques are not mutually exclusive; for example, some nanoparticles, viruses, prodrugs, and cells use transcytosis to enter the BBB. While prodrugs, transcytosis, nanoparticle systems, and viruses have ongoing clinical trials, cell-mediated drug delivery is in the preclinical stage[2,85–88]. With rapid advances in bioengineering, the application of device-based technology for overcoming the challenges presented by the BBB in CNS drug delivery is becoming increasingly promising. Current device-assisted methods for drug delivery to the CNS increase the BBB's permeability by either disrupting it or circumventing the BBB altogether. BBB disrupting techniques include magnetic resonance-guided focused ultrasound, laser interstitial thermal therapy, electrical fields, and electroporation, while BBB circumventing techniques include convection-enhanced delivery and sustained delivery. Below, we discuss the mentioned device-assisted delivery technologies. For more information on pharmaceutical non-invasive delivery systems, please refer to refs. 2 and 89.

Magnetic resonance-guided focused ultrasound (MRgFUS) utilizes ultrasound to transiently increase BBB permeability in targeted areas of the CNS which enables drug delivery across the BBB (Fig. 3)[90]. Magnetic resonance imaging (MRI) is used to navigate the application of ultrasonic energy and provide real-time feedback with submillimeter accuracy[91,92]. The application of ultrasound for GBM treatment initially focused on high-intensity ultrasound[90] which can increase BBB permeability and create necrosis-inducing thermal effects that eradicate tumor cells. However, the thermal effects of high-intensity ultrasound damage the CNS[90,93]. Low-intensity ultrasound has minimal thermal effects and produces mainly mechanical effects (e.g., cavitation, acoustic microstreaming, and acoustic radiation forces). These mechanical effects can increase BBB permeability reversibly and with fewer side effects than high-intensity ultrasound[90,94,95]. MRgFUS with low-intensity ultrasound has been investigated in preclinical in vivo models and clinical research[96–98]. Mainprize et al., introduced the first clinical trial to quantitatively assess the use of MRgFUS for site-specific trans-BBB delivery of intravenously infused Doxorubicin and Temozolomide in brain tumor patients[99]. A 7.7-fold rise in drug levels in targeted versus non-target regions was observed; MRgFUS was well tolerated and without adverse effects. Also, the duration of increased BBB permeability did not exceed 24 hours[99]. This study demonstrated the utility of MRgFUS for CNS drug delivery, paving the way for further clinical trials. Table 1 summarizes this clinical trial along with all other current clinical trials involving device-assisted methods to permeabilize or circumvent the BBB in GBM patients.

Although MRgFUS's mechanical effects are safer than thermal effects, excessive mechanical force can lead to capillary defects and erythrocyte extravasation[100]. Intravenous injection of microbubbles can be utilized to fine-tune the cavitation effect of MRgFUS[90]. Microbubbles are gas-filled polymeric microspheres, 1–5 μm in diameter, that display periodic expansion and contraction when located in an ultrasonic field. This repetitive oscillation induces the flow of liquids surrounding the microbubbles, generating shear forces that cause transient permeability of endothelial cell membranes[101]. Using microbubbles can lessen the acoustic energy required to produce a BBB-disrupting effect, reducing damage to healthy neighboring CNS tissue[100,102,103]. Microbubbles can also be loaded with therapeutics (e.g., chemotherapeutics, recombinant proteins, genetic material, or whole cells) and/or imaging agents[91,92,104–107] which are released into the targeted area upon applying ultrasound. Thus, MRgFUS with therapeutic-loaded microbubbles allows targeted drug delivery to the CNS. One study showed that by identifying the best ultrasonic parameters for safe cavitation in vitro, replication of the same parameters in rodents injected with Carmustine bubbles did not show erythrocyte extravasation, demonstrating the safety of therapeutic-loaded microbubbles[100]. More recently, smaller nanodroplets have been developed for use with MRgFUS, which might further increase drug delivery efficiency and safety, due to their smaller size[102,108–110]. However, more research is needed to better understand their mechanism of action which will help identify safe and efficient ultrasonic settings. Encapsulation of therapeutics in both microbubbles and nanodroplets can increase treatment efficacy by increasing the circulation half-life of the encapsulated compound, increasing the drug dose at the delivery site, and reducing systemic side effects. Most studies using therapeutic-loaded microbubbles or nanodroplets with MRgFUS are in the preclinical stage[90]. In one such study, GBM tumors in mice were targeted by packaging HIF-1α inhibitors into $Cu_{2-x}Se$ nanoparticles coated with the tumor cell membrane and using MRgFUS to increase the permeability of BBB and achieve targeted drug release[108]. This treatment alone led to a slight decrease in GBM tumor size. When combined with oral disulfiram (an alcohol abuse medication under evaluation as a potential anti-cancer agent), this regimen resulted in a significant reduction in tumor size. The strong anti-cancer effects of this combination treatment are perhaps due to significantly reduced hypoxia in the tumor microenvironment as well as anti-proliferation and anti-angiogenesis effects[108].

Another aspect of MRgFUS to consider in clinical application is the ultrasound device. The particular ultrasound instrument used plays a crucial role in defining the accessibility, cost, and efficacy of MRgFUS treatment[102]. Ultrasound transducers are categorized as therapeutic versus diagnostic and extracranial versus implanted. Therapeutic ultrasound devices have a greater focus than diagnostic ultrasound devices, making them better suited for most MRgFUS applications. Extracranial devices are non-invasive and are preferable for treating small target areas deep in the brain; however, they are not user-friendly, and, when compared to implantable devices the BBB opening procedure can be time-consuming. On the other hand, implantable ultrasonic devices require invasive surgery and a short treatment duration, making them a better option for tumors that require multiple rounds of opening of the BBB. Implantable ultrasonic devices are also better suited for large, superficial target areas in the brain[90]. Moreover, even in patients for whom extracranial devices might be the optimal option, the application of implantable devices, after treatment with the extracranial device, might decrease the chances of recurrence; due to the larger scope of BBB opening caused by implantable devices, there is a higher probability of targeting cancer cells that have migrated out of the tumor margin. However, more research on the rate of recurrence post-treatment with implanted MRgFUS is needed to validate this hypothesis. The first clinical study of MRgFUS using an implanted ultrasonic device (SonoCloud) combined with microbubbles demonstrated the safety and increased sensitivity of patients to Carboplatin, with patient survival increasing significantly, from 6-9 months in historical controls to 13 months in treated patients[111]. The BBB remained permeable for a minimum of 30 minutes[111]. Results from ongoing randomized phase II clinical trials (Table 1) can further shed light on the safety of implanted MRgFUS devices.

Since MRgFUS technology is not yet widely used, safety remains a concern. Microhemorrhages have been observed in preclinical MRgFUS studies, and there is evidence that MRgFUS can induce sterile inflammation, possibly dependent on microbubble size[112–114]. Nonetheless, the few clinical MRgFUS trials have shown promise for the feasibility and safety of these

