Novel delivery methods bypassing the blood-brain and blood-tumor barriers

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Glioblastoma (GBM) is the most common primary brain tumor and carries a grave prognosis. Despite years of research investigating potentially new therapies for GBM, the median survival rate of individuals with this disease has remained fairly stagnant. Delivery of drugs to the tumor site is hampered by various barriers posed by the GBM pathological process and by the complex physiology of the blood-brain and blood–cerebrospinal fluid barriers. These anatomical and physiological barriers serve as a natural protection for the brain and preserve brain homeostasis, but they also have significantly limited the reach of intraparenchymal treatments in patients with GBM.

In this article, the authors review the functional capabilities of the physical and physiological barriers that impede chemotherapy for GBM, with a specific focus on the pathological alterations of the blood-brain barrier (BBB) in this disease. They also provide an overview of current and future methods for circumventing these barriers in therapeutic interventions. Although ongoing research has yielded some potential options for future GBM therapies, delivery of chemotherapy medications across the BBB remains elusive and has limited the efficacy of these medications.

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GLIOMASTOMA (GBM) is the histological type of World Health Organization Grade IV astrocytic malignant tumors of the brain. According to the Central Brain Tumor Registry of the United States, with 45.6% of all malignant brain tumors, GBM was the most common malignant brain tumor from 2006 to 2010, and it represents 15.4% of all primary tumors.¹²⁵ The median age of patients at GBM diagnosis is 64 years, and the male/female predominance ratio is 1.6.¹²⁵ Although brain tumors are relatively rare compared with the most prevalent types of cancer, GBM disproportionately carries high rates of morbidity and mortality. The current standard of care includes cytoreductive surgery or biopsy combined with adjuvant chemoradiation using temozolomide (TMZ).¹³ With these standard interventions, the 5-year survival rate of patients with GBM is < 5%, the mean survival is approximately 14 months, and the disease nearly universally

recurs.^{25,125,140} Survival rates worsen with increasing age, such that in patients > 65 years the mortality rate at 5 years is greater than 98%.¹²⁵ This grim prognosis is due in part to barriers to efficacious delivery of pharmaceutical therapy, and these barriers are discussed in this review.

The relatively limited access of chemotherapy medications to the microenvironment of a malignant brain tumor is a major obstacle to their delivery. This limited access is due to 2 major structural barriers: the blood-brain barrier (BBB) and the blood–cerebrospinal fluid barrier (BCSFB). Both barriers provide biomechanical and cellular resistance to pharmaceutical extravasation and to the exposure of the parenchymal tissues of the central nervous system (CNS) to chemotherapy agents. Experimental manipulation to circumvent these obstacles has been a major focus of research into the treatment of GBM and other malignant diseases of the CNS.

ABBREVIATIONS BBB = blood-brain barrier; BCSFB = blood–cerebrospinal fluid barrier; BTB = blood-tumor barrier; BV = bevacizumab; CNS = central nervous system; CSC = cancer stem-like cell; CSF = cerebrospinal fluid; GBM = glioblastoma; LTH = liposomal Trojan horse; TMZ = temozolomide; VEGF = vascular endothelial growth factor.

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The BBB: Anatomy and Physiology

Anatomical Features

The BBB constitutes the interface between the brain parenchyma and its vascular supply vessels. It is one of several systems that defend against the intrusion of foreign substances into the CNS, and it also maintains CNS homeostasis, including ionic and fluid balances. Thus, the BBB shields CNS neurons from systemically circulating hormones, inflammatory mediators, waste products, strongly fluctuating ionic concentrations, and toxic compounds.^{67,147} It also prevents the egression of CNS neuropeptides and neurotransmitters into the general circulation.⁶⁷ Therefore, the BBB serves to isolate both CNS and systemic fluid contents via a multifaceted regulatory process.

Structurally, the BBB comprises capillary endothelial cells with adjoining tight junctions overlying a continuous, nonfenestrated basal lamina (Fig. 1A).¹⁴⁸ The tight junctions restrict paracellular substrate flux and separate the apical and basal cellular domains with associated peripheral membranous protein complexes to establish specific functional domains.³ Invested pericytes are bounded by the basal lamina of the endothelium, and a second layer of basal lamina adjacent to the astrocytic interdigitating end-foot processes of the perivascular space forms a sheathed neurovascular structure.^{1,3} This structural barrier to solute movement is the rigid frame on which physiological modification will mediate transport of solutes across the BBB.

Detailed evaluation of the BBB has shown poor permeability for solutes > 500 D.¹⁰³ Molecules such as ethanol

(46.1 D), nicotine (162.2 D), and caffeine (194.2 D) are passively transported across the luminal and abluminal membranes, and the efficiency of this transport is determined by the lipophilic characteristics and molecular sizes of these compounds.¹⁷⁴ Other less lipophilic and larger molecules such as glucose (180.2 D), insulin (6 kD), and albumin (6.6 kD) use solute carriers (GLUT-1), receptor-mediated transport, and absorption-mediated transport, respectively.¹⁷⁴ Orally dosed TMZ (194 D) has approximately 20% relative penetration across the normal BBB because of the small molecular size, lipophilic properties, and efflux pump specificity of TMZ.^{7,124} In summary, the BBB permeability to macromolecules is determined by a multifactorial anatomical and physiological milieu.

