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Advances in Blood-Brain Barrier Disruption to Facilitate Drug Delivery for Infiltrative Gliomas

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KEYWORDS
• Blood–brain barrier • Malignant glioma • Glioblastoma • Low-intensity pulsed ultrasound • Chemotherapy
• Enhanced drug delivery

KEY POINTS
- The blood-brain barrier (BBB) is complex, and it involves tight junctions and active transporters that limit the penetration of most circulating drugs into the brain.
- The BBB is conserved across many different species.
- The use of ultrasound waves paired with circulating microbubbles can transiently disrupt and open the blood-brain barrier, and enhance the permeability of the brain to circulating drugs.
- In humans this can be accomplished through the use of transcranial devices, or skull implantable ultrasounds where the ultrasound waves either penetrate the skull or bypass it.
- Several clinical trials have shown the safety of of this approach, and demonstrated BBB opening using gadilunium and MRI.

INTRODUCTION

The blood–brain barrier (BBB) poses a major obstacle to the effective pharmaceutical treatment of central nervous system (CNS) tumors. The BBB is a network of various interacting cell types (astrocytes, pericytes, microglia and endothelium), and features that are unique to the cerebral endothelium (tight junctions and transport proteins). The BBB effectively seals the CNS from most molecules in the systemic circulation, except for lipophilic molecules smaller than 400 Da [1]. This impermeability protects the brain from many toxins and pathogens but also prevents the delivery of most systemically administered chemotherapies. This is of particular importance when treating infiltrative malignant gliomas like glioblastoma (GBM), which infiltrate to the brain beyond the margins of surgical resection. The residual disease after surgery is

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shielded from systemically administered therapies, leading to predictable patterns of recurrence in the brain that persist beyond the periphery of the resection cavity [2].

Facilitating the delivery of drugs across the BBB would be of great benefit to patients suffering from these cancers. Low-intensity pulsed ultrasound (LIPU) combined with intravenous administration of microbubbles (MB) is an emerging technology with the ability to locally and temporarily open the BBB, enhancing delivery to the brain of systemically administered drugs. Preclinical studies using this technology have extended survival in animal models of glioma [3–6]. As a result, several trials showing the safety and feasibility of repeat LIPU/MB-mediated BBB opening with systemic chemotherapy have been initiated, translating this technology into the clinic [7–9]. Larger trials are currently underway to assess efficacy.

Given the growing interest that this technology has generated, this review summarizes key findings from recent clinical testing of LIPU/MB to open the BB to treat GBM. This will include a summary of the basic principles of LIPU/MB-enhanced drug delivery, its mechanisms of action, evidence for its safety and efficacy, and the current understanding of the pharmacokinetics of this drug delivery technology and its use in the human brain. A summary of ongoing clinical trials that are using LIPU/MB for drug delivery to treat malignant glioma is provided and future directions are posed for consideration.

MECHANISM OF ACTION AND TECHNICAL CONSIDERATIONS

The basic principle of LIPU/MB-mediated BBB opening is to target a region of the brain with low-intensity ultrasound waves while systemically administering inert, gas-filled MB as a cavitation agent. Within the target region, the oscillating acoustic pressure induces the cavitation agent to rapidly expand and contract, thereby exerting a mechanical force upon the vessel wall. This force alters the configuration of the tight junction proteins that normally seal gaps between adjacent endothelial cells, allowing for the temporary paracellular diffusion of drugs into the parenchyma Fig. 1 [10]. This mechanism was elucidated by electron microscopic studies conducted in rabbits, but these experiments also posit that LIPU/MB promotes caveolar transcytosis across the cerebral endothelium, suggesting a complementary transcellular route of drug delivery [11,12].