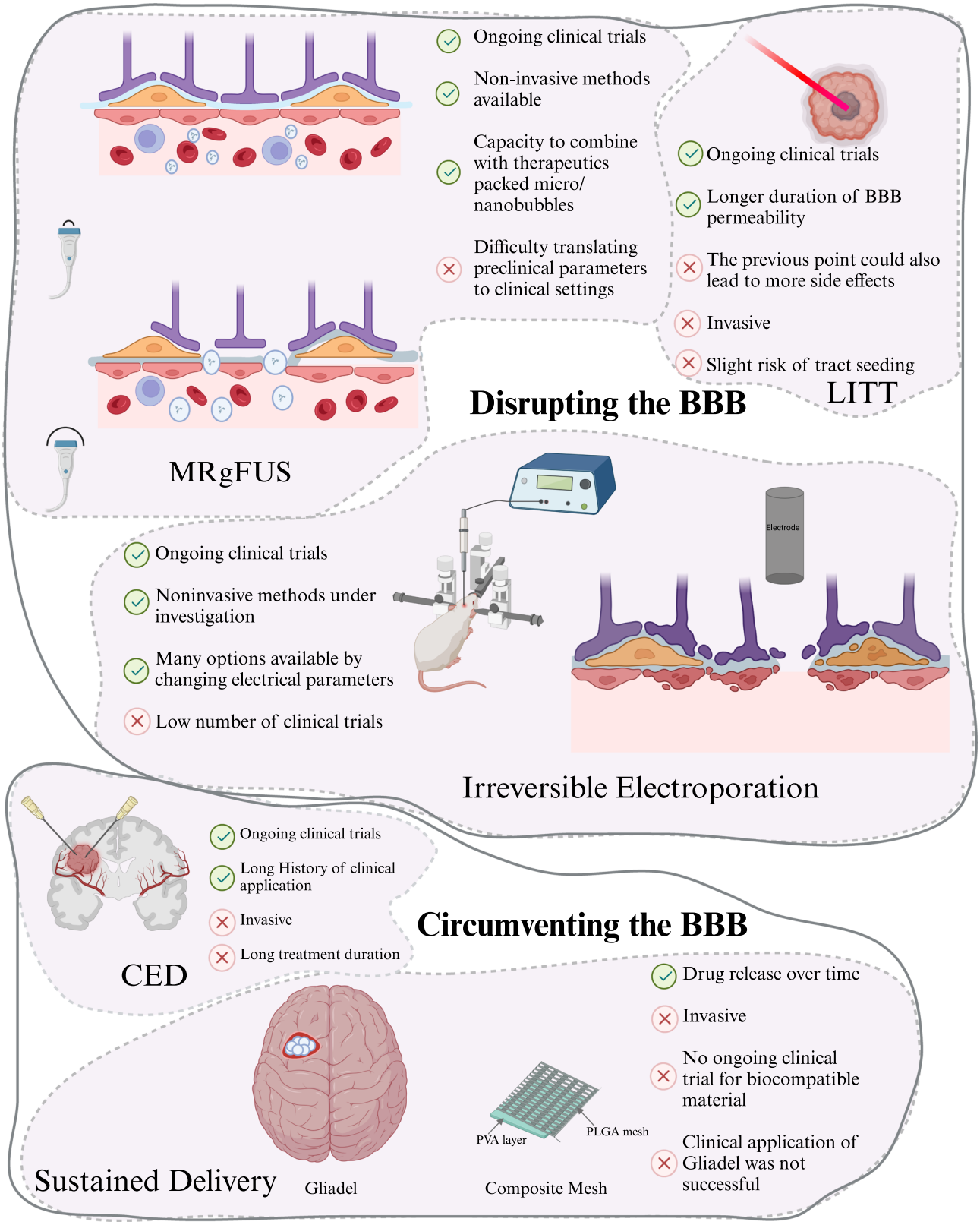

Fig. 3 | Device-assisted drug delivery methods applicable to GBM. Promising device-assisted methods to deliver drugs across the BBB include MRgFUS, LITT, electroporation, CED, and sustained delivery. These methods either disrupt or circumvent the BBB. Device-assisted methods that disrupt the BBB include MRgFUS, LITT, electroporation, and electrical fields. Methods that circumvent the BBB include CED and sustained delivery methods (e.g., Gliadel wafers and composite meshes). Each of these technologies presents opportunities and challenges for translation to clinical application. GBM, glioblastoma; BBB, blood-brain barrier; MRgFUS, magnetic resonance-guided focused ultrasound; LITT, laser interstitial thermal therapy; CED, convection-enhanced delivery; PVA, polyvinyl alcohol; PLGA, poly(lactic-*co*-glycolic acid). Figure created in BioRender. Lab, B. (2024) https://BioRender.com/a63u637.

Table 1 | Current clinical trials applying device-assisted methods to permeabilize or circumvent the BBB in GBM patients

Identifier	Trial Title	Sponsor	Clinical Phase	BBB Disruption Method	Drug Intervention	Recruitment Status	Publications from Trial
NCT02343991	Blood-Brain Barrier Disruption Using Transcranial MRI-Guided Focused Ultrasound	InSightec	Not Applicable	MRgFUS	Doxorubicin	Unknown	99
NCT02253212	Safety of BBB Opening With the SonoCloud	Assistance Publique - Hôpitaux de Paris	I/II	MRgFUS	Carboplatin	Completed	111,218,219
NCT03744026	Safety and Efficacy of Transient Opening of the Blood-brain Barrier (BBB) With the SonoCloud-9 (SC9-GBM-01)	CarThera	I/II	MRgFUS	Carboplatin	Completed	220
NCT04614493	Innovative SonoCloud-9 Device for Blood Brain Barrier Opening in First Line Temozolomide Glioblastoma Patients. (SonoFIRST)	Assistance Publique – Hôpitaux de Paris	II	MRgFUS	Temozolomide	Unknown	Not published yet
NCT04528680	Ultrasound-based Blood-brain Barrier Opening and Albumin-bound Paclitaxel and Carboplatin for Recurrent Glioblastoma (SC9/ABX)	Northwestern University	I/II	MRgFUS	Albumin-bound paclitaxel	Recruiting	221–224
NCT03616860	Assessment of Safety and Feasibility of ExAblate Blood-Brain Barrier (BBB) Disruption for Treatment of Glioma	InSightec	Not Applicable	MRgFUS	Temozolomide	Completed	116
NCT03551249	Assessment of Safety and Feasibility of ExAblate Blood-Brain Barrier (BBB) Disruption	InSightec	Not Applicable	MRgFUS	Temozolomide	Completed	Not published yet
NCT04998864	Assessment of Safety and Feasibility of ExAblate Blood-Brain Barrier (BBB) Disruption in GBM Patients	InSightec	Observational	MRgFUS	Temozolomide	Unknown	Not published yet
NCT04988750	Evaluate the Safety and Preliminary Efficacy of the Combination of NaviFUS System With Re-irradiation for rGBM Patients	NaviFUS Corporation	Not Applicable	MRgFUS	Not Applicable	Recruiting	225
NCT04440358	Exablate Blood-Brain Barrier Disruption With Carboplatin for the Treatment of rGBM	InSightec	I/II	MRgFUS	Carboplatin	Active, not recruiting	Not published yet
NCT04417088	Exablate Blood-Brain Barrier Disruption for the Treatment of rGBM in Subjects Undergoing Carboplatin Monotherapy	InSightec	I/II	MRgFUS	Carboplatin	Active, not recruiting	Not published yet
NCT03712293	ExAblate Blood-Brain Barrier Disruption for Glioblastoma in Patients Undergoing Standard Chemotherapy	InSightec	Not Applicable	MRgFUS	Temozolomide	Completed	115,226
NCT04446416	Efficacy and Safety of NaviFUS System add-on Bevacizumab (BEV) in Recurrent GBM Patients	NaviFUS Corporation	Not Applicable	MRgFUS	Bevacizumab	Completed	Not published yet
NCT03626896	Safety of BBB Disruption Using NaviFUS System in Recurrent Glioblastoma Multiforme (GBM) Patients	NaviFUS Corporation	Not Applicable	MRgFUS	Not Applicable	Completed	Not published yet
NCT05370508	Study of Sonodynamic Therapy Using SONALA-001 and Exablate 4000 Type 2 in Subjects With Progressive or Recurrent GBM	SonALAsense, Inc.	II	MRgFUS	Not Applicable	Terminated due to funding challenges and not due to safety concerns	227
NCT05383872	Blood-Brain Barrier Disruption (BBBD) for Liquid Biopsy in Subjects With GlioBlastoma Brain Tumors	InSightec	Not Applicable	MRgFUS	Not Applicable	Recruiting	228
NCT03322813	ExAblate Blood Brain Barrier Disruption (BBBD) for Planned Surgery in Suspected Infiltrating Glioma	InSightec	Not Applicable	MRgFUS	Not Applicable	Suspended; “study temporarily suspended until priorities are reassessed”	229

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Table 1 (continued) | Current clinical trials applying device-assisted methods to permeabilize or circumvent the BBB in GBM patients