Physiological Features

The physiological contribution of astrocytes to the BBB involves modulating the cytoarchitecture of the endothelial tight junctions, determining the polarization of transport systems, and enhancing the expression of enzymatic mechanisms.¹⁷⁴ Because astrocytes maintain the anatomical and physiological function of the BBB, diseases affecting astrocytes can, in turn, dramatically affect the BBB. For example, water transport across the BBB is primarily mediated by aquaporin 4, which is expressed along astrocytic endfeet of the neurovascular unit and controls the flows of ions, macromolecules, and fluids.^{121,166}

The endothelial lining of the neurovascular unit also possesses a multitude of transport proteins, including ABCB1 (P-glycoprotein), ABCC (MRP) transporters, ABCG2

FIG. 1. Barriers to CNS access. **A:** The neurovascular unit is composed of the endothelial lining, the basal lamina, ensheathed pericytes, and astrocytic endfeet. **B:** Solute transporters on the endothelial surface are major barriers to extravasation of chemotherapy agents. **C:** The blood-CSF barrier serves as the interface between the vasculature and the CSF and is composed of the choroid plexus epithelium. Copyright Aaron Cohen-Gadol. Published with permission.

(BCRP), OAT3 (SLC22a8), OATP2, and OATP3¹⁴⁸ (Fig. 1B). These transporters have major physiological significance for resistance to chemotherapeutic agents in GBM therapy. They mediate efflux of xenobiotics from the endothelium or into the endothelium away from the intraparenchymal fluid compartment, effectively clearing these agents into the luminal compartment.

In addition to the transport barrier at the BBB, intracellular and extracellular catabolic enzyme processes also contribute to the barrier within both the endothelium and astrocytes.² One enzymatic process of particular importance is the O-6-methylguanine-DNA methyltransferase reaction, whose increased activity generates TMZ resistance.⁸⁷ Therefore, for an agent to traverse the BBB, the agent must circumvent the anatomical barrier, avoid efflux transport, and evade enzymatic conversion to an inactive metabolite.

Representing another interface with the brain parenchyma, the cerebrospinal fluid (CSF) compartment may serve as an additional avenue for drug delivery. The composition of the CSF is highly regulated through a modifiable barrier at the choroid plexus epithelium, referred to as the blood-CSF barrier (BCSFB) (Fig. 1C). The BCSFB is composed of choroid plexus epithelial cells, a basal membrane, apical microvilli, and a fenestrated endothelial lining of the choroidal vasculature.¹⁴⁸ To facilitate secretory control, the choroid plexus epithelium is adjoined by tight junctions that are absent from the adjacent ependymal and pial epithelial linings.¹⁵⁸ To produce and secrete CSF, the choroid plexus has an array of papillary structures within the brain's ventricular system. As shown in rat models, the choroid plexus receives the highest local blood flow in the brain, despite a surface area (75 cm²) similar to that of the BBB.¹⁴⁸ Given that its permeability is greater than that of the BBB, with a flux rate inversely proportional to the molecular weight of the solute^{128,132} and a permeability generally limited to molecules smaller than 400 D, the physical properties of the choroid plexus offer a potential delivery route for chemotherapy through the BCSFB.⁶⁰

However, for each millimeter of depth within the leptomeningeal space, the distribution of a solute via diffusion decreases logarithmically. For small lipophilic molecules, a distance of 500 microns results in a 10-fold drop in diffusion, and diffusion of larger molecules is even less efficient in this space.¹²⁸ The leptomeningeal space provides 2 anatomical locations for the exchange between the CSF and brain parenchyma. One exchange conduit is through the ependymal cell surface within the ventricles, and the other via the subarachnoid space because there is no substantial permeability barrier along the pial surface.^{148,172} Neither of these surfaces has tight junctions for control of paracellular permeability. Although the pial surface is not a formidable barrier to the passage of molecules, a network of glial cells forms the glia limitans, which regulates the diffusion of macromolecules.⁶⁸ The CSF-to-parenchyma penetration by macromolecules occurs at rates faster than can be explained by passive diffusion alone; therefore, it has been hypothesized that translocation across the CSF-parenchymal interface involves active transport mechanisms.¹²⁷ By modifying these specific secretory and permeability mechanisms involved in the production of

CSF, a tumor may alter the brain's biochemical microenvironment.^{88,171}

In summary, the BBB/BCSFB represents a controlled entry system for the CNS, accessible along the neurovascular unit via 2 cellular transport processes: simple diffusion of lipophilic compounds across the endothelium and transporter-mediated delivery of macromolecules and ions.^{15,148,174} both are possible conduits for strategic drug delivery.¹⁴⁷ However, the systems described above are not the settings through which most drug delivery is preferred, because the environment of a malignant tumor undergoes significant changes, which will be discussed in the next sections.

Effects of Malignant Neoplasia on the BBB

A malignant tumor results in many changes that contribute to specific pathological disruptions of the BBB. These disruptions include an alteration within the tumor mass referred to as the blood-tumor barrier (BTB) and diffuse, remote changes within the neurovascular system.⁷³ Those discussed in this review include alterations in vascular permeability, hypoxia-induced changes, and modifications in solute-carrier proteins.

Pathological vascular changes within the GBM can be attributed to the astrocytic diffuse upregulation and redistribution of aquaporin 4,^{3,121} decreased claudin and occludin expression within the tight junction complex,¹⁰² basement membrane disruption,⁵¹ and leaky neovascularization.¹⁵⁰ These changes all enhance the permeability across the BTB, facilitating influx of proteins and inflammatory mediators into the brain parenchyma. The BTB displays a heterogeneous breakdown in control of permeability,¹⁵⁰ which, in theory, is localized primarily to the tumor core. The adjacent BBB, as well as the tumor periphery, appears to retain permeability-resistance properties.^{45,168,169} A focal edematous state in the surrounding parenchyma, enhancing hydrostatic interstitial pressure, would theoretically compensate for the enhanced BTB permeability and potentially decrease the time-concentration exposure of chemotherapy agents delivered across the BTB.¹¹⁸ Overall, the enhanced BTB permeability does not ensure optimal pharmacokinetics of these agents because of a multifactorial dependence on efflux transporters and altered dynamics of cerebral fluid flow that accompany the BTB state.

