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# Applications of transcranial focused ultrasound for primary brain tumors

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**Abstract**

Individuals diagnosed with primary brain tumors (PBTs) experience major morbidity and mortality, compounded by the risks associated with standard-of-care brain-directed therapeutic approaches. Recurrence remains inevitable in patients with high-grade gliomas (HGG), due to their brain-infiltrative growth hindering surgical removal, immunosuppressive tumor-microenvironment, dynamic disease evolution from their high cellular and molecular heterogeneity, and multi-faceted challenges to brain drug delivery, primarily stemming from the blood–brain barrier (BBB). This expert narrative review describes the current state of transcranial focused ultrasound (FUS), and its multi-modal applications for PBTs, including delivery of therapeutic agents and sono-liquid biopsy (via BBB opening), immunomodulation, radio-sensitization, and direct destruction of tumor cells/tissue via thermoablation, histotripsy, and sonodynamic therapy. BBB opening-based approaches have the most promising clinical evidence so far, warranting randomized comparative evalutions. Further translation will require standardized FUS treatment protocols, translational investigations nested into trials, coordinated global efforts, strategic trial design incorporating methodological advances, and implementation approaches enabling broader participation. Worldwide efforts to advance FUS will be aided by ongoing device evolution, support from professional societies, and the development of FUS research consortia (like ReFOCUSED). FUS applications open a potential combinatorial path forward with systemic therapies for "adaptive theragnostic" tumor management, thus targeting the root causes of therapeutic failure for HGG patients.

## Key Points

- Transcranial MR-guided MB-FUS with real-time acoustic emissions closed-feedback-loop capabilities allows for safe, controlled, transient BBB opening, enabling precision drug delivery and sono-liquid biopsy.
- Multiple FUS devices are being tested for different indications with different adjustable sonication parameters, warranting efforts to enable comparative evaluation and standardization.

Individuals diagnosed with primary brain tumors (PBTs) experience substantial morbidity and mortality, compounded by the risks associated with the current standard-of-care (SOC) brain-directed therapeutic approaches.[1,2] While the outcomes of patients with low-grade PBTs have steadily improved over the past few decades,[3] patients with high-grade gliomas (HGGs) have limited effective therapeutic options, despite decades of drug discovery efforts and thousands of patients enrolled in clinical trials of novel therapies.[4–6] Glioblastoma (GBM), the most common malignant PBT has a five-year

OS < 10%,[4,5] where median overall survival (mOS) has improved by only a few months over the past three decades.[4–6] Breakthrough therapeutic paradigms are thus needed to advance beyond the current SOC approaches.[5,6]

Transcranial focused ultrasound (FUS) has emerged as a novel non-invasive, brain-directed energy delivery approach, with multiple applications (**Figure 1**), being explored for multiple PBT types (**Figure 2**).[7–9] This multidisciplinary, expert, narrative review describes the current state of both FUS and its multi-modal applications for PBTs, including delivery of therapeutic agents[10,11] and plasma sono-liquid biopsy,[12–15] immunomodulation,[16,17] radiosensitization, and direct tumor cell/tissue destruction using thermoablation,[18,19] histotripsy,[20–25] and sonodynamic therapy (SDT)[26–28] (**Figure 1**, **Table 1**).[29] Our companion review, in this supplement, examines FUS for brain metastases,[30] while another expert group has discussed implantable ultrasound approaches.[31] Discussed below are preclinical and clinical insights, as well as challenges and opportunities for the field. Completed and ongoing FUS trials for PBTs are described in the Supplementary Appendix (**Supplementary Tables S1 and S2**).