Identifier	Trial Title	Sponsor	Clinical Phase	BBB Disruption Method	Drug Intervention	Recruitment Status	Publications from Trial
NCT05879120	Randomized Study of Neo-adjuvant and Adjuvant Pembrolizumab With and Without Targeted Blood Brain Barrier Opening Using Exablate MRI-guided Focused Ultrasound (Exablate MRgFUS) for Recurrent Glioblastoma	M.D. Anderson Cancer Center	II	MRgFUS	Pembrolizumab	Withdrawn due to "PI Request"	Not published yet
NCT01851733	MRI-Guided Laser Surgery and Doxorubicin Hydrochloride in Treating Patients With Recurrent Glioblastoma Multiforme	Washington University School of Medicine	I	LITT	Doxorubicin	Completed	119,125
NCT03022578	Laser Interstitial Thermal Therapy and Lomustine in Treating Patients With Recurrent Glioblastoma or Anaplastic Astrocytoma	M.D. Anderson Cancer Center	II	LITT	Lomustine	Termination, due to "slow accrual"	No publication
NCT03341806	Avelumab With Laser Interstitial Therapy for Recurrent Glioblastoma	Icahn School of Medicine at Mount Sinai	I	LITT	Avelumab	Completed	230
NCT05318612	Effectiveness of MR-guided LITT Therapy in Irresectable Glioblastoma (EMITT)	Radboud University Medical Center	III	LITT	Chemotherapy not specified	Active, not recruiting	231
NCT02372409	Using MRI-Guided Laser Heat Ablation to Induce Disruption of the Peritumoral Blood Brain Barrier to Enhance Delivery and Efficacy of Treatment of Pediatric Brain Tumors	Washington University School of Medicine	II	LITT	Doxorubicin, Etoposide	Terminated due to "Low accruals and competing clinical trials"	Not published yet
NCT02311582	MK-3475 in Combination With MRI-guided Laser Ablation in Recurrent Malignant Gliomas	Washington University School of Medicine	I/II	LITT	Pembrolizumab	Completed	232
NCT05663125	LITT Combined With Early Use of Temozolomide for Recurrent Glioblastomas	Beijing Tiantan Hospital	I/II	LITT	Temozolomide	Active, not recruiting	Not published yet
NCT03176160	LITT Palliative Treatment for Patients With Malignant Gliomas	Duke University	Not Applicable	LITT	Temozolomide	Withdrawn, due to "failure to enroll"	Not published yet
NCT02880410	Feasibility Study on LITT for Newly Diagnosed Glioblastoma (FLAG)	Monteris Medical	I	LITT	Temozolomide	Terminated due to "failure to enroll"	Not published yet
NCT03277638	Laser Interstitial Thermotherapy (LITT) Combined With Checkpoint Inhibitor for Recurrent GBM (RGBM)	Case Comprehensive Cancer Center	I/II	LITT	Pembrolizumab	Recruiting	Not published yet
NCT03491683	INO-5401 and INO-9012 Delivered by Electroporation (EP) in Combination With Cemiplimab (REGN2810) in Newly-Diagnosed Glioblastoma (GBM)	Inovio Pharmaceuticals	I/II	Electroporation	INO-5401, INO-9012, Cemiplimab, Temozolomide	Active, not recruiting	233
NCT02283944	TMS Electrochemotherapy for Glioblastoma Multiforme	University of Aarhus	II	Electroporation	Chemotherapy not specified	Withdrawn, due to "device for multi-coil TMS no longer supported"	No publication
NCT05743595	Neoantigen-based Personalized DNA Vaccine With Retifanlimab PD-1 Blockade Therapy in Patients With Newly Diagnosed, Unmethylated Glioblastoma	Washington University School of Medicine	I	Electroporation	Personalized Neoantigen DNA vaccine, Retifanlimab	Recruiting	No publication
NCT01317212	Dose-Escalation Study of Carboplatin Administration Into the Brain for Glioblastoma Multiforme	North Bristol NHS Trust	I	CED	Carboplatin	Withdrawn, due to no funding for study	Not published
NCT02858895	Convection-Enhanced Delivery (CED) of MDNA55 in Adults With Recurrent or Progressive Glioblastoma	Medicenna Therapeutics, Inc.	II	CED	MDNA55	Completed	234
NCT00076986	The PRECISE Trial: Study of IL13-PE38QQR Compared to GLIADEL Wafer in Patients With Recurrent Glioblastoma Multiforme	INSYS Therapeutics Inc	III	CED	IL13-PE38QQR	Completed	153

Table 1 (continued) | Current clinical trials applying device-assisted methods to permeabilize or circumvent the BBB in GBM patients

Identifier	Trial Title	Sponsor	Clinical Phase	BBB Disruption Method	Drug Intervention	Recruitment Status	Publications from Trial
NCT02869243	A Dose Escalation Phase I Study Of Human-Recombinant Bone Morphogenetic Protein 4 Administrated Via CED In GBM Patients	Stemgen	I	CED	hrBMP4	Completed	148
NCT01582516	Safety Study of Replication-competent Adenovirus (Delta-24-rgd) in Patients With Recurrent Glioblastoma	Erasmus Medical Center	I/II	CED	Delta-24-RGD adenovirus	Completed	Not published yet
NCT00797940	Convection Enhanced Localized Administration of PRX321 With Real-time Imaging for Therapy of Recurrent Glioblastoma (CLARITY-1) (CLARITY-1)	Sophiris Bio Corp	II	CED	IL-4PE	Withdrawn, due to "no funding for study"	No publication
NCT05324501	A Study of Intra-tumoral Administered MTX110 in Patients With Recurrent Glioblastoma (MAGIC-G1)	Midatech Ltd	I	CED	MTX110	Active, not recruiting	Not published yet
NCT04608812	Convection-enhanced Delivery of OS2966 for Patients With High-grade Glioma Undergoing a Surgical Resection	OncoSynergy, Inc	I	CED	OS2966	Terminated due to "Slow enrollment and financial constraints"	Not published yet
NCT00089427	IL13-PE38QQR Infusion After Tumor Resection, Followed by Radiation Therapy With or Without Temozolomide in Patients With Newly Diagnosed Malignant Glioma	INSYS Therapeutics Inc	I	CED	IL13-PE38QQR	Completed	Not published yet
NCT04599647	EAP for the Treatment of Glioblastoma With PVSRIPO	Istari Oncology, Inc	Expanded Access	CED	PVSRIPO	No longer available	No publication
NCT00104091	Safety and Efficacy Study to Treat Recurrent Grade 4 Malignant Brain Tumors	Teva Branded Pharmaceutical Products R&D, Inc.	II	CED	TP-38	Completed	Not published yet
NCT01491893	PVSRIPO for Recurrent Glioblastoma (GBM) (PVSRIPO)	Istari Oncology, Inc.	I	CED	PVSRIPO	Completed	235
NCT00761280	Efficacy and Safety of AP 12009 in Patients With Recurrent or Refractory Anaplastic Astrocytoma or Secondary Glioblastoma (SAPPHIRE)	Isarna Therapeutics GmbH	III	CED	AP 12009	Terminated, due to "inability to recruit the projected patient number"	No publication
NCT00431561	Phase IIb Clinical Trial With TGF-β2 Antisense Compound AP 12009 for Recurrent or Refractory High-grade Glioma	Isarna Therapeutics GmbH	II	CED	AP 12009	Completed	236
NCT03043391	Phase 1b Study PVSRIPO for Recurrent Malignant Glioma in Children	Istari Oncology, Inc.	I	CED	PVSRIPO	Completed	237
NCT01082926	Phase I Study of Cellular Immunotherapy for Recurrent/Refractory Malignant Glioma Using Intratumoral Infusions of GRm13Z40-2, An Allogeneic CD8+ Cytolitic T-Cell Line Genetically Modified to Express the IL 13-Zetakine and HyTK and to be Resistant to Glucocorticoids, in Combination With Interleukin-2	City of Hope Medical Center	I	CED	ALLOLYMPH, Aldesleukin	Completed	Not published yet
NCT00024570	Interstitial Infusion of IL13-PE38QQR Cytotoxin in Recurrent Malignant Glioma	INSYS Therapeutics Inc	I/II	CED	IL13-PE38QQR	Completed	Not published yet
NCT00024557	Histologic Effect/Safety of Pre/Post-Operative IL13-PE38QQR in Recurrent Resectable Supratentorial Malignant Glioma Patients	INSYS Therapeutics Inc	I	CED	IL13-PE38QQR	Completed	Not published yet
NCT04160494	D2C7-IT With Atezolizumab for Recurrent Gliomas	Darell Bigner	I	CED	D2C7-IT, Atezolizumab	Active, not recruiting	Not published yet
NCT04547777	Phase 1 Trial of D2C7-IT in Combination With 2141-V11 for Recurrent Malignant Glioma	Darell Bigner	I	CED	D2C7-IT, 2141-V11	Recruiting	238