The BTB neovascularization is of particular relevance to therapy because this neovascularization significantly determines the permeability of the local tumor neurovascular unit. This microvasculature can be classified as 1 of 3 endothelial types: continuous nonfenestrated endothelium, continuous fenestrated endothelium, or discontinuous endothelium.¹⁴³ The first exhibits permeability similar to that of the normal neurovascular unit, and the latter 2 types exhibit permeability dependent on pore area and the molecular weight of the fluxing macromolecule.¹⁴³ In addition, microvessel diameter and density are compounding variables that result in a highly heterogeneous angioarchitecture of the GBM.⁵² This heterogeneity may be explained by the fact that the pericyte precursors for neovascularization are derived from glioma stem cells.⁴⁰ Theoretically, profiling the vascular cell population of a

tumor before chemotherapy could predict the efficacy of the intervention and suggest certain BTB circumvention mechanisms to increase chemotherapy delivery.

Interrelated with vascular changes associated with GBM, hypoxia and acidotic microenvironmental conditions all contribute to BTB permeability through induction of hypoxia inducible factor 1 (HIF1).¹²² Induced production of vascular endothelial growth factor (VEGF) contributes directly and indirectly to hypoxia via neoangiogenic tortuous and leaky vasculature that enhances BTB permeability caused by gaps in the basement membrane and endothelium,^{86,150} as described above. The HIF α 1 receptor and VEGF expression are precipitous within GBM stem and non-stem cells.^{39,51} This modifiable physiological response to hypoxia is the target for GBM therapy via antiangiogenic pharmaceuticals such as bevacizumab (BV).^{30,37}

Through tumor-induced alteration in the expression of transporter proteins, solute-carrier proteins operate in the BTB of a GBM, being expressed in neoplastic cells and along the neurovascular endothelium.^{51,107} These proteins impede GBM therapy because they reflux chemotherapy agents that have reached the parenchyma or endothelial cells back into the vasculature.¹⁴⁸ The pathophysiology of GBM often involves the upregulated expression of these efflux systems resulting in chemotherapeutic resistance; chemotherapy agents to which this reflux phenomenon applies include paclitaxel, docetaxel, imatinib, topotecan, and TMZ.^{24,69,100,116} The x_c^- antiporter promotes elution of glutamate from tumors, and this excess glutamate is hypothesized to contribute to the excitotoxic death of the surrounding healthy parenchyma and to promote GBM extension into a zone free of contact inhibition.^{50,51} These solute-transport pumps provide pharmaceutical targets that could be tailored to individual tumors according to the dominant tumor transcriptome, to minimize resistance and promote chemotherapeutic efficacy, as has been shown in murine models.¹¹⁶

Surgical Implications of the BBB and BTB

The pathologic condition of the BTB, as described above, exhibits enhanced permeability due to leaky neovasculature and disruption of structural barriers.¹⁵⁰ This altered state facilitates the use of the photosensitive agent fluorescein to guide resection of gliomas through a preferential tropism of this agent for penetrating pathological BBB locations,¹⁴² such as the BTB. Although lacking a control comparison group, a recent study demonstrated an 80% complete resection rate of contrast-enhancing tumors when the resection was assisted by fluorescein guidance.⁵ Fluorescein guidance relies on BBB permeability and not on uptake by glioma cells, as is the case with the tumor marker 5-aminolevulinic acid;⁵ therefore, the presence of cells resistant to permeability within the tumor periphery could decrease the sensitivity of fluorescein's marking capability in regions with a peripherally infiltrating cell population.^{45,168,169} By, in theory, containing the greatest BBB disruption, the tumor core would be less likely to contribute to errors in fluorescein marking. Despite the possibility of not adequately delineating the tumor periphery, fluorescein showed 94% marking sensitivity when compared

with biopsy data.⁵ However, the knowledge that the tumor periphery is likely to maintain barrier permeability could justify a more conservative wider margin of resection.

Methods for Circumventing the BBB

The BBB/BTB provides a unique challenge for researchers to target malignant disease in the CNS because these barriers prohibit entry of chemotherapy agents into this region. Various strategies have been explored to circumvent the BBB with the goal of effective chemotherapeutic penetration. These methods include prodrug formulations, chemical barrier disruption, biochemical drug modification, intraarterial injection, surgical circumvention, hyperosmotic barrier disruption, lipophilic carrier molecules, nanoparticle-delivery vehicles, convection-enhancement delivery, disruption of efflux transporters, thermotherapy, viral vehicles, and stem cell-mediated delivery (Table 1). Several of these circumvention methods that have shown promise or are historically important are discussed in the following. We caution against implying therapeutic efficacy of the abovementioned methods when they have been tested *in vitro* or when nonprimary tumors were used as a model system for human GBM.

Prodrug-Mediated Therapy

Prodrug-formulated agents are a theoretical approach to BBB penetration that would involve administering an agent that becomes chemotherapeutically active through enzymatic modification after it has crossed the BBB. This enzymatic modification would render the chemotherapy agent less permeable and thus less prone to removal from the parenchyma. This increased residence time would increase the time-concentration product, while leaving the total body exposure unchanged.⁷³ To our knowledge, this approach has not been investigated thus far for GBM therapy *in vitro* or *in vivo*. Therefore, it remains a theoretical method for delivery of chemotherapy agents.