## Principles of FUS

### Technical Advances Enabling Transcranial FUS

In the 1950s, the brothers William and Francis Fry pioneered the use of high-intensity focused ultrasound (HIFU) for brain lesioning, which did require skull removal.[9] Lars Leksell later improved this system with a stereotactic frame and a new transducer.[32–36] Since then, several advancements have made noninvasive, precisely targeted FUS possible:

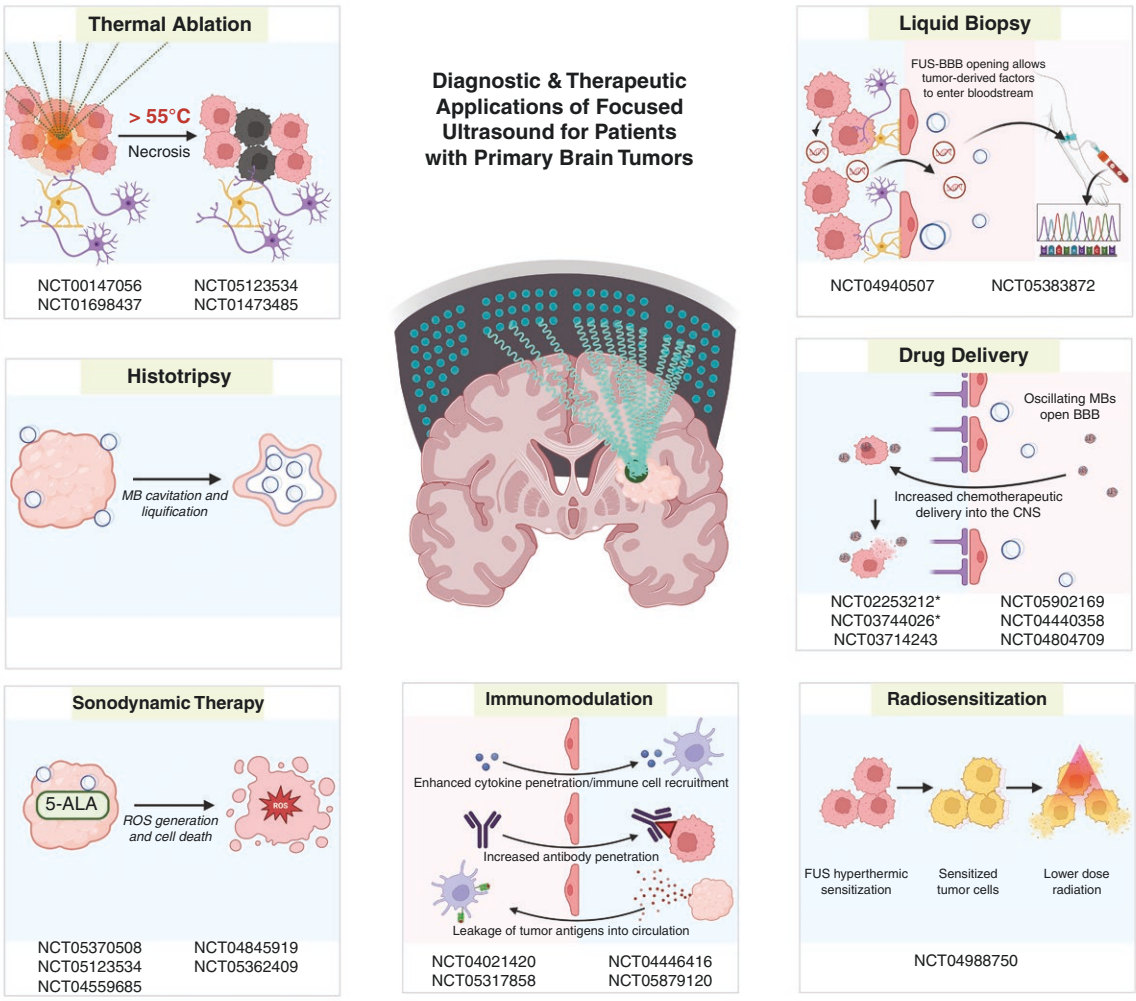


**Figure 1. Diagnostic and therapeutic applications of FUS for patients with brain tumors.** The translational readiness of these applications has been described in Table 1. Created in https://BioRender.com