Table 1 (continued) | Current clinical trials applying device-assisted methods to permeabilize or circumvent the BBB in GBM patients

Identifier	Trial Title	Sponsor	Clinical Phase	BBB Disruption Method	Drug Intervention	Recruitment Status	Publications from Trial
NCT01906385	Maximum Tolerated Dose, Safety, and Efficacy of Rhenium Nanoliposomes in Recurrent Glioma (ReSPECT)	Plus Therapeutics	I/II	CED	Rhenium-186 NanoLiposome	Recruiting	239
NCT05734560	D2C7-IT and 2141-V11 in Newly Diagnosed GBM Patients	Darell Bigner	I/II	CED	D2C7-IT, 2141-V11	Recruiting	Not published yet
NCT06177964	Lerapolturev (PVSRIPO) in GBM	Darell Bigner	II	CED	Lerapolturev	Recruiting	Not published yet
NCT04479241	LUMINOS-101: Lerapolturev (PVSRIPO) and Pembrolizumab in Patients With Recurrent Glioblastoma	Istari Oncology, Inc.	II	CED	Lerapolturev	Completed	Not published yet

BBB blood-brain barrier, *BTB* blood-tumor barrier, *MRgFUS* magnetic resonance-guided focused ultrasound, *LITT* laser interstitial thermal therapy, *CED* convection-enhanced delivery.

methods in the context of intracranial tumors, even when performed repeatedly on the same patient[115]. One such clinical trial demonstrated the feasibility of repetitive BBB opening, indicating the potential utility of MRgFUS for liquid biopsy applications, as disrupting the BBB via MRgFUS increased the concentration of brain-derived proteins, extracellular vesicles, and cell-free DNA—potentially useful sources of disease biomarkers for liquid biopsies—in the peripheral blood[116]. MRgFUS is thus promising, and the multiple ongoing clinical trials involving MRgFUS (Table 1) will help verify its safety and efficacy for enhanced drug delivery and possibly liquid biopsy in GBM patients.

Laser interstitial thermal therapy (LITT) is an ablative therapy in which a target site is identified via intraoperative MRI, an optical fiber is introduced stereotactically to the target site, and laser light is delivered to thermally ablate the target tissues (typically >40 °C as monitored by MRI thermometry, for over 2 min) (Fig. 3)[117]. LITT has attracted attention because it requires minimal surgery (a ~ 3.2-mm laser probe is inserted through a cranial burr hole) and has a rapid recovery time. Moreover, LITT is potentially helpful for deep-seated or difficult-to-access lesions and incurs minimal damage to off-target healthy tissue[118–120]. The US Food and Drug Administration has approved two LITT devices: the Medtronic Visualase system was approved in 2007 and the Monteris NeuroBlate system in 2008[119,121].

In addition to tumor ablation, LITT can be used for local BBB disruption to improve chemotherapeutic targeting[122–124]. In the first clinical study on BBB disruption using LITT, the permeability of the BBB increased after hyperthermia, with higher permeability peaking a few days to a few weeks after the procedure[119]. The increased permeability persisted for 4 to 6 weeks as measured by dynamic contrast-enhanced MRI and by tracking serum levels of a brain-specific factor (Table 1)[119]. Based on MRI observations, LITT increased BBB permeability from the center of ablation to a 1-2 cm radius beyond the viable tumor rim. Patients did not manifest any LITT-related side effects[119]. Subsequent research with a larger cohort showed that administration of Doxorubicin, with one group receiving Doxorubicin immediately after LITT and another 7-9 weeks after LITT, increased survival in both groups of GBM patients compared to historical controls receiving Bevacizumab[125]. The procedure and drug administration were well tolerated (Table 1)[125]. In other studies, the BBB has been reported to remain permeable for as long as 6 months after LITT[126]. The variability in the duration of observed permeability may be due to differences in the molecular size of the therapeutics used and in the method used to measure permeability[119]. The prolonged permeability following LITT allows extra-neural inflammatory factors to leak into the brain possibly resulting in side effects[127], but longer permeability is useful for administering drugs that require slower or continuous delivery for efficient treatment and to mitigate drug toxicity.

Despite the abundance of clinical data on the tumor-ablating effects of LITT, the biological mechanisms underlying the increased permeability of BBB caused by LITT are mostly unknown. In a preclinical study, LITT increased BBB permeability (proteins as large as 150 kDa entered the brain parenchyma) by reducing TJ integrity and increasing transcytosis in BBB endothelial cells[124]. Understanding the mechanisms underlying LITT-induced BBB permeability is crucial for developing LITT-assisted BBB-opening procedures, which necessitates more studies on this topic.

LITT is becoming increasingly popular in neurosurgery; however, this procedure may be impeded by ventricles or large arteries in the path from scalp to lesion, and adverse effects are common. LITT can cause neurological deficits, cerebral edema requiring postoperative steroid use, seizures, and intracranial hemorrhage possibly from the introduction of the fiber cable[118]. The danger of side effects increases with the number of ablations required. Large or diffuse tumors requiring multiple rounds of ablation are not suggested for LITT[118,128]. Cancer recurrence from tract seeding of cancer cells during LITT surgery is also a concern. A retrospective clinical study indicated a slight risk of tract seeding associated with LITT (5.4% of the cohort)[129]. The risk of tract seeding during LITT was associated with ablation protocol (superficial to deep and vice versa) and surgeons' level of

experience. LITT ablation for all patients with tract seeding occurrence followed the superficial to deep ablation protocol, but tract seeding did not occur in all patients undergoing superficial to deep ablation. Moreover, LITT procedures were accompanied by tissue biopsy, which raises the question of whether the observed tract seeding resulted from the biopsy and not from LITT[129].

Despite these side effects, LITT is gaining popularity as a substitute for resection surgery, particularly in cases for which surgery is high-risk, as LITT is primarily employed as an ablative technique. LITT does not entail a significantly higher incidence of adverse events than resection surgery[130]; therefore, taking advantage of the post-LITT permeability by combining it with chemo- and immuno-therapeutics is promising. To realize the potential of LITT treatment, suitable candidate drugs for post-LITT treatment should be identified, and additional research on the risk of tract seeding is needed. Also, future LITT clinical trials should assess drug penetration into tumor tissue.

Electrical fields and electroporation can be used to precisely target increases in BBB permeability; electroporation can also be used for tumor ablation. The tumor-ablative effects of electroporation are non-thermal and involve high-voltage, short-duration electrical pulses that disrupt cell membranes[131]. Depending on the voltage level, the effect can be reversible or irreversible (Fig. 3)[132]. For in vivo applications, electrodes are introduced stereotactically to provide a localized electrical current that can be modulated or guided to limit the impact on surrounding blood vessels and tissues. The applied voltage corresponds to the affected volume as well as the amount of BBB disruption and has been shown to persist for several days post-treatment in rats[133,134]. Preclinical studies using canine glioma models have shown safe tumor ablation and increased peritumoral BBB permeability using non-thermal irreversible electroporation including high-frequency modalities[131,135,136]. Electroporation-mediated drug delivery across the BBB is the focus of very few current clinical trials (Table 1), and to the best of our knowledge, there are no previous clinical studies of this method.

Adverse reactions such as muscle tetany and cardiac asynchrony were frequently observed initially when applying electroporation. To circumvent these adverse reactions, high-frequency methods using ultra-short bipolar pulses have been developed to remove the need for paralytics and to allow the use of sub-ablative voltages. In a rodent model, high-frequency irreversible electroporation alone or combined with liposomal Doxorubicin was shown to elicit transient BBB disruption for up to 72 hours and to increase survival[137]. The group receiving high-frequency electroporation without drugs exhibited significant infiltration of immune cells into the tumor microenvironment, which could explain the increased survival of this group. Interestingly, groups treated with only liposomal Doxorubicin had worse survival than the sham control group, likely due to systemic drug toxicity, illustrating the need for therapeutics that specifically target the tumor cells[138].