Chemical-Mediated Disruption

Chemical disruption involves exposing the BBB to an agent that transiently increases BBB permeability. Research into this approach has yielded results with intraarterially infused vasoactive compounds such as leukotriene C₄,¹⁷ alkylglycerols,⁵⁸ interleukin-2,⁷⁵ RMP-7,⁶³ bradykinin,^{46,83} and tumor necrosis factor- α /interferon- γ .¹⁰⁴ These agents induce a transient inflammatory reaction within the endothelium, resulting in enhanced paracellular permeability and increased chemotherapeutic concentration in the parenchyma. Black and Chio¹⁷ encountered initial obstacles to this approach in 1992, such that leukotriene C-4 opened the BBB only enough to facilitate passage of small hydrophilic molecules and not of larger dextrans. Subsequent *in vivo* attempts have used the bradykinin and kinin analog RMP-7, which has been shown to generate greater disruption than leukotriene C-4; however, RMP-7 produces side effects, including headache, nausea, vomiting, hypertension, and tachycardia.⁶³ Moreover, only a very modest increase in BBB enhancement was observed in *in vivo* models of cisplatin with leukotriene C-4¹⁸ and of carboplatin with RMP-7.^{63,71,109}

TABLE 1. Methods for circumventing the blood-brain barrier in GBM treatments

Delivery Method	Description	Most Recent Clinical Trial
Chemical-mediated BBB disruption	Transient inflammatory response in endothelial lining, investigated w/ LTC4, alkylglycerols, IL-2, RMP-7, bradykinin, & TNF- α /IFN- γ	Warren et al., 2001 & 2006; Grant et al., 2002
Hyperosmotic BBB disruption	Endothelial dehydration, cell body shriveling, & tight junction disruption; mannitol primarily investigated	Boockvar et al., 2011; Guillaume et al., 2010
IA delivery	IA injection, often combined w/ hyperosmotic disruption	Burkhardt et al., 2012 ²⁸
Convection-enhanced delivery	Intratumoral infusions from surgically placed micro-pumps	White et al., 2012; Bogdahn et al., 2011; Kunwar et al., 2010; Carpentier et al., 2010
Intraventricular & intrathecal injection	Agent injection bypasses BBB; low parenchymal penetration	Witham et al., 1999
Intranasal delivery	Agent is inhaled & follows olfactory & trigeminal nerve pathways	da Fonseca et al., 2008 & 2013
Gliadel wafer	Surgically implanted carmustine-loaded polymer	Kunwar et al., 2010; Attenello et al., 2008
Efflux pump inhibitors	Suppress transporters to increase intraparenchymal drug presence	Brown et al., 2008
Focused ultrasound-mediated disruption	Thermomechanical disruption of the BBB	None
Molecular Trojan horses	Engineered ligand coupled to drug of interest shuttled across BBB via receptor-mediated transcytosis	Drappatz et al., 2013
Liposomal Trojan horses	Lipophilic vehicle loaded w/ Tx agent for delivery across BBB	Caraglia et al., 2006; Fabel et al., 2001
Nanoparticle vehicular delivery	Nanoscale vehicular devices often delivered via CED & intrathecal or intraventricular injection	None
Viral vector-mediated delivery	Vector for transport & transduction of gene Tx	Rainov, 2000
Stem cell-mediated delivery	Cells serve as carriers w/ tropism for cancer cells	None

CED = convection-enhanced delivery; IA = intraarterial; IL-2 = interleukin-2; INF γ = interferon- γ ; LTC4 = leukotriene C4; TNF- α = tumor necrosis factor- α ; Tx = therapy.

Effectiveness of the RMP-7 and carboplatin regimen was investigated in Phase I (2001) and Phase II (2006) clinical trials, which concluded that this combination was ineffective.^{164,165} Recent research indicates that glioma invasion can be achieved through a bradykinin-mediated chemotactic process,^{112,144} and this finding questions the overall efficacy of using a kinin agent. Nevertheless, kinin agents continue to remain an active area of translational research,⁴⁶ particularly because of the advantage of preferential BTB disruption observed with these vasoactive compounds.^{57,83,109}

Hyperosmotic Disruption

Transient hyperosmotic disruption of the BBB to facilitate its penetration involves the use of an intraarterially delivered osmotic agent, such as mannitol. Mannitol acts by drawing water from the intra- or extracellular compartments into the vasculature, effectively decreasing interstitial and intracellular cerebral volumes. This mechanism of BBB disruption may involve endothelial dehydration, cell body shriveling, and a resultant opening of the spaces between the tight junctions.¹¹⁴ This method was pioneered by Neuwelt and colleagues in 1979,¹¹⁹ and the effectiveness of a 25% mannitol solution was investigated in clinical trials in 1981.¹²⁰ Further analysis in rat models indicated

that this disruption of the BBB is maintained for approximately 30 minutes^{82,179} and that permeability is achieved for between 15 minutes and 4 hours, depending on the molecular weight of the agent used. Current in vivo research efforts involve intraarterial injection of the hyperosmotic agent that induces BBB disruption and subsequent intraarterial injection of the drug of interest. These approaches have indicated that the intraarterial route significantly improves BBB penetration compared with intravenous injection of the drug.^{35,74,117} Additionally, recent research indicated that volume, not flow rate, of the mannitol injection is an important factor influencing the magnitude of the BBB disruption.⁶² In addition to enhancing BBB permeability to drugs, use of mannitol in vivo has been shown to increase permeability of intraarterially injected viral vectors,^{62,113} stem cells,⁷⁰ liposomal vehicles,¹⁰⁸ and antibodies such as BV.²³