In preclinical models and in clinical trials a common method to confirm ultrasound-mediated BBB opening is by MR imaging. Gadolinium-based contrast enhancement agents can be administered after treatment and subsequent MRI scans show hyperintensity where the contrast agent extravasates across the BBB and into the parenchyma. This localized contrast enhancement is often used as a surrogate marker for the enhanced delivery of systemic therapies. MRI at later timepoints after "sonication", commonly 24 h, show little to no parenchymal enhancement indicating that BBB integrity has been reestablished [8,13,14]. Similar kinetics of BBB closure have been reported in animal models of LIPU/MB; however, there are also studies showing that the BBB begins to close within 2 to 6 h after sonication [15,16]. To date, the exact rate of BBB closure/restoration after ultrasound-based disruption in humans remains unknown.

Opening of the BBB is dependent upon the dose and nature of the cavitation agent and requires reaching a sufficient mechanical index—a metric based on acoustic pressure and frequency of the ultrasound. Modeling of this phenomenon in rodents reveals that there is a minimum threshold of the mechanical index needed to open the BBB. Exceeding this threshold has the potential to generate adverse effects such as T2* hyperintensity and red blood cell extravasation [17,18].

A phase 1 dose escalation study confirmed the importance of mechanical index in humans and showed that BBB opening highly depends on acoustic pressure. Recurrent GBM patients were treated with LIPU/MB of increasing acoustic pressures, ranging from 0.5 to 1.1 MPa. Following sonication, the patients received systemic chemotherapy and contrast-enhanced MRI to confirm adequate BBB opening. The extent of BBB opening was measured according to the change in pixel intensity in the target region (roughly 1 mL in volume) and graded on a scale of 0 to 3 (0 being no observable enhancement and 3 being marked enhancement observed in grey, white, and subarachnoid tissue). Lower acoustic pressures (0.4 to 0.66 MPa) resulted in lower grade or no BBB opening, whereas higher acoustic pressures (0.8 to 1.1 MPa) yielded more extensive BBB opening graded as 2 to 3 [19].

Multiple animal studies have shown that regular sonication (two to three times a week) at this threshold is well tolerated, yet frequent treatment with higher mechanical indices can result in tissue damage, cellular apoptosis, microhemorrhage, and even temporary behavioral changes [18,20–23]. Negative side effects and adverse events in humans include transient neurological deficits and/or edema. Protocols of LIPU/MB-enhanced chemotherapy in recent clinical trials have sonicated infrequently, typically once a month to coincide with cycles of chemotherapy, and therefore perhaps spared patients from the negative consequences observed in animals [7,9]. Regimens that require weekly or daily treatments with chemotherapy may be too frequent to also involve