Electrical fields utilized in electroporation cause various cellular and physiological phenomena[139]. The increase in BBB permeability induced by electric fields that cause electroporation is thought to result from a reduction in TJ integrity due to cytoskeletal remodeling in endothelial cells rather than pore formation in the cellular membrane[138,140]. Consistent with this idea, pulsed electrical fields applied using non-invasive electrodes adhered to the skull have been shown to increase BBB permeability without causing electroporation[141,142]. Similarly, BBB disruption has been shown to occur in regions receiving electrical field intensities less than what is required for electroporation[139], suggesting that BBB disruption by electric fields can be independent of electroporation. Electroporation and electrical fields have received less clinical attention as a strategy to disrupt the BBB than MRgFUS or LITT, but the use of non-invasive scalp-mounted electrodes in humans is a promising approach that warrants further research. To this end, more studies on the mechanisms underlying BBB opening resulting from electrical fields should be conducted, and more information regarding the precision of the BBB opening due to electroporation and electrical fields is needed; to this end, the path and distribution of the electrical fields within the CNS should be further investigated since this can offer insight into the range and precision of BBB opening.

Convection-enhanced delivery (CED) involves inserting one or more catheters intracranially and infusing chemotherapeutics using a pump to generate a positive pressure gradient and drive the infusate into the brain (Fig. 3)[143]. The effects of CED are largely determined by the choices of cannula style and placement, infusion rate, drug encapsulation method, and proximity of the target to brain substructures with low-flow resistance, such as white matter tracts, white matter edema, or ventricles[144,145].

CED for treating brain tumors via intratumoral delivery of therapeutic compounds has been studied extensively preclinically and clinically (Table 1)[146,147]. In one example of a phase I clinical trial, human recombinant bone morphogenetic protein (hrBMP4), a cancer stem cell suppressor, was delivered via CED to the tumor and peritumoral area of GBM patients[148]. In patients that had tumor recurrence after CED treatment, the recurrent mass was rarely in the areas infused with hrBMP4, demonstrating the effectiveness of CED-delivered drug penetration in the infusion area[148]. Advances in CED technology such as improved catheter design for reducing reagent reflux, algorithms for optimal placement, and implantable systems that allow continuous and refillable administration of chemotherapeutics are gradually increasing the therapeutic efficacy and safety of CED[149–151]. Also, new methods combining real-time imaging, such as MRI, with CED can help visualize the infusate; monitoring the infusate is valuable since predicting drug distribution by CED is challenging, especially in GBM tumors in which the BTB is leakier in some parts than others[148,152].

Although many clinical trials on CED have been conducted (Table 1), and gradual improvements have been observed, the clinical application of this approach is not yet widely used. Headaches are common following treatment with CED and can indicate induced cerebral edema, hemorrhage, or hydrocephalus. Corticosteroids administered prior to CED reduce edema-related side effects but may inhibit therapeutic agents that rely on immune-mediated anti-tumoral effects. The procedure for CED can be long (a few hours to a few days) and uncomfortable for patients. The prolonged CED procedures can also increase the risk of infection and pulmonary embolism[127,153,154]. In the first randomized phase III clinical trial of CED which compared CED to Gliadel wafers, while the CED group had a higher occurrence of pulmonary embolism (8% for CED and 1% for Gliadel wafer)[153], the CED group did not display a significant increase in survival compared to the Gliadel group. One of the key limitations of this study was catheter positioning, with more than 30% of catheters not placed per protocol[153,155]. Therefore, the optimal time for catheter placement should also be considered. Catheters placed after resection surgery are more likely to be positioned at the intended location[156]; however, post-resection CED also increases the risk of infusate leakage, which is also a major limitation observed in CED studies[148]. Improvements in the capacity to monitor the infusate increase the potential for progress in the clinical application of CED for drug delivery to treat GBM; further, the novel refillable systems can help with decreasing the duration of hospital stay, reducing the risks and discomfort associated with longer hospital stays; However, further research to enhance the catheter and CED protocol should also be conducted.

Sustained delivery approaches aim to release therapeutics gradually and locally to avoid the toxicity of systemically injected chemotherapeutics (Fig. 3). FDA approval of Gliadel wafers for sustained local release of Carmustine for GBM treatment was an exciting prospect, but the wafers demonstrated only slightly increased survival[157] and were associated with a high rate of complications[158]. Gliadel wafers had detrimental effects on surrounding tissue including cerebral edema, intracranial infection, and necrosis[159–161], possibly due to the degradation of the polymer in the wafer. In addition, Gliadel wafers could not preserve a slow and controlled release rate[157,162].

Other approaches to sustained delivery that use more biocompatible materials such as hydrogels and composite meshes are in the preclinical research phase. Soft hydrogels are more quickly degraded than Gliadel