In addition to opening the BBB, mannitol may decrease intratumoral hydrostatic pressure, which may enhance chemotherapy delivery by passive diffusion. However, it is important to remember that although BBB disruption with mannitol has been demonstrated, the data about the success of in vivo BTB disruption with mannitol are conflicting.^{35,73,145,179} In addition, earlier research with mannitol has shown that it may preferentially permeate healthy BBB,

thereby increasing neurotoxicity,^{78,84} which is in contrast to the effects seen with vasoactive mediators.^{57,83,109} Despite these observations, Phase I clinical trials with patients who had malignant gliomas have reported significant radiological evidence for tumor reduction and improved progression-free survival after mannitol-mediated BBB disruption followed by treatment with intraarterially injected BV and melphalan/carboplatin, respectively.^{23,74} In summary, these findings suggest that mannitol mediates BBB disruption *in vitro* and *in vivo* for certain tumors, but its efficacy is largely agent dependent. More research is necessary to determine the efficacy of mannitol as a method for delivering chemotherapy in GBM.

Intraarterial Infusion

Intraarterial infusion provides a more favorable approach for BBB penetration because this method avoids the limitations of systemic dilution and toxicity of chemotherapy agents. Initial use of intraarterial infusions for malignant tumors of the brain began in the 1950s following the identification of the BBB.⁶⁶ Recent research with intraarterial infusions has focused on BV because the VEGF plays an important role in cancer stem-like cell (CSC) signaling at the perivascular niche, the heterogeneity of which generates chemotherapeutic resistance of GBM. The perivascular niche, also described as the intratumoral neoangiogenic margin, is a microenvironment housing multiple cell types including CSCs.³⁶ Importantly, CSCs secrete VEGF, and in GBM, the CSCs may differentiate into endothelial precursors, which in response to VEGF signaling can further differentiate into mature endothelial cells, leading to angiogenesis.^{29,163} Therefore, perivascular inhibition of VEGF signaling by BV represents an important method for disrupting GBM progression by limiting the blood supply to CSCs. This may be achieved by selective intraarterial niche disruption by intraarterially infused BV.²⁹ The BBB/BTB would maintain the highly regulated signaling environment in the perivascular niche by limiting drug exposure and retaining tumor-derived signaling molecules within this niche through the abovementioned processes. The upper pore size constraint of the BTB is approximately 12 nm,¹³⁹ which necessitates barrier disruption to enable translocation of BV across the neurovascular complex and accumulation in the intratumoral signaling environment.²⁹ This targeted translocation was achieved in a mouse model in which intraarterial infusion with BV resulted in significantly greater BBB penetration of the medication than with intravenous infusion; an even greater intratumoral BV presence was observed when intraarterial infusion was combined with mannitol injection.²⁷ Burkhardt et al.²⁸ also observed improved progression-free survival in a clinical trial with BV designed to investigate the effect of a single intraarterial infusion of BV followed by standard-of-care intravenous BV therapy to treat recurrent GBM.

Additional clinical trials have evaluated intraarterial injection of cisplatin,⁵⁵ carboplatin,^{64,65,85,96} or methotrexate^{10,64,65,85} to treat additional CNS tumors, and many of these trials utilized BBB disruption with mannitol. To evaluate the use of intraarterial infusion as a single modality for GBM treatment, Theodotou et al.¹⁵² performed

a literature review in 2014, which showed that although intraarterial chemotherapy for GBM was associated with lower median overall survival (14.02 months) than intravenous infusion (16.3 months), it decreased the risk of adverse side effects (1.08 and 1.54 recorded events per patient, respectively).

In summary, the efficacy and safety of intraarterial infusion for treating GBM has been extensively studied. These studies have indicated at best marginal efficacy compared with control treatments. However, as research expands to study newer therapies, intraarterial infusion will likely continue to have a role in providing BBB circumvention.

Surgical Approaches

Surgical circumvention of the BBB involves placing a pharmaceutical agent or eluting system directly into the tumor, brain, or CSF. These delivery agents include controlled-release medications (Gliadel), brachytherapy, intrathecal delivery, intraventricular delivery, intranasal delivery, or a catheter convection system for drug delivery.

Distribution of agents via convection-enhanced delivery involves microinfusions of an agent with micropumps for targeted delivery to the brain parenchyma, and this approach depends on hydrostatic pressures, diffusion, and efflux from the capillaries.⁷³ Examples of delivery agents used are nanoparticles, liposomes, viral vectors, and immunotoxins.¹⁶⁰ Convection systems, though intuitively attractive, result in heterogeneous distribution of these agents, impeding high interstitial fluid pressure, rapid efflux from the injection site,^{118,128} and they may also result in neurotoxicity.⁴³

A study by Sampson et al.¹³⁸ showed that a bolus dose of stereotactic injection into GBM tumors provided no advantage in the distribution volume of chemotherapy agents compared with continuous bulk flow by convection-enhanced delivery. However, this delivery method has been shown to improve survival in a murine model of intratumoral etoposide¹⁴⁶ and has mediated targeted GBM therapy with the immunotoxins DTAT and DTATEGF.⁸⁰ Convection-enhanced delivery was not able to sufficiently deliver O-6-methylguanine-DNA methyltransferase-siRNA/liposome complexes to the brain in rat and porcine models because of a limited radial liposome distribution attributed to the cationic nature of these liposomes.¹⁵⁷ However, this delivery method has been implemented in numerous clinical trials to assess the efficacy of using multiple chemotherapy agents to treat GBM.^{22,33,34,95,161,170} Recently, convection-enhanced delivery was implemented with a transcatheter bone anchor port in an *in vivo* human trial of carboplatin for recurrent GBM; the results suggested use of the method in further clinical trials.¹² In summary, convection-enhanced delivery is currently an active research frontier in the delivery of GBM therapy and will likely be implemented in clinical trials in the near future.