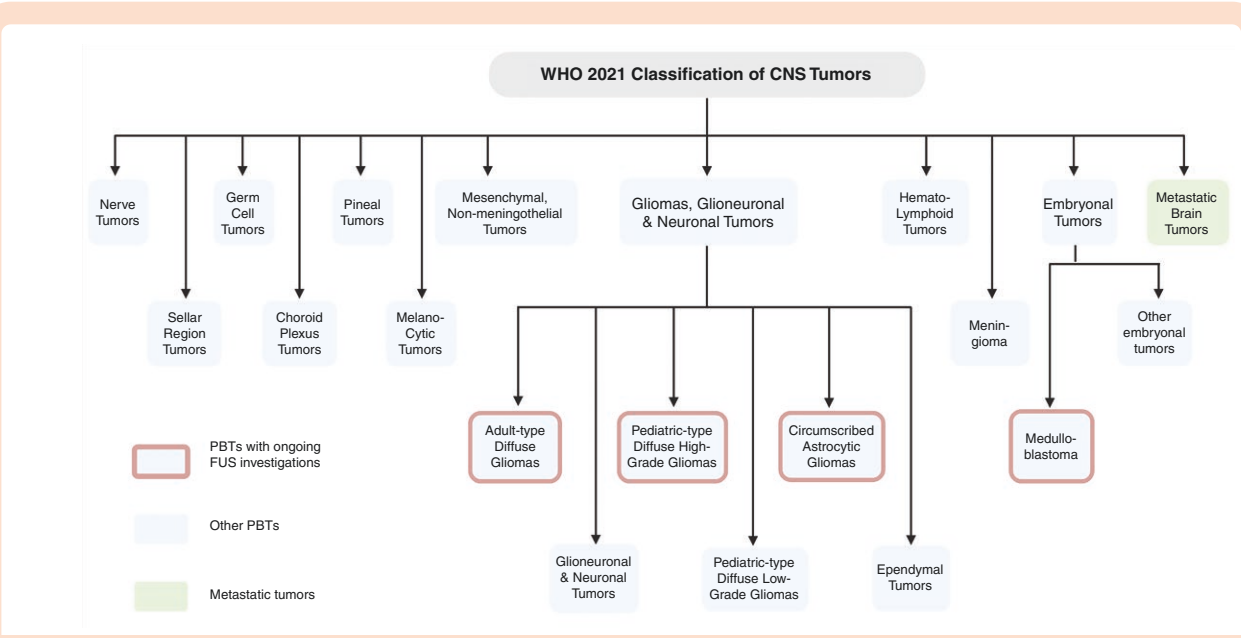


**Figure 2.** **Brain tumor types for which focused ultrasound is primarily being investigated.** Figure created in the context of the WHO 2021 Classification of CNS Tumors (WHO CNS5). Created in https://BioRender.com.

- **Phased Array Transducers**: Enabled precise control of ultrasound beams,[9] allowing beam focusing without energy loss or beam distortion.[37–39]
- **Aberration Correction Algorithms**: Helped address skull-induced distortions[40] using imaging and computational methods,[40–42] with open source developments.[40,43]
- **Integration with Real-Time Image-Guidance**: Use of MR-guidance or neuronavigation enhanced targeting accuracy and clinical feasibility.[9,44,45]
- **Real-time Closed-Feedback-Loop (CFL) Power Control**: Ensured safety and effectiveness by providing a paradigm for real-time monitoring and adaptive power-cycling, initially based on temperature monitoring on MR thermometry for HIFU,[46] and later through acoustic emissions monitoring (AEM) through passive cavitation detectors (hydrophones),[47,48] for low-intensity focused ultrasound (LIFU).[49]

These innovations made the delivery of acoustic energy through noninvasive transcranial focused ultrasound (FUS) clinically feasible. Broadly, acoustic energy can be utilized in either thermal mode or mechanical mode, both having multiple downstream applications (**Figure 1**).[50]