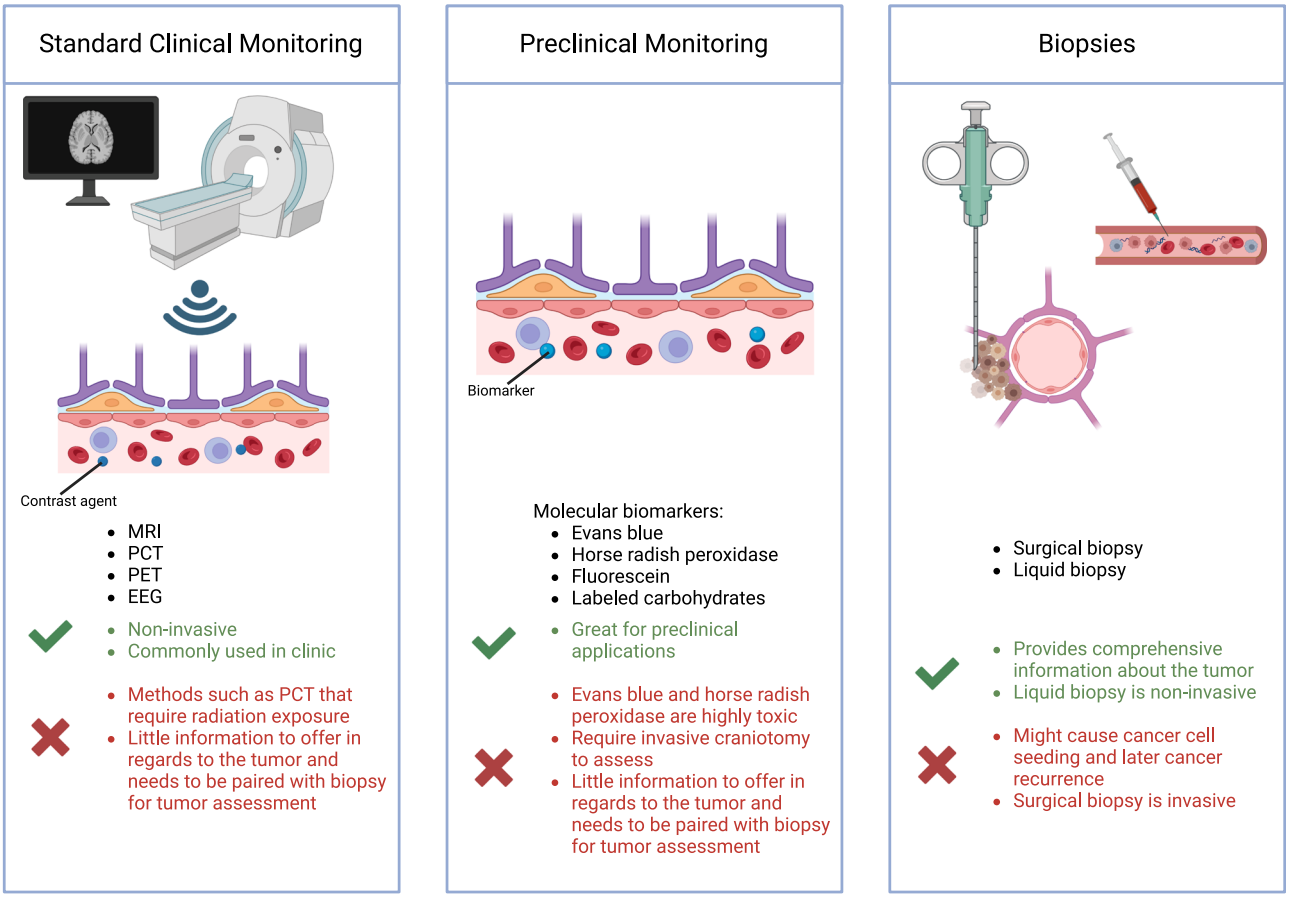

Fig. 4 | BBB and tumor monitoring and assessment methods. BBB monitoring methods can be categorized by application and invasion into clinical and preclinical monitoring methods. These monitoring methods can also be used to provide some data on the tumor and BTB conditions; however, BBB monitoring methods are not sufficient to assess the tumor and taking biopsies is necessary. BBB, blood-brain barrier; BTB, blood-tumor barrier; MRI, magnetic resonance imaging; PCT, perfusion computed tomography; PET, positron emission tomography; EEG, electroencephalography. Figure created in BioRender. Lab, B. (2024) https://BioRender.com/o56x349.

wafers, though this short half-life can be limiting if the treatment is longer than one month[159,163]. Composite meshes may provide a better option for a longer duration of drug release. A composite mesh consisting of poly(lactic-*co*-glycolic acid) (PLGA) edges surrounding arrays of polyvinyl alcohol (PVA) pillars imbued with Docetaxel, Paclitaxel, or a nanoencapsulated form of these chemotherapeutics exhibited sustained release for over 150 days in mice. All four treatment groups were associated with increased survival; greater survival was observed in groups containing meshes with the macro format of the drug[162]. The composite mesh has the additional advantage of flexibility in the types of compounds it can carry since the mesh is composed of two compartments, one which is well-suited for hydrophobic compounds (PLGA) and the other for water-soluble compounds (PVA)[162]. Composite meshes are mostly biodegradable, and unlike Gliadel wafers, the remaining polymer shell should not have adverse effects; additional research is needed to test this hypothesis.

Monitoring the BBB to assess permeability

Monitoring the BBB is essential in developing CNS disease treatments and novel drug delivery methods that cross the BBB. Monitoring the BBB can assist in the guided delivery of therapeutic compounds to reduce adverse systemic effects. Non-invasive and invasive BBB-monitoring techniques have been introduced and often work in tandem to provide a holistic view of BBB integrity and functional status. Non-invasive monitoring techniques are favored for clinical use, while invasive techniques are mostly limited to preclinical applications. Here, we will present an overview of both preclinical and clinical BBB monitoring methods (Fig. 4).

Preclinical techniques

Molecular biomarkers are used in preclinical research to assess physiological abnormalities of the BBB or pharmacokinetic drug properties, but not in humans due to the requirement for invasive craniotomy to evaluate the biomarkers. Several of these molecular biomarkers provided initial insights into BBB structure and function (Fig. 4).

Evans blue dye (EBD) is a high molecular weight marker that was used in early studies of BBB capillary and cell membrane permeability[164]. EBD is visible to the naked eye following intraperitoneal or intravenous injection within the tissue, making it simpler to use than other markers. The molecule binds strongly but reversibly to albumin, making it a useful marker for BBB extravasation[164,165]. EBD is used to assess BBB disruption as the dye permeates the barrier and aggregates at sites of damaged lesions and is commonly used in preclinical studies of the devices described in this review[166]. However, EBD also binds plasma proteins other than albumin, and this nonspecificity must be accounted for to prevent overestimation of BBB permeability[167]. The dye also binds tissues outside the CNS, causing cell toxicity and organ dysfunction, limiting its clinical application[167,168].

Horseradish peroxidase (HRP) is widely used in BBB imaging in preclinical studies. The reaction product of HRP and its substrate 3,3′-diaminobenzidine (DAB) can be made electron-dense for electron microscopy[166,167] and can be visualized macroscopically based on its dark brown color[166]. Assessing HRP activity can provide insights into differential permeability and structural variations within the BBB[166,167,169]. HRP must be used cautiously as it induces mast cell degranulation and release of histamine and serotonin, which can cause cytotoxic immune activity and

dysfunction in adjacent tissues[167,170]. As a result, HRP is limited to preclinical BBB assessment, and concurrent administration of antihistamines has been explored to augment its utility in animal models[6].

Fluorescein was one of the first fluorescent molecules used to visualize BBB integrity and is a popular molecular biomarker for studying BBB permeability[167,171]. Fluorescein can be assayed by fluorescence spectroscopy and microscopy[166], which are relatively low-cost and accessible measurement techniques. Antibody-conjugated fluorescein exhibits greater specificity than EBD due to its weaker nonspecific binding to plasma proteins, making its systemic distribution highly dependent on the plasma concentration of the antibody target molecule[167]. Fluorescein is a marker of choice in preclinical BBB disruption studies due to its sensitivity and safety[166,167] and is used clinically for guided resection of malignant tumors such as GBM[172]. However, due to its invasive nature, its clinical application for BBB monitoring outside of surgery is limited.

Labeled carbohydrates have also been used to assess BBB permeability. Radiolabeled (^{14}C) sucrose was used in early quantitative determinations of BBB permeability[6,173]; however, the stable isotope of sucrose (^{13}C) is more accurate, with reduced sensitivity to impurities[174]. Sucrose is preferable to other carbohydrate markers as it is uncharged, exhibits low-protein binding, is metabolically stable after parenteral administration, and is not a substrate for active transporters in animals[6,167]. Sucrose permeability markers are assessed using liquid chromatography–tandem mass spectrometry (LC-MS/MS) and intracranial microdialysis[174]. Dextrans are an alternative to sucrose and have been increasingly used for BBB permeability assessment. Dextrans are complex, branched glucans derived from repeated condensation reactions with glucose molecules[167,175]. Biotin- and fluorophore-labeled dextrans have been used to quantify BBB extravasation[167,176]. The rates of diffusion of these modified dextrans across the BBB are often compared to those of sucrose and insulin. These biomolecules can be detected with high sensitivity, allowing them to be used at low concentrations[176,177], thus limiting side effects and toxicity in BBB animal models[166,167].

Clinical techniques

Magnetic resonance imaging (MRI) is the most practiced technique for visualizing CNS anatomy and identifying diseases. The integration of MRI with focused ultrasound, LITT, and CED has contributed to the rise in clinical trials involving these device-assisted methods[99,119,148]. Moreover, combining MRI with devices that deliver therapeutics to the CNS helps increase the efficiency and targeting ability of these devices. Specialized MRI methodologies such as dynamic contrast-enhanced MRI (DCE-MRI) have been developed for monitoring the BBB and assessing BBB disruption due to disease or acute injury. DCE-MRI improves the quantification of perfusion and fluid flow due to an increased sensitivity and ability to evaluate changes invisible to conventional MRI methods[178–180]. The increased sensitivity is achieved by tracking the movement of contrast agents such as gadolinium across the BBB, expressed as the influx constant K_i[181]. Disruption of the BBB initiates extravasation of MRI contrast agents which accumulate in the extracellular space, causing an increase in signal intensity in T_1-weighted images, which is then used to detect and evaluate regions of BBB disruption[178]. Assessment of the influx constant and spatial accumulation of contrast agents by DCE-MRI has proven a reliable, non-invasive method for assessing BBB permeability[178,181–183]. However, the relatively low signal-to-noise ratio (caused by sensitivity to acquisition variables) and the necessity to adopt a 3D, fast, low-angle shot sequence to achieve adequate spatial and temporal resolution are disadvantages of this technique[179,184].