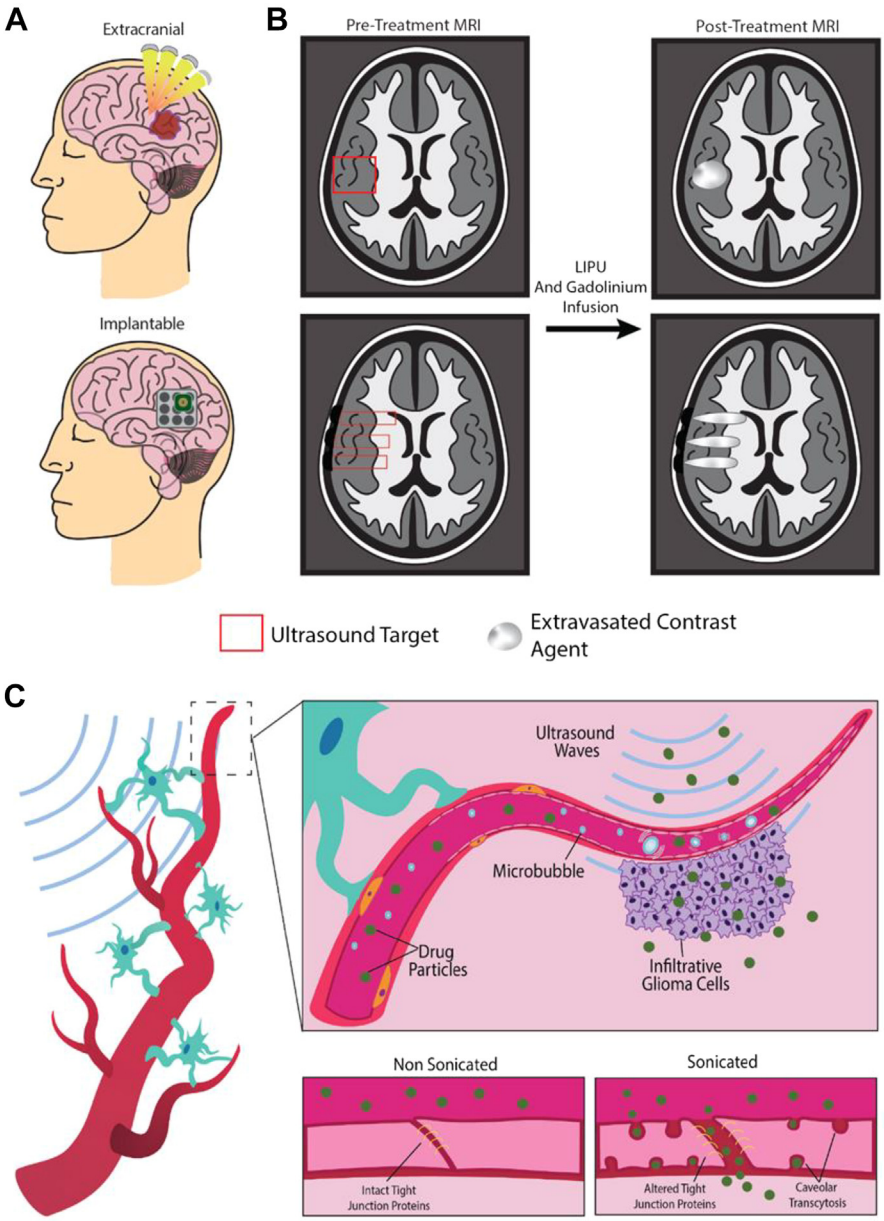

FIG. 1 LIPU/MB-mediated opening of the blood–brain barrier. (**A**) Cartoon illustration of the human patient undergoing focused ultrasound BBB opening. Extracranial ultrasound emitters are targeted toward a region of brain and emit low-intensity ultrasound waves. Lower panel shows the same patient, with an implantable ultrasound device positioned over the target tissue. (**B**) MRI imaging is often used to confirm BBB opening. The extravasation of contrast enhancement agents on a posttreatment MRI is often taken as an indicator of effective BBB opening. (**C**) Illustration of LIPU/MB-mediated BBB opening at the level of the cerebral vasculature. The mechanical force exerted on the endothelium by the oscillating microbubbles alters the confirmation of tight junctions, allowing for paracellular diffusion of circulating drugs into the parenchyma. Caveolar transcytosis can also be a mechanism of enhanced drug delivery following FUS.

LIPU/MB. Integrating this technology into new treatment regimens will require balancing the frequency of treatment, with mechanical index, and sufficient chemotherapy exposure to maximize the benefit to patients.