Approaches very similar to convection-enhanced delivery are intraventricular and intrathecal drug administrations into the CSF. These approaches are also dependent on bulk flow as the driving force for distribution within the leptomeningeal space, but do not rely on BBB or BCSFB penetration. Intraventricular dosing of chemotherapy for

GBM has been previously studied.⁴⁹ However, because this delivery route facilitates only very limited exposure of the parenchyma to chemotherapy agents, it is not effective for treating parenchymal disease. Moreover, the large volume of CSF turnover leads to significant diffusion-related declines in leptomeningeal drug concentrations. These factors make these modalities not feasible for treating intraparenchymal GBM.⁷³ However, in clinical trials, leptomeningeal drug concentrations have been shown to be high enough in brain diseases such as carcinomatous meningitis, meningeal gliomatosis, and lymphomatous meningitis whose treatments do not require parenchymal penetration of chemotherapy agents.^{123,173,185} Carcinomatous meningitis may co-occur with multiple systemic malignant tumors, in which case the leptomeningeal space serves as a safe haven for these tumors because of the BBB, which shelters these tumors from exposure to systemic chemotherapy.⁴⁴ The CSF in carcinomatous meningitis shows pleocytosis, elevated protein levels, and glycorrhachia,⁴⁴ which likely are the result of the decreased control over permeability at the BCSFB. In carcinomatous meningitis, tumors may occupy the perivascular spaces and increase the interstitial compartment pressure,¹¹⁴ which limits solute translocation across the BBB, BCSFB, and CSF-parenchymal interface and therefore poses a challenge for treatment. In summary, therapies utilizing the CSF as a means to circumvent the BBB/BCSFB are effective for leptomeningeal disease; however, restricted parenchymal exposure limits its clinical applicability for GBM therapy.

Intranasal delivery of chemotherapy has been hypothesized to result in sufficient concentrations of antineoplastic agents within parenchymal tumors. Clinical trials that have tested intranasal administration of perillyl alcohol to treat CNS tumors have indicated significant clinical tumor remission: a cohort composed of > 80% patients with GBM had a 4-year tumor remission rate of 19%.^{47,48} The chemotherapy agents translocate into the CNS via olfactory and trigeminal nerve pathways.⁷⁶ In addition, intranasal delivery has been recently studied for the delivery of neural stem cells and curcumin-loaded, nanostructure lipid carriers and has shown good results in localizing to tumors.^{106,133} In summary, intranasal administration represents an under-researched method for circumventing the BBB, and early clinical results suggest a potential role for this modality in future clinical trials.

An alternative to injectable chemotherapy agents is the Gliadel wafer. It represents an adjunctive method to surgery for localized chemotherapy delivery; the wafer consists of a surgically implanted, carmustine-loaded polymer for continuous dosing of a medication to a potentially neoplastic environment within a resection cavity. The chemotherapy delivery achieved with Gliadel relies on simple diffusion, falls off sharply within a few millimeters, and lacks precise control of the concentration-time exposure.⁷³ Despite these drawbacks, a systematic literature review of 19 studies with 795 patients reported that using adjuvant Gliadel resulted in a mean survival of 16.2 months, whereas patients receiving the standard of care had a mean survival of approximately 14 months.^{25,149} This study also reported that use of Gliadel resulted in a 42.7% complication rate.²⁵ However, a review of the outcomes in 1013 craniotomies for malignant astrocytoma in which 288 patients received Gliadel wafers observed no significant increase in perioperative complications compared with the standard of care.¹¹ With only a marginal increase in survival and a potentially increased risk of complications, this therapy is at best only marginally indicated for treating GBM.

Efflux Pump Inhibitors

As discussed above, the cerebral endothelium and tumor cells possess a multitude of active efflux transporters,⁶ which may transport compounds that have crossed the BBB back into the neurovascular system. Inhibitors to these transporters could conceivably increase the net flux across the BBB. The previously mentioned ATP-dependent ABCB1 (P-glycoprotein) transporter actively pumps chemotherapy agents such as methotrexate, vinca alkaloids, anthracycline analogs, various monoclonal antibodies, and TMZ out of neoplastic and endothelial cells and into the vascular lumen.^{6,116} Upregulation of the MDR1/ABCG2 transporter contributes to tumor resistance against TMZ.¹³⁵ The proposed mechanism involves a TMZ-induced, active-state conformational change in the MDR1 protein, which generates signaling via epidermal growth factor that serves as an autocrine stimulator of GBM cells to induce expression of the *MDR1* gene.¹¹⁶ The expanding recognition of the role of efflux transporters in chemoresistance suggests that modulating their function could be an appealing strategy in chemotherapy.

One such molecular modulator is erlotinib, an inhibitor of epidermal growth factor receptor kinase. Using a mouse model, investigators have shown that erlotinib prevents TMZ-induced, MDR1-mediated tumor resistance.¹¹⁶ However, this finding was not replicated in a clinical trial, which indicated that adding erlotinib to TMZ therapy did not increase survival of patients with GBM.²⁶ The P-glycoprotein is also the target of an inhibitor, PSC-833 (valspodar), application of which resulted in an increased permeability of the BBB to the chemotherapy agent litaxel and in a 90% reduction in GBM volume in a xenograft mouse model.⁶¹ The scarcity of research into this modality reflects the complex milieu of efflux pumps involved in the BTB/BBB function and the cost involved in the development of these target-specific agents.