Perfusion computed tomography (PCT) has also been optimized for BBB imaging. PCT is an adaptation of traditional CT imaging based on the central volume principle which relates cerebral blood flow to cerebral blood volume and mean transit time[185]. This technique utilizes serial CT images to assess changes in contrast agent concentration over time[185,186] which are used to estimate BBB permeability[187]. This technique has been implemented in brain tumor imaging to non-invasively detect increased BBB permeability[187]. Limitations of PCT include variability between institutions including variability in post-processing methods and permeability assessments[186,188]. Section selection is also critical as many CT systems are limited in the area covered during a single acquisition[189]. This limitation can be overcome by sequential imaging or by transposing the CT scanner during acquisition[186]. Another limitation is the low resolution of PCT maps which makes smaller disruptions difficult to visualize[189]. Finally, the radiation exposure inherent to CT systems can contraindicate its usage or limit the frequency of imaging, especially in children.

Positron emission tomography (PET) is another imaging technique used to assess BBB status[190]. PET imaging involves the intravenous injection of a positron-emitting radioisotope and the subsequent detection of emitted photons in vivo[191]. PET allows accurate quantification of BBB permeability, identification of lesions where the barrier is compromised, and cell surface receptor density mapping[190]. Radiotracers such as 2-amino-[3-^{11}C]isobutyric acid ([3-11 C]AIB) and [^{68}Ga]ethylenediaminetetraacetate (68Ga-EDTA) are used in BBB imaging[192,193]. PET radiotracers play a crucial role in investigating transporter function, with ABCB1 efflux transporters being among the most extensively studied[194]. In the examination of transporter functionality, researchers apply radiotracers that either constitute the transporter's substrates themselves or are transformed into such substrates during metabolism. Additionally, compounds capable of penetrating the brain with the capacity to be subsequently metabolized into radiotracers that act as substrates for efflux transporters also offer a valuable approach to studying these specific transporters[194]. Moreover, since PET is non-invasive, it allows for a parallel drug uptake or expulsion comparison between in vivo models and humans. PET is often used in conjunction with MRI or molecular biomarkers to produce a quantitative, multimodal image of BBB health and function[195]. Combining MRI and PET reduces the motion artifacts and partial-volume effects that limit PET imaging while maintaining the quantitative capability of PET, thus augmenting the capabilities of MRI[196,197].

Electroencephalography (EEG) is another evolving technique for real-time BBB monitoring. EEG exploits the difference in electrical potential between the blood and brain tissue maintained by the BBB[198,199]. When the BBB is disrupted due to disease or injury, this potential can be locally altered, which is detected non-invasively by scalp EEG[198,200]. This was confirmed by a clinical study assessing EEG signal shifts in response to changes in cerebral blood volume which was measured by near-infrared spectroscopy[198,199]. These EEG signatures have also been explored for the dynamic characterization of BBB extravasation[201]. Due to the variable operating parameters of EEG (e.g., electrode choice, frequency, and computing technique), the EEG is combined with other imaging modalities such as MRI, to identify BBB disruption or changes in permeability consistently[201]. The low-cost and non-invasive nature of EEG makes it a viable auxiliary tool for BBB assessment despite its minimal sensitivity and low resolution[201]. A related technique, electrocorticography (ECoG), has been used successfully for the determination of BBB permeability and disruption[202]. This technique utilizes the same principles as EEG but requires electrodes to be placed intracranially. ECoG allows better spatial and temporal resolution than EEG, but the invasive nature of ECoG severely limits its clinical application[203]. However, the emergence of novel catheters that deliver drugs and take ECoG measurements enables the coupling of CED with this monitoring technique[152].

Although the mentioned monitoring methods can provide valuable information on the conditions of the BBB, in the case of GBM, these methods provide little information on the tumor. Therefore, clinical monitoring needs to be coupled with biopsies, most commonly surgical biopsies, to give more details on the tumor, BTB, and BBB (Fig. 4).

Outlook

As the focus shifts towards larger biopharmaceuticals in CNS therapy, overcoming the challenges posed by the BBB has become even more essential. An indispensable future research step is to develop effective techniques to disrupt or circumvent the BBB and increase the efficiency of novel therapeutics for CNS diseases. Device-assisted techniques are promising for achieving this objective. The testing and approval process for

devices is generally faster than for pharmaceuticals[204]. Devices are also less probable than pharmaceutical drugs of becoming obsolete due to the development of drug resistance. Device parameters are also customizable, making them suitable for the practice of personalized medicine. Moreover, some of the devices mentioned here have ablative functions, rendering them with the dual ability to ablate the tumor and increase the permeability of the BBB, simultaneously helping to optimize surgery and drug delivery. Considering the benefits of BBB opening devices, it is encouraging to conduct further research on these technologies.

An exciting and highly discussed technology in the evolving landscape of BBB opening device-assisted methods is MRgFUS[205,206]. MRgFUS was initially recognized for its success in the treatment of essential tremor which led to its other applications including for drug delivery to the CNS[207]. MRgFUS has exhibited great potential as an emerging clinical device that temporarily opens the BBB. Also, the ability to obtain samples through liquid biopsy with MRgFUS[116] highlights this method's dual capacity for diagnostic and treatment applications. Safety studies of MRgFUS have generally reported low rates of complications; however, more studies are needed to confirm the safety of this technology. Moreover, further long-term studies in larger patient populations are required to assess the effect of drug delivery via MRgFUS on survival and recurrence rates in patients with GBM. In the clinical application of MRgFUS, it is critical to identify and implement the appropriate ultrasound parameters for each patient to ensure precise targeting of areas for BBB opening, preventing damage to the tissue, or excessive expansion of the microbubbles, leading to blockage of blood flow and ischemia-like effects[208]. The recent advances in AI will assist with the development of accurate simulations with the capacity to model and predict the optimal ultrasonic parameter for each patient. Overall, this technology shows great potential; however, its establishment as a clinical device for drug delivery in GBM is contingent on the positive results of current ongoing and future clinical studies on the safety and efficacy of MRgFUS.

Ablative devices offer the dual benefit of tumor eradication and drug delivery; however, when compared to devices that are not ablative, such as low-intensity MRgFUS, ablative devices carry a higher risk of complications. Ablative devices such as LITT provide a longer duration of high BBB permeability[90,118], though long intervals of high BBB permeability may result in adverse effects from toxic material entering the CNS. In fact, some side effects observed after LITT, such as edema, could be attributed to this phenomenon, similar to previous studies on CED that demonstrated increased edema in response to macromolecules entering the CNS parenchyma[127]. However, the longer durations of permeability might be more suitable for cell cycle phase-specific agents, which require longer exposure times[209], or therapeutics that have a longer blood circulation time and/or a later onset of action. For MRgFUS, which allows for a shorter duration of BBB permeability, therapeutics with a faster onset of action might be the best option. Also, in the case of implanted MRgFUS, therapeutics administered in short intervals may be more suitable since these devices can open the BBB repeatedly. It is important to gain a better understanding of the optimal time of administration for each type of therapeutic being coupled with devices, designing the best treatment regimen, and having optimal drug and device pairs.

CED benefits from a long history of clinical application. Nonetheless, its application is limited by catheter design, placement, and drug penetration into the surrounding tissue. With the advent of real-time monitoring and AI, optimal catheter location and drug diffusion patterns in brain parenchyma can be predicted before surgery and visualized in real-time. Future research on optimal catheter design is also encouraged. While the preclinical studies on opening the BBB, non-invasively, with electrical fields appear promising, more studies are needed to elucidate the mechanism of action and precision in targeting of BBB opening by these devices. The transition of this device into clinical settings is an exciting prospect. With sustained delivery, identification of the candidate material for the drug delivery platform is key. This material should be biocompatible, should not induce significant glia or fibrotic scarring, and should have a stiffness similar to the brain parenchyma. If the material is biodegradable, the byproducts of degradation should be non-toxic. The material should also allow for extended-release (3-6 months) for most therapeutics[159]. Another key aspect of sustained delivery is the accurate prediction of drug release and diffusion. Therefore, more preclinical research with interdisciplinary teams involving physicians, material, and biomedical scientists is required to identify the best material for the device and optimize drug release parameters.