SAFETY AND FEASIBILITY OF LOW-INTENSITY PULSED ULTRASOUND/MICROBUBBLE-MEDIATED BLOOD–BRAIN BARRIER OPENING IN HUMANS

Two strategies of LIPU/MB predominate for human testing, which can be broadly classified as either extracranial or implantable Fig. 1. Devices like the ExAblate System developed by InSightec use large external arrays of ultrasound emitters that open the BBB within a focused volume of tissue. Targeting the array requires target guidance with MRI, meaning this technique is often referred to as Transcranial Magnetic Resonance Guided Focused Ultrasound (TcMRgFUS). The obvious advantages of this approach are that it is noninvasive and able to target brain structures with an impressive resolution, even in deep and eloquent tissues [24,25]. However, the primary obstacle to this approach is the human skull. The thick cranial bone diminishes and alters the ultrasound waves, requiring multiple ultrasound generators to be focused on the target for extended periods of time. The ultrasound focal spot is much smaller than the targeted region. The targeted region has to be scanned with the focal spot, which requires an accurate registration between the external ultrasound emitter, the patient, and MR images, and can take several hours [26]. In contrast to extracranial devices, implantable LIPU/MB devices like the SonoCloud devices developed by Carthera are designed to be surgically implanted in a bone window in the patient's skull, sitting directly over the meninges and underlying brain. Although this carries the inherent risks of intracranial surgery, the device could be implanted during a neurosurgical procedure for resection or biopsy, avoiding the need for multiple surgeries. The advantage of the implantable approach is that the power of the ultrasound waves is unimpeded by the bones of the skull, allowing for protocols that use low-energy waves that last for minutes rather than hours. This approach also obviates the need to coordinate with MRI as the device is fixed in the position directly over the target tissue [19,27]. Once implanted, the sonication field of the device is fixed and as such the target can't be altered, but this can be somewhat overcome by using devices with multiple ultrasound emitters, covering a wider volume of tissue. The skull-implantable ultrasound technology might allow for rapid adoption into the community setting, as there is little infrastructure needed for repeated, outpatient ultrasound-based BBB opening, which therefore can take place in standard chemotherapy infusion suites. Second-generation larger skull implantable device called the Sonocloud-9 has been developed by Carthera and is currently undergoing testing in clinical several trials led by our group.

Studies in human patients have shown that both the extracranial as well as the implantable ultrasound devices can elicit LIPU/MB-mediated BBB opening, and are well-tolerated when performed as frequently as once every few weeks with no evidence of neurological deficits or local toxicity [24,25,28]. Clinical trials have already used both kinds of devices in combination with systemic chemotherapy to treat malignant gliomas. Mainprize and colleagues [8] successfully treated five patients with recurrent GBM with TcMRgFUS combined with either temozolomide ($n = 4$) or liposomal doxorubicin ($n = 1$). Following treatment, patients were observed for 24 h before undergoing intracranial surgery, with no adverse events. Park and colleagues [9] treated six patients of newly diagnosed GBM regularly with monthly TcMRgFUS-enhanced temozolomide for up to 6 months. A total of 145 sonications were completed across the whole study, with no adverse events attributed to treatment. Idbaih and colleagues [7] conducted a phase I dose-escalation study, the largest to date to use an LIPU/MB-enhanced chemotherapy regimen, treating 21 recurrent GBM patients with up to 12 treatments of LIPU/MB enhanced delivery of carboplatin (NCT02253212). This study used an implantable, single-emitter LIPU/MB device designated the SonoCloud-1. A total of 65 sonications with chemotherapy were conducted over the study, with a median of three sonications per patient. Only two serious adverse events were ruled in relation to the sonication procedure–two instances of Grade 4 cerebral edema that resolved with treatment.

Thus far, regular LIPU/MB-based delivered chemotherapy appears to be a safe and feasible treatment for patients with malignant glioma. Recent clinical trials have shown that regular LIPU/MB by itself is well-tolerated in non-tumor-bearing patients, and there appears to be no additional neurological toxicity in glioma-bearing patients when systemic chemotherapy is added. Ongoing clinical trials with expanded patient cohorts will provide a better sense of the safety of LIPU/MB-based-delivered chemotherapy.

EVIDENCE FOR ENHANCED DRUG DELIVERY

Several preclinical studies have directly quantified the concentrations of various drugs in the brains of animals

following LIPU/MB. Rather than rely upon solely MRI to map opening of the BBB, these studies may use colorful or fluorescent tracer molecules to map the distribution of drugs in the parenchyma ex vivo. This allows researchers to easily identify sonicated and nonsonicated brain tissues, so they can be interrogated for differences in drug concentration.