### Thermal Modes of FUS

Initial efforts to use brain-directed FUS began with investigations into the thermal mode of ultrasound.[18,19,51–53] This can be harnessed for direct thermoablation, where high temperatures cause irreversible localized tissue damage,[18,19,51] or for hyperthermia, where moderate temperature increase enhances the effectiveness of radiation therapy (RT),[29,51] or increases local perfusion.[54] HIFU delivery is supported by image guidance (either MR-guided or neuronavigation-guided) and real-time feedback using MR thermometry, with the critical metric of CEM43.[55]

### Mechanical Modes of FUS

Using LIFU, directed ultrasound waves can be used to excite intravascularly administered microbubble (MB) in targeted regions, leading to phenomena like stable cavitation and microstreaming. MBs, such as DEFINITY® (Lantheus, North Billerica, MA, USA), Sonoview (Bracco, Milan, Italy), and USphere (Trust Bio-Sonics, Hsinchu County, Taiwan), amongst others, are micron-sized inert gas-filled bubbles (phospholipid or polymer lipid shells), initially developed as contrast agents for ultrasound imaging approaches like echocardiography. Their excitation causes local biomechanical perturbation, including transient disruption of tight junctions of the endothelium, a primary component of BBB.[49] This approach is referred to as MB-enhanced FUS (MB-FUS), with the interactions being tunable by adjusting factors like ultrasound frequency, power, sonication time, MB dose, and the critical metric of AEM-based sonication dose. Key recent milestones in the clinical application of MB-FUS BBBO are described in **Figure 3**.

## PBT-Related Challenges: Opportunities for FUS

Effectively managing aggressive PBTs like high-grade gliomas (HGGs) is hindered by multiple challenges—whilst some are shared across solid tumors, many others are distinct to HGGs.[56] Many of these, as discussed below and in

**Table 1.** Current State of Applications of Transcranial FUS for Primary Brain Tumors

| Transcranial FUS application | Sufficient preclinical evidence? | Safety shown Clinically? | Feasibility shown Clinically? | Utility# shown clinically? | RCT data? | Translational readiness of approach |
|---|---|---|---|---|---|---|
| Delivery of Therapeutic Agents | ✔ | ✔ | ✔ | ✔ | - | High |
| SLB/Plasma-Based Diagnostics | ✔ | ✔ | ✔ | ✔ | - | High |
| Immunomodulation | ✔ | ✔ | ✔ | - | - | Moderate |
| Sono-Dynamic Therapy | ✔ | ✔ | ✔ | - | - | Moderate |
| Radiosensitization | ✔ | ✔ | ✔ | - | - | Moderate |
| Thermoablation% | ✔ | ✔☎ | -% | - | - | Low |
| Histotripsy* | ✔ | Unclear$ | - | - | - | Low |

Table prepared based on review of indexed and grey literature, based on advances up until April 2025.
#Utility seen in clinical studies, being either diagnostic accuracy for diagnostic applications (like sono-liquid biopsy or SLB) and clinical efficacy for therapeutic applications (like thermoablation). Refer to text for NCT numbers.
$Transcranial histotripsy, in its currently delivered format, has unclear safety for clinically relevant tissue volumes.
%Thermoablation has been reported to be safe for small brain volumes and low procedure times, increasing which leads to risk of skull heating and/or hemorrhage when performed without real-time thermometry. With use of thermometry, the temperature rise is insufficient to cause tissue ablation.

specific sections, are ripe for disruption through various FUS applications.