Thermotherapy

Thermotherapy can encompass many modalities for producing changes to the BBB and to the tumor mass. They include focused ultrasound, radiofrequency microwaves, laser-induced interstitial thermotherapy, and magnetic disruption.⁹⁹ These modalities induce intracranial hyperthermia, which facilitates potentiation of radiotherapy and chemotherapy, exhibits preferential cytotoxicity to glioma cells, increases BBB permeability, and induces heat-shock protein-mediated cytotoxicity within tumor cells.^{99,167} Most relevant to this discussion is focused ultrasound-mediated disruption of the BBB, which is appealing because it is noninvasive and economical to perform. Focused ultrasound thermomechanically disrupts the BBB. A decrease in ultrasonic intensity coupled with intravenous injection of albumin-coated octafluoropropane

microbubbles produces only a transient opening of the BBB, which decreases the risk for permanent tissue damage.^{81,92} The microbubbles enhance the thermal effects of the ultrasonic energy and decrease the ultrasonic intensity required for disruption of the BBB.⁸¹ Refinement of this process has facilitated a protocol for reliable BBB disruption, and no histological or functional evidence has suggested any damage *in vivo* in nonhuman primates.¹¹⁰

In animal models, ultrasound-mediated disruption has delivered several agents across the BBB, including trastuzumab,⁹⁰ TMZ,¹⁶⁸ doxorubicin,^{93,155} poly (ethylene glycol)-poly (lactic acid) nanoparticles,¹⁸² AP-1 lipoplatin,¹⁷⁷ and self-complementary adeno-associated virus.¹⁵³ The upper size limit for transfer across the BBB through focused ultrasound is 2000 kD,^{38,42} large enough to facilitate transfer of a multitude of therapeutic agents. Notably, experiments in a rat model reported a 38.6% CSF-to-plasma ratio for TMZ transferred with focused ultrasound compared with only 22.7% in control modalities.¹⁶⁸ Disruption of the BBB via ultrasound exhibits sufficient translational research success to warrant further evaluation of this delivery method in clinical trials.

Vehicular Delivery Devices

Vehicular delivery can serve as a stand-alone or adjuvant method for improved delivery of chemotherapy, gene therapy, or gene-silencing RNA across the BBB. The first 2 delivery methods have been called Trojan horses—one relies on molecular targeting and the other on lipophilic features to cross the BBB. Lastly, nanoparticle and viral vector vehicles will be discussed in the context of gene therapy.

Antibodies that facilitate transcytosis of carrier molecules have been called molecular Trojan horses.¹⁹ Using imaging data in a nonhuman primate model, researchers have shown that molecular Trojan horse-mediated recombinant protein transport was effective for translocating antibodies across the BBB.¹⁹ A study by Farrington et al.⁶⁰ involved a therapeutic antibody possessing a previously described BBB-transcytosing arm called FC5,⁴ and a bivalent therapeutic arm fused with the human Fc protein. This antibody increased transfer of 2 natively impermeable molecules, neuropeptide Y and neuropeptide darginin, 30-fold across the BBB.⁶⁰ The FC5 antibody targets the epitopes of the BBB-enriched transporter, and this targeting facilitates receptor-mediated transcytosis.⁴ The low-density lipoprotein receptor-related protein-1 was the target in a Phase I clinical trial for recurrent malignant GBM in which GRN1005, a peptide drug conjugate with paclitaxel, yielded sufficient results to warrant further investigation in Phase II trials.⁵⁴ Other targets also have been used in molecular Trojan horse approaches, such as the transferrin,^{21,184} glutathione,¹⁶ and insulin receptors.^{20,21} However, these targets are less BBB specific and therefore less effective at enhancing BBB permeability. The molecular Trojan horse method was recently investigated for RNA interference via transferrin receptor targeting to gliomas.¹⁸⁴ The molecular Trojan horse approach enables very target-specific focal delivery of recombinant therapy, but a more diffusely dispersed Trojan horse method involves a liposomal vehicle.

The second class of Trojan horse devices involves liposomal vehicles, termed liposomal Trojan horses. These vehicles possess a highly lipophilic character and are therefore more efficient at penetrating the BBB; accordingly, liposomes may be used to deliver agents that cannot cross the BBB to their intraparenchymal targets and help maintain a controlled release of chemotherapy or gene-therapy agents.¹⁸⁶ Liposomal Trojan horse-mediated passage across the BBB was first described in 1980,¹²⁹ and since then, it has been extensively researched for pharmaceutical delivery. The liposomal Trojan horse approach to test efficacy of delivery in treating GBM has been applied in many animal models such as canine,⁹⁴ rat,^{94,136,176} and nonhuman primate models.¹³⁷ Two clinical trials with liposome-loaded doxorubicin for treatment of solid tumor metastasis³² and malignant glioma⁵⁹ reported more successful outcomes than in a control treatment, although in the glioma trial, the median overall survival with the standard of care exceeded that of the liposome-loaded approach. We also note that much of the recent research into liposomal vehicles has focused on its application in gene therapy.

The use of nanoparticles involves a delivery concept similar to that of liposomal Trojan horses, except the method of BBB circumvention involves simple diffusion from a convection-enhanced delivery, intraventricular, or intrathecal source. Experiments in animal models have shown that nanoparticles improved *in vivo* trafficking of chemotherapy agents, such as camptothecin, TMZ, doxorubicin, irinotecan, and vincristine^{14,141,154} and also of gene-therapy agents.¹⁰¹ Nanoparticle-based approaches can uniquely serve in targeted thermotherapy, for example, via stereotactically injecting magnetic nanoparticles and inducing particle vibration via MRI.⁹⁹ This field represents a novel approach to vehicular delivery of chemotherapy with promise for future research and clinical trials.