In 2020, our group published a study wherein tumor-bearing mice were treated with LIPU/MB-based delivery of paclitaxel. Rather than rely upon MRI, fluorescein was co-injected with chemotherapy and imaged ex vivo within 45 min of sonication by fluorescent microscopy. Sonicated brain showed intense fluorescence (resulting from the leakage of fluorescein into the parenchyma), making it readily distinguishable from the surrounding tissue. Downstream drug quantification also showed that these tissues had three- to four-fold higher concentrations of paclitaxel compared with nonsonicated brain regions from the same mice. In nontumor mice, therapy was well-tolerated with no impact on animal body weight or additive toxicity. Four treatments of LIPU/MB enhanced paclitaxel also extended survival in mice with patient-derived xenograft (PDX) tumors compared with tumor-bearing mice treated with systemic chemotherapy alone [5]. A similar study published the year before used Evan's Blue as a tracer molecule for LIPU/MB enhanced delivery of carboplatin. LIPU/MB increased the concentrations of carboplatin in sonicated brain fourfold and significantly extended survival in mice bearing PDX-derived glioma compared with glioma-bearing animals treated with just systemic chemotherapy [4].

Studying the pharmacokinetics of LIPU/MB enhanced drug delivery of systemically administered drugs will be vitally important for future studies. A solid understanding of the factors that affect drug accumulation and clearance from the brain following sonication could help clinicians to select chemotherapies knowing the effective dose that will reach the brain. A preclinical study conducted by Chen and colleagues [29] provides some early insight into LIPU/MB-based pharmacokinetics that could inform future treatment protocols. The researchers sonicated a group of mice before administering an array of fluorescently labeled dextrans of increasing size (3, 70, and 2000 kDa). Twenty minutes after treatment the mice were euthanized, and the relative concentrations and distributions of each dextran were estimated by immunofluorescent imaging. Within the sonicated brain, the smallest dextrans were highly concentrated and well-distributed throughout the whole tissue, whereas 70 kDa dextrans were less concentrated and localized around blood vessels. At the acoustic pressure used (0.56 MPa), the 2000 kDa dextrans failed to cross into the parenchyma at all. A similar study by the same group treated mice with an identical array of fluorescent dextrans and varying acoustic pressures (0.31 to 0.84 MPa) to determine the effects of increasing the mechanical index on the size of drugs delivered. At 0.31 MPa, only 3 kDa dextrans were able to cross into the parenchyma, but elevated acoustic pressures were associated with the delivery of larger and larger dextrans up to a maximum of 2000 kDa at 0.84 MPa [30]. These studies indicate that drug accumulation and distribution after LIPU/MB likely depends upon drug size, but can be adjusted for using higher-intensity ultrasound protocols. This has obvious implications for the clinical translation of this technology, as smaller therapeutics may more readily reach therapeutically relevant concentrations in the brain after LIPU/MB, whereas regular sonication at higher intensity could have negative consequences for patients.

Although there is no shortage of animal studies proving that LIPU/MB can quickly enhance intracerebral drug concentrations, this achievement has yet to be recapitulated in humans. Recent clinical trials have focused on safety and efficacy over validating preclinical pharmacokinetics. As a result, there is scarce evidence for the concentrations of drugs in the human brain following LIPU/MB. The extravasation of gadolinium-based contrast agents (approximately 1 kDa) following treatment is typically used as a surrogate for enhanced delivery of concomitant chemotherapies, but this obviously does not provide a direct quantification of other drugs in the brain, particularly if they are of a larger size than the contrast agent.

Mainprize and colleagues [8] (NCT02343991) were able to biopsy the non-eloquent peritumoral brain, both sonicated and nonsonicated, from patients who had received TcMRgFUS the day before. Biopsies were collected for all five patients, but chemotherapy was detected in only two (one treated with temozolomide and the other with liposomal doxorubicin). In both cases, the sonicated biopsies had marginally higher concentrations of chemotherapy than the nonsonicated (0.22 ng/mg in sonicated brain versus 0.15 ng/mg in nonsonicated brain in the case of liposomal doxorubicin, 3.47E–4 ng/mg in the sonicated versus 0.45E–4 ng/mg in non-sonicated brain in the case of temozolomide). Anastasiadis and colleagues [31] (NCT03322813) conducted a similar experiment and indirectly measured the abundance of fluorescein several hours after TcMRgFUS using confocal microscopy. In this study, 4 patients of glioma were treated with TcMRgFUS before undergoing a fluorescence-assisted surgical