HGGs display aggressive, brain-infiltrative growth, which often extends several centimeters beyond the contrast-enhancing region. This peri-tumoral infiltration precludes surgical resection from complete tumor elimination both at diagnosis,[57–59] and recurrence,[60] howsoever supramaximal the resection may feasibly be. The challenge is compounded for tumors located in deep-seated or "eloquent" brain regions, where oncological principles need to be balanced against functional preservation (onco-functional balance). Post-resection, remnant cells (which drive recurrence) remain shielded from systemic therapies, due to the blood–brain barrier (BBB) and blood–tumor barrier (BTB).[61,62] Even the most widely used systemic therapy for HGGs, that is, temozolomide (TMZ), achieves a brain interstitium/plasma ratio of < 20%.[63] For HGGs, spatially targeted FUS offers an opportunity for peri-tumoral, targeted BBB opening (BBBO) for precision drug delivery against infiltrating tumor cells. It also allows for exploring drugs beyond TMZ and lomustine/CCNU (either single-agent or given as PCV regimen), whose survival benefit for GBM is in months.[5]

HGGs also have a uniquely locally immunosuppressive ("cold") tumor microenvironment (TME), with further systemic immunosuppressive effect of SOC therapies like TMZ and corticosteroids.[64–66] The tumor-TME interactions distinctly inhibit the desired immune response against the HGG cells, as well as systemic efforts to bolster the response, such as through immune checkpoint inhibitors (ICIs). Randomized trials of immunotherapies have overall not found success for HGG patients in improving survival.[67–69] FUS-enabled immunomodulation offers an opportunity for reactivating this cold TME.

HGGs possess high cellular and molecular heterogeneity, with multiple neoplastic subclones within the tumor.[70,71] A rapid evolution of HGGs' molecular attributes occurs under therapeutic pressures (thought to be faster than most solid tumors).[72–74] The dynamic disease evolution has been recently recognized to be driven by extra-chromosomal DNA (ecDNA).[75] Of note, the expression of epidermal growth factor receptor (EGFR), a key oncogenic receptor tyrosine kinase, gets reduced under therapeutic pressure from anti-EGFR therapies, due to ecDNA dynamics (such as high-frequency of EGFR amplification and variants).[73–79] These challenges are underscored by sobering failures of trials of molecularly targeted therapies,[80] such as EGFR inhibitors and VEGF inhibitors, for HGGs.[73,81–84] Because of rapid evolution, longitudinal molecular evaluation is needed[74,85,86] but is limited (a) through repeated tissue collection by the morbidity and complexity of repeat brain biopsy,[85–87] as well as (b) through standard blood liquid biopsy (LBx). Conventional LBx using circulating tumor cells (CTCs) or cell-free DNA (cfDNA) have seen limited success for HGGs, due to impeded outflow of CTC and cfDNA into bloodstream.[1,88] FUS-enabled plasma biomarker profiling offers an opportunity for longitudinal molecular evaluation, detection of changes in tumor receptor status, and downstream modification of systemic therapy regimen.

Recurrence of HGGs, due to the infiltrating tumor cells, is considered inevitable, for which effective treatment options are lacking. Radiotherapy is frequently utilized, albeit the benefit is modest[56,89]—FUS-mediated radiosensitization could enhance efficacy.

Additionally, disease progression is often detected when tumor has regrown to a certain radiographic extent, or has begun producing clinical features again. At this point, the tumor is not able to be effectively targeted with systemic therapy and/or radiation, while repeat resection is often too morbid to pursue. Unlike many other malignancies with disease surveillance performed through multiple modalities, here, interval MRI remains the primary approach. FUS-enabled plasma ctDNA monitoring offers an opportunity for longitudinal disease surveillance and early intervention.

Disrupting the BBB safely and transiently has been a long-standing effort in neuro-oncology.[90] While previous attempts to open the BBB through drugs like mannitol, RMP-7, and regadenoson had limited success and minimal spatial control,[90] BBBO has now become clinically feasible through therapeutic ultrasound (delivered via FUS or implantable approach).[91] Here, a patient is administered microbubble (MBs) intravascularly (preferably continuous