Combining other BBB opening methods with devices can increase the potency of drug delivery to the CNS. This includes the application of nanoparticulate systems, which have been studied in combination with multiple device-assisted delivery methods[108,137,162]. Nanoparticles can be adjusted to target cancer cells[108]. This combination yields more specific and efficient drug delivery while being safer than one of these methods alone. Combination drug delivery methods using CED have undergone clinical trials, and further trials will likely explore their efficacy in conjunction with other device-assisted approaches. Another important consideration is the effect of the efflux transporters on therapeutics that have entered the CNS; a study by Goutal et al. demonstrated the ability of efflux transporters to hinder drug delivery to the CNS even in the presence of MRgFUS[210]. Therefore, for therapeutics that are targets of efflux transporters, combining device-assisted opening of the BBB with efflux transporter blockers should be considered. More studies on the effects of efflux transporters on therapeutics that enter the CNS via assistance from LITT or electroporation are also needed. The mentioned study was not performed in a cancer model; conducting such studies in cancer models is also recommended. Overall, a combination of device-assisted drug delivery with other methods of drug delivery to the CNS, such as nanoparticles and efflux transporter inhibitors is highly recommended.

When utilizing MRgFUS and electrical fields for BBB opening, since the BTB is more permeable in some areas, it might be best to target the areas of the BBB and BTB that display low permeability. The permeability of the targeted BBB or BTB can be assessed with the clinical monitoring devices mentioned earlier. This further emphasizes the importance of coupling monitoring devices with drug delivery devices to improve specificity. An emerging paradigm addressing both the monitoring and drug-delivery challenges posed by the BBB is continuous sampling or repeated tissue collection[211]. This concept has garnered significant attention, as evidenced by discussions led by key opinion leaders in neuro-oncology[211,212]. Accordingly, devices facilitating continuous access across the BBB and repeated access to intracortical tumor tissue are an increasingly important area of research[213–215]. Such approaches facilitate the important molecular profiling derived from tissue biopsy, enable targeted drug delivery across the BBB, and synergize with existing imaging techniques (Fig. 5). Moreover, with continuous tissue sampling, the drug concentration in the tissue after treatment can be analyzed, which is an important data point absent from many of the clinical trials mentioned in this manuscript, as tissue biopsy is not standard practice in neuro-oncology clinical trials[211]. These on-demand sampling devices have the potential to augment both drug delivery and discovery efforts as well as sharpen the molecular picture of an individual's GBM over the course of the disease.

Local BBB permeability via device-assisted methods might be less effective for diffuse cancer cells or GBM cells that have migrated outside the tumor margin, than for aggregate tumors. Although the application of these devices can help increase drug delivery to the regions targeted by the device, or in the case of CED and sustained delivery, in the vicinity of the device, the possibility of recurrence will not be eliminated due to scattered cancer cells positioned in areas that have not received the proper dose of therapeutics and were not affected with increased BBB permeability. In the case of CED and sustained delivery, increasing drug penetration can help decrease local recurrence, but it might be less effective in preventing distant recurrence. For MRgFUS and electrical fields, having multiple opening foci and widening the focal spot size will increase the span of the area with increased BBB permeability without the need to increase the intensity of these devices. However, this could potentially aggravate the rate of complications due to an

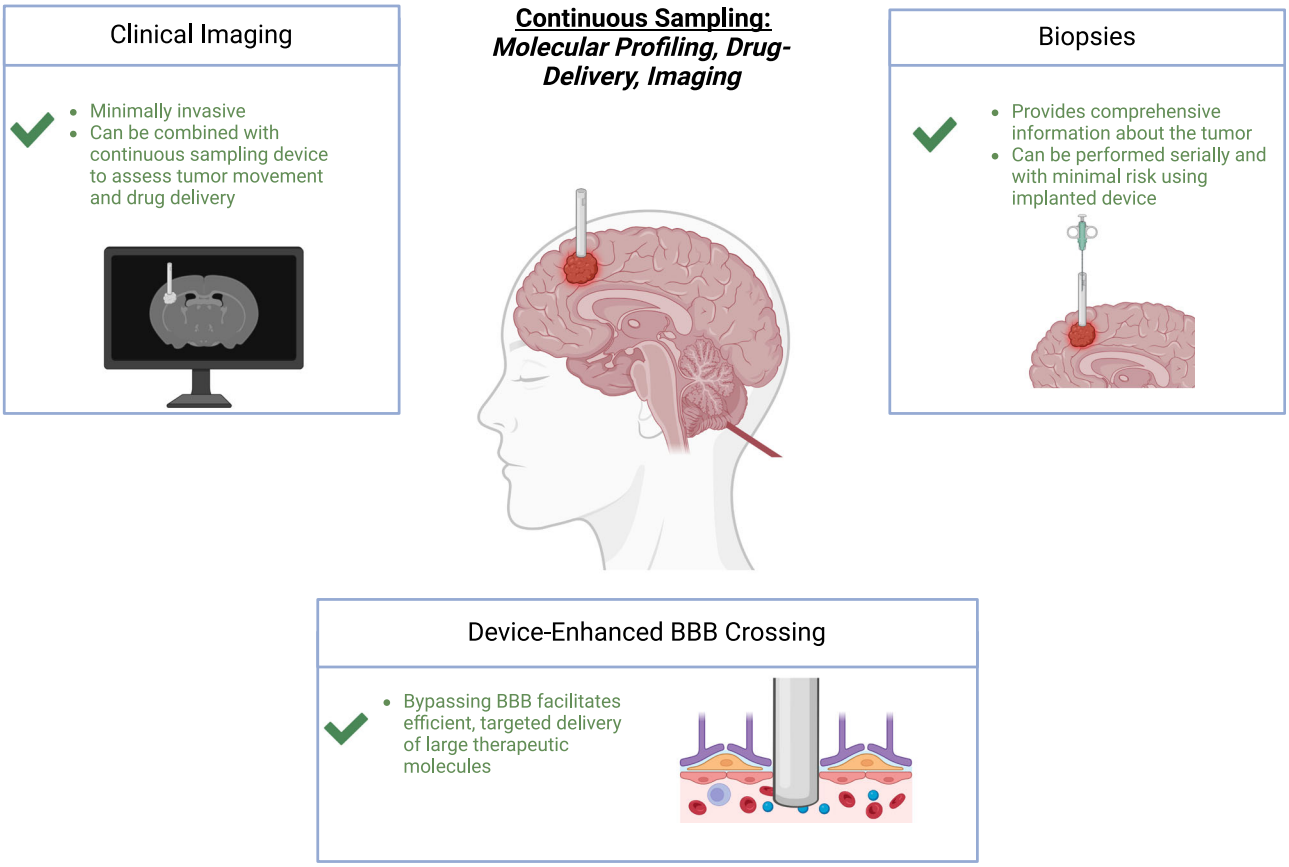

Fig. 5 | Continuous sampling. Continuous sampling can encompass the benefits of other BBB crossing and tumor-monitoring strategies in a single platform with reduced risks to patients. On-demand biopsy facilitates continuous tumor phenotyping and assessment of drug concentration at the tumor site. Relatedly, the sampling conduit also enables targeted drug delivery via assisted BBB crossing. Finally, clinical imaging techniques such as MRI can be used in conjunction with an implanted device to evaluate tumor movement into the device as well as BBB disruption at the interface with the implant. BBB, blood-brain barrier; MRI, magnetic resonance imaging. Figure created in BioRender. Case, A. (2024) https://BioRender.com/o69z127.

increase of macromolecules and toxins entering the CNS, and more healthy cells being exposed to higher concentrations of therapeutics. Combining BBB opening methods with cell traps that utilize taxis-based methods to move GBM cells to a specific location in the brain may have the potential to address this challenge in the future[216,217]. However, more investigation is needed as most of the studies conducted to date for trapping GBM cells have been preclinical research.

Ultimately, while more clinical work on validating and improving the safety and performance of these devices is needed, the future of drug delivery to the CNS for GBM patients is progressing toward combination therapies that are personalized and minimally invasive, and device-assisted drug delivery has the potential to be a key player in this progression. Devices with the ability to be combined with real-time monitoring will allow for improved precision and targeting, while the advancements in serial biopsy that these devices allow for will help improve the design of treatment plans and prognosis. With the continued advances in nanotechnology, biomaterials, monitoring, in silico, and in vitro modeling, the field of device-assisted drug delivery to the CNS is poised to deliver more effective treatments for GBM, potentially transforming the outlook for this challenging disease.

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