resection of their tumors. Sonicated and non-sonicated non-eloquent peritumoral brain tissue was biopsied during the surgery and the intrinsic fluorescence of each tissue sample was measured by confocal microscopy. The enhancing tumor tissues had the highest mean intrinsic fluorescence overall, but the fluorescence of the sonicated peritumoral tissues was significantly greater than that of the nonsonicated tissues. A more recent study by Meng and colleagues [32] used TcMRgFUS to enhance the delivery of radiolabeled trastuzumab, a monoclonal antibody ($\sim$150 kDa) in 4 patients with Her2 Positive breast cancer brain metastases. The researchers estimated the penetrance of the antibody in enhancing disease and adjacent nonenhancing peritumoral tissue, by comparing gamma emission values between pre- and post-treatment SPECT imaging. The Standardized Uptake Value Ratio (SUVR) between both scans was then used to estimate the effect of TcMRgFUS on antibody delivery. On average, TcMRgFUS significantly increased the SUVR of the radiolabeled isotope in the volume of sonicated tissue, from 1.41 to 2.29 ($P < 0.001$, paired t-test) at 4 h after treatment. At an even later timepoint of 48 h after treatment, this value further increased from 2.44 to 4.36 ($P < 0.001$, paired t test).

These three studies represent the evidence to date of the effect of LIPU/MB on drug concentrations in the human brain. Although the concentrations reported by Mainprize and colleagues are too low to be therapeutically relevant, it should be noted that these tissues were sampled a whole day after sonication. Animal studies indicate that the fold changes in drug concentrations are much higher at earlier timepoints (within 1 h of sonication) and so it is possible that the concentrations reported by Mainprize and colleagues are a consequence of the delay between sonication and biopsy [4–6]. The data presented by Meng and colleagues [32] would suggest the opposite, as the highest estimates of radiolabeled antibodies were achieved two days after sonication. However, the authors of this study estimated drug delivery within a volume of tissue that encompassed both radiographically enhancing disease and non-enhancing peritumoral tissue. Given that the baseline (nonsonicated) SUVR values were also higher at the 48-h timepoint compared with the 4-h timepoints, it is possible that the diffusion of the drug from enhancing disease to non-enhancing tissue plays a role in drug delivery over time, but that this is enhanced by LIPU/MB.

To characterize the effect of LIPU/MB on drug concentrations in the brain it would be important to sample sonicated and non-sonicated tissues at varying timepoints after sonication, at distance from enhancing disease and using therapeutics of different size. This would provide insights into the pharmacokinetics of LIPU/MB-enhanced chemotherapy. Such findings could be used to refine treatment regimens used in ongoing or future clinical trials.

CURRENT CLINICAL TRIALS

Idbaih and colleagues [7] conducted the largest study to date using an implantable LIPU/MB device to treat patients with recurrent GBM. Although this was a Phase I dose escalation study that focused on safety and feasibility, the authors did note a marginal difference in survival between two subsets of patients: one group of 11 patients who were sonicated with higher acoustic pressures and another group of 8 patients sonicated at lower acoustic pressures. The higher-pressure group showed marginally extended progression-free survival (4.11 vs 2.73 months) and overall survival (12.94 vs 8.64 months) compared with the lower-pressure group. The authors also noted that when progression was observed on MRI, it often occurred outside of the sonication field of the device. This could also hint that LIPU/MB is enhancing chemotherapy concentrations within the target tissue, with no effect on disease outside of the focus of sonication. Although this study was not powered to determine the significance of these observations, this is a tantalizing hint at the potential efficacy of implantable devices for LIPU/MB-based BBB opening.