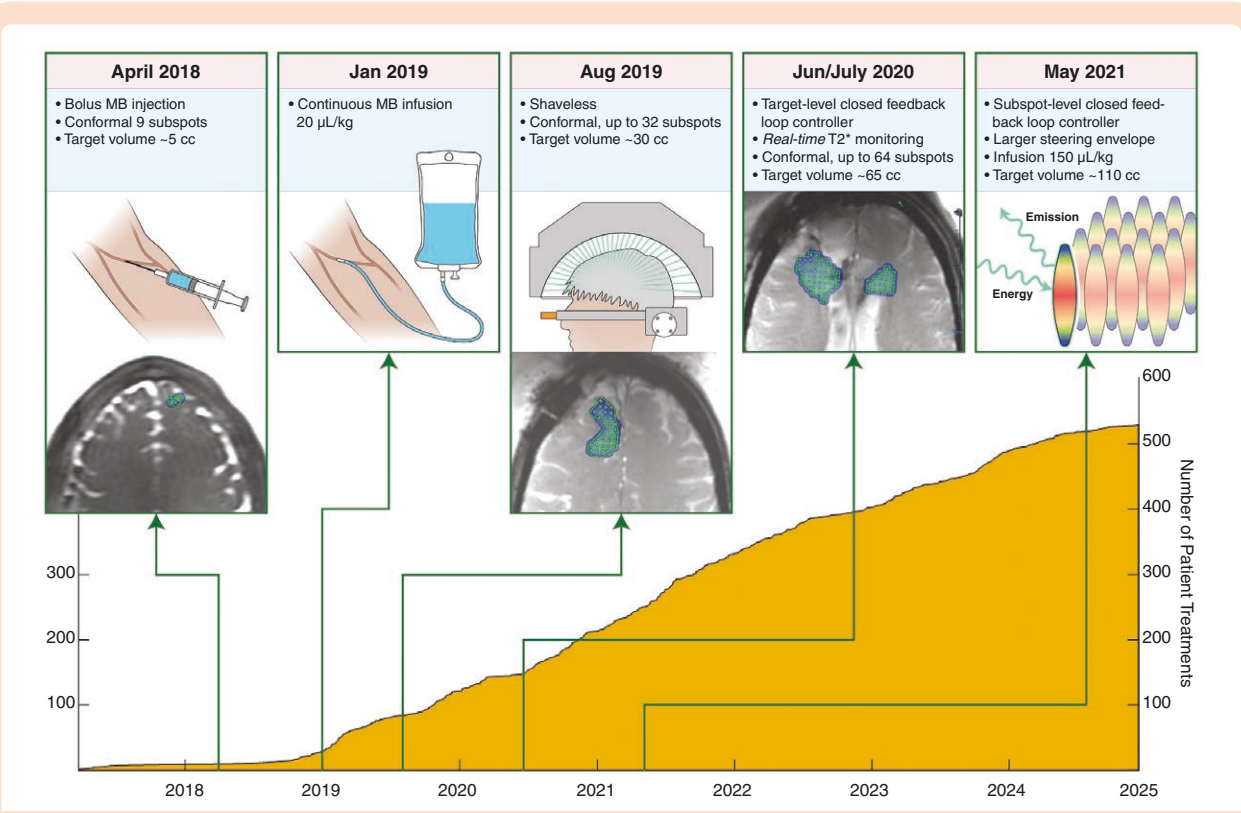


**Figure 3. Key milestones in technological and device evolution for MR-guided MB-FUS.** The yellow-shaded graph represents the cumulative number of MB-FUS number of treatments worldwide, with scale on the y-axis, indicating that the landmark of over 500 MB-FUS BBBO treatments was crossed in 2025. The timeline, as depicted on the x-axis, captures the key milestones in the evolution of the technology. Key developments included approval for the delivery of microbubbles (MBs) as bolus injections (as per on label for DEFINITY) to continuous MB infusions, which enabled more homeostatic MB concentrations in the brain microvasculature and also enabled easier intra-procedural coordination. While head shaving was initially mandatory, a shaveless option became available allowing patients to maintain their hair up to 10 cm in length. Most critically, the conformal treatment subspots (subspots in the target grid) increased majorly from initially 9 subspots per grid to 32 per grid in 2019 to 64 subspots in 2020 (subspots in a grid at a distant of 2.5 or 3.0 mm from each other). Both the regulatory approval for continuous MB infusion as well as the improvements in the device corresponded with the increase in the treatment volume, beginning initially from 5 cc to 30 cc in 2019 to 65 cc in 2020 and finally 110 cc in 2021, that is, allowing large-volume, conformal BBBO (ie, clinically relevant BBBO for adequate brain targeting). Important safety improvements were added in 2020 with target-level closed feedback loop (CFL) power control and real-time T2* MR imaging. These were followed in 2021 by a larger steering envelope and more advanced CFL algorithm working at the subsonication level, with the latter allowing for more efficient power delivery with more homogeneous dose distributions.