Vehicular delivery has now expanded to include gene therapy as a cargo. Current research has shown that adeno-virus,^{31,159} adeno-associated virus,^{41,77,91} vesicular stomatitis virus,¹²⁶ retrovirus,¹³⁰ herpes simplex virus,¹³⁰ liposomal Trojan horses,^{89,180,181} Semliki Forest virus,¹³⁴ and synthetic nanoparticles¹⁰¹ can all be used as vehicles for BBB transit. Expansion of gene-therapy approaches to treat neurological disease has included the use of viral vectors for GBM therapy. However, viral vectors, although efficient and customizable by tropism and replication method, are problematic because they may pose a risk for catastrophic immunogenic responses and may not effectively reach tumor cells that have disseminated throughout the brain parenchyma.¹⁸⁶

The side effects of viral vectors have led to greater adoption of synthetic delivery systems, as described above, such as liposomal Trojan horses and nanoparticle-mediated delivery for gene therapy. The advantages of these synthetic delivery systems are that they target tumor cells and can carry large genetic payloads. In addition, many of these delivery methods elicit little or no immune response,¹⁸⁶ making them ideal vehicles for DNA and siRNA transfer. Initial synthetic systems for gene therapy had excessive cationic density and induced significant toxicity by disrupting normal cell functioning.^{105,156} In light of these findings, studies with high-molecular weight hydrophobic

compounds with lower cationic densities have yielded a polymer vehicle with minimal adverse effects from in vivo exposure.¹⁸⁶

Uses of molecular and liposomal Trojan horses, viral vectors, and nanoparticles have involved combinations with hyperosmolar disruption, convection-enhanced delivery,^{134,162} receptor-mediated substrate tagging,⁹⁷ and ultrasound-mediated BBB disruption^{177,182} to provide even better circumvention of the BBB. Results have shown that protocols that combine different delivery modalities statistically significantly improve the penetration of the BBB.^{177,178,182} The efficacy of an approach consisting of a liposome-based, focused ultrasound–assisted approach to open the BBB was measured via dynamic microSPECT/CT imaging of ¹¹¹In-labeled liposomes containing doxorubicin.¹⁷⁸ This approach yielded maximal doxorubicin concentrations with focused ultrasound–assisted BBB disruption that were twice those of a control treatment in a mouse model of GBM.¹⁷⁸

In summary, vehicular delivery for BBB circumvention represents another research frontier and provides a targeted delivery mechanism with application for delivery of many GBM therapies. It also highlights the need for further research into how the aforementioned methods could be further combined to optimize synthetic vehicular delivery across the BBB.

Stem Cell–Mediated Delivery of Therapies

The principle of stem cell–mediated chemotherapy delivery is similar to that of viral vectors. The tropism of the stem cells toward cancer cells may facilitate targeted delivery of chemotherapy, gene therapy, prodrugs, or siRNA agents to brain tumors. Researchers have largely focused on mesenchymal stem cells as delivery agents because of the lack of allogeneic barriers to therapy and because neural stem cells require an autologous source.⁸ Stem cell–based approaches fuel active research in a number of fields, including neurotrauma, neurodegenerative diseases, and neurooncology. The tumor-homing ability of the mesenchymal stem cells is the driving force, indicating their potential utility in glioma diagnosis and therapy.^{53,131} It has been suggested that selecting the mesenchymal stem cells for the presence of specific chemokine receptors could enable control of their homing capability.¹⁸³

The use of mesenchymal stem cells in GBM has included delivery of gene therapy in murine^{9,56} and canine¹⁷⁵ hosts, as well as delivery of synthetic miRNAs in vitro⁹⁸ and in a murine system.¹¹⁵ Neural stem cells have been used to deliver lanatoside C in a murine host with a xenograft human GBM¹⁵¹ and for delivery of carboxylesterase-activated CPT-11 (irinotecan) therapy to xenograft human glioma;¹¹¹ both approaches showed therapeutic efficacy and feasibility. In combination, the results from these studies suggest a progression of stem cell–assisted delivery of chemotherapy in experimental models to the clinical trial stage for GBM treatment.

However, we note some reservation about stem cell–mediated delivery because it has been associated with tumor formation and increased neuroinflammation, despite the innate immunosuppressive character of stem cells.⁷⁹ It has also been reported that mesenchymal stem cells may

support the tumors that they are targeting for therapy.⁷² Despite these challenges, mesenchymal stem cell therapy is being investigated in clinical trials for many diseases, including cancers of the ovary, prostate, and head and neck, as well as for hematological cancers.⁸ Stem cell–assisted chemotherapy may therefore offer a viable clinical option for treating some CNS tumors after the efficacy and safety of this approach have been established in human subjects.

Conclusions

The BBB serves an essential function in the CNS, but its presence necessitates complex therapeutic solutions for treating malignant tumors of the CNS. In this review, we have discussed several current, theoretical, and animal-model methods for circumventing this obstacle to chemotherapy. In view of the high morbidity and mortality rates associated with GBM, many promising methods for its treatment have emerged from years of laboratory research to address the impediments to improved BBB permeability. By targeting the physiological processes at the BBB or at the BTB, research into these new methods and techniques has provided the groundwork for future combinatory therapies and removed hurdles to administering therapeutic agents that were previously ineffective in treating CNS tumors. Researchers and clinicians have great hope that these evolving techniques will contribute to a more effective standard of care for patients with GBM.

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Author Contributions

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