There are three active clinical trials using implantable devices to treat malignant glioma (NCT04446416, NCT04528680, and NCT03616860). In all three of these studies, the device is the SonoCloud-9, the successor to the SonoCloud-1 used by Idbaih and colleagues As the name suggests, this device has 9 individual ultrasound emitters rather than just 1, all united in a 3×3 square-shaped array on a single biocompatible mesh. Each emitter can be activated individually, allowing for multiple fields of sonication which collectively target a larger volume of tissue shaped to fit the unique anatomy of the patient's disease. All three studies will use treatments of LIPU/MB that coincide with cycles of chemotherapy, which include carboplatin (NCT03744026), paclitaxel (NCT04528680), or temozolomide with concomitant radiation therapy (NCT04614493). Similarly, there are four ongoing trials using extracranial LIPU/MB devices such as the ExAblate System by InSightec or the NaviFUS system. The largest of these trials (NCT04440358 and NCT04417088) are using LIPU/MB with monthly treatments of carboplatin. Table 1 summarizes ongoing clinical trials using ultrasound to enhance the delivery of systemically administered therapies to the brain for infiltrative gliomas.

TABLE 1
Summary of Ongoing Clinical Trials Using Low-Intensity Pulsed Ultrasound-Enhanced Chemotherapy to Treat Malignant Glioma

Clinical Trial ID	Study Title	Phase	Estimated No. of Patients	Disease	External /Internal (Device)	Therapy
NCT04614493	Innovative SonoCloud-9 Device for Blood–Brain Barrier Opening in First Line Temozolomide Glioblastoma Patients.	II	66	Newly Diagnosed GBM	Implantable (SonoCloud-9)	RT/TMZ
NCT04528680	Ultrasound-Based Blood–Brain Barrier Opening and Albumin-Bound Paclitaxel for Recurrent Glioblastoma	I/II	39	Recurrent GBM	Implantable (SonoCloud-9)	Paclitaxel
NCT03744026	Safety and Efficacy of Transient Opening of the Blood–Brain Barrier (BBB) with the SonoCloud-9 (SC9-GBM-01)	I/II	33	Recurrent GBM	Implantable (SonoCloud-9)	Carboplatin
NCT04440358	Exablate Blood–Brain Barrier Disruption with Carboplatin for the Treatment of Recurrent GBM	I/II	50	Recurrent GBM	Extracranial (InSightec ExAblate)	Carboplatin
NCT04417088	Exablate Blood–Brain Barrier Disruption for the Treatment of Recurrent GBM in Subjects Undergoing Carboplatin Monotherapy	I/II	30	Recurrent GBM	Extracranial (InSightec ExAblate)	Carboplatin
NCT04998864	Assessment of Safety and Feasibility of ExAblate Blood–Brain Barrier Disruption in GBM Patients	I	5	Newly Diagnosed GBM	Extracranial (InSightec ExAblate)	Temozolomide/ RT
NCT04446416	Efficacy and Safety of NaviFUS System add-on Bevacizumab in Recurrent GBM Patients	I	10	Recurrent GBM	Extracranial (NaviFUS)	Bevacizumab

CONCLUDING REMARKS AND FUTURE CONSIDERATIONS

The BBB remains the foremost obstacle to effective pharmaceutical treatment of malignant glioma. LIPU/MB is an emerging technology capable of focally, temporarily, and reversibly opening the BBB and enhancing drug delivery to the brain. Preclinical studies have successfully used this technology to treat animal models of glioma while also elucidating the basic principles of ultrasound-mediated BBB opening and enhanced drug delivery. Clinical testing of LIPU/MB with systemic chemotherapy is still in the early stages, yet relevant clinical trials from the last several years have shown the safety and feasibility of this approach.

Both implantable and extracranial devices have been used in combination with systemic chemotherapies to treat malignant glioma with few serious adverse events. Phase I/II clinical trials with expanded patient cohorts, are currently underway to determine the efficacy of this technology in extending patient survival.

Animal studies have directly quantified the concentrations of chemotherapies in the sonicated and nonsonicated brain, yet this topic remains relatively unexplored in humans. Mainprize and colleagues have given the most direct measurement to date of chemotherapy concentrations in the brain following sonication, but the concentrations in the brain soon after sonication remain unknown. Animal models of LIPU-enhanced pharmacokinetics indicate that intracerebral drug concentrations are highest immediately following sonication. The size of a drug also appears to impact distribution and accumulation in the brain following sonication, with larger molecules requiring sonication at elevated acoustic pressures. Ongoing in-human studies are performing biopsy peritumoral tissues soon after drug administration and sonication to provide the most accurate measurement of peak intracerebral drug concentrations. MRI and SPECT imaging in humans and animals has shown that the BBB is reestablished within 24 h, but other animal models estimate a rapid rate of closure that begins soon after sonication. Future treatment protocols will likely have to be customized according to the drug of choice, balancing factors like acoustic pressure, size of the therapy, and time of administration, to maximize the benefit offered to patients.

Other relevant questions that remain unanswered are the functional and ultrastructural consequences on the BBB and brain tissue following LIPU. The accepted mechanism of LIPU/MB-enhanced drug delivery was developed in animal models and revolves around mechanically induced alterations to tight junction conformation, enhancing the paracellular diffusion of drugs through the cerebral vasculature. These findings, reported in rabbits, used LIPU devices, acoustic pressures, and cavitation agents that are different from what is used today in human patients. Given these differences, observations made in animals may not readily translate to the human BBB. The same animal studies also implicate a time-dependent mechanism of caveolar transcytosis, as a secondary route of entry into the brain. Considering that transcytosis is tightly regulated at the BBB, this would mean that the mechanical stress induced by the ultrasound has broader, unrecognized molecular impacts on the cerebral endothelium, which could be exploited by future treatment regimens and maximize the benefit to patients [33].

CLINICS CARE POINTS

- The blood brain barrier (BBB) prevents the delivery of most systemically-administered chemotherapies and contributes to the progression of CNS malignancies. Low-Intensity Pulsed Ultrasound with Microbubbles (LIPU/MB) is an emerging technology capable enhancing the delivery of circulating drugs to the brain.
- Preclinical models of LIPU/MB-enhanced chemotherapy have successfully extended survival in animal models of glioma. Clinical trials have shown monthly LIPU/MB-enhanced chemotherapy to be safe and feasible in patients.
- The extent to which LIPU/MB-enhances drug delivery across the human BBB is unknown. Preclinical models have shown three to fourfold increases in drug concentration within an hour of sonication. Human studies have estimated much lower concentrations 24 hours after treatment.
- Extracranial LIPU/MB devices are less invasive and can be highly targeted, but require longer treatment times MRI guidance. Skull-implatable devices require surgery and have fixed positioning, yet require significantly less time to activate and can be adopted into outpatient chemotherapy infusion settings.

DISCLOSURES

M. Canney, G. Bouchoux, and C. Desseaux are employees of Carthera, inventors of patents related to the technology, and have stock ownership in Carthera. A.M. Sonabend, D.Y. Zhang, and R. Stupp are coauthors of a patent filed by Northwestern related to this technology. R. Stupp has acted or is acting as a scientific advisor or has served on advisory boards for the following companies: Alpheus Medical (formerly Craniovation), AstraZeneca, Boston Scientific, Carthera, Celularity, GT Medical, Insightech, Lockwood (BlackDiamond), Northwest Biotherapeutics, Novocure, Inc., Syneos Health (Boston Biomedical), TriAct Therapeutics, Varian Medical Systems. A. Carpentier is the inventor of the SonoCloud device patented by Sorbonne University and developed by Carthera Inc, has ownership interest in Carthera Inc and is a paid consultant to Carthera Inc. A.M. Sonabend and R. Stupp have received in-kind (drug) support from BMS, in kind (ultrasound devices) and research support from and Carthera.

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