MB infusion), followed by sonication, which leads to MB oscillation. Resonant MB causes mechanical endothelial perturbation, leading to BBB opening, which can be utilized for multiple applications.

Finally, HGG care may potentially need a paradigm-changing approach to killing tumor cells/tissues. FUS also offers the opportunity for direct destruction of tumor cell/tissue, through either (1) thermoablation via HIFU, (2) SDT via LIFU, or (3) histrotripsy. While these three approaches have less translational readiness (**Supplementary Table S2**), they do not require a highly active systemic therapy for combination, which the MB-FUS BBBO approach does require, but currently lacks.

## FUS-Enabled Delivery of Therapeutics

Amongst its diverse applications, it is ultrasound-enabled (through either implantable and focused approaches) delivery of therapeutic agents that, so far, has most substantial body of preclinical and clinical evidence that can be applied to PBTs (**Table 1**, **Supplementary Table S1**),[7,8] given its immediate relevance to and demonstrated potential in multiple other brain diseases, such as amyotrophic lateral sclerosis (ALS)[92,93] and Alzheimer's disease.[94–96] FUS allows for precision drug delivery in targeted brain regions,[96] predominantly through MB-FUS BBBO (ie, via LIFU), although the mechanism of gentle hyperthermia (i.e. via HIFU) for drug delivery has seen some recent interest.

### Preclinical Evidence for MB-FUS BBBO

Work in animal models two decades ago demonstrated the safety and feasibility of MB-FUS BBBO. In 2001, Hynynen et al. demonstrated in rabbits using a much more primitive version of MR-guided MB-FUS transient BBB opening without damage to neuronal tissue.[38] There was a lack of contrast enhancement one day after the initial FUS

procedure.[38] MB-FUS for the delivery of chemotherapy was first demonstrated in rats treated with doxorubicin, where sonicated regions were found to have enhanced drug penetration.[97] Follow-up preclinical studies showed significant promise across numerous species, therapeutics, and disease contexts, with findings of > 50-fold increases in drug delivery following MB-FUS.[98,99] These findings were confirmed in larger animal models, including canine, sheep, and non-human primates, that better recapitulate human cranial characteristics.[17,100–102]

The technical efficacy of MB-FUS BBBO for drug delivery across a range of molecule sizes was first exemplified in its success with the much larger-sized anti-VEGF agent bevacizumab, a monoclonal antibody (MAb), based on seminal work by Kinoshita, McDannold, Jolesz, and Hynynen.[99,103,104] Later, successful MAb delivery through MB-FUS was also validated for anti-EphA2 $^{89}$Zr-radiolabeled antibody,[103–105] anti-EGFR antibody[106,] $^{89}$Zr-radiolabeled anti-CD47 antibody,[107] and $^{89}$Zr-radiolabeled cetuximab,[108] with the latter two studies using immunoPET for precise mAb delivery visualization. Antibody delivery has been safely demonstrated with 1.15MHz MB-FUS in orthotopic syngeneic mice model of high-risk medulloblastoma (**Figure 2**).[109]

## Clinical Investigations of MB-FUS BBBO

The translation of MB-FUS-enabled CNS drug delivery from animals[45,110–114] to humans, given their higher skull density and larger brain sizes,[115] has required careful optimization of adjustable sonication parameters for BBBO. Clinically, MB-FUS is being delivered via a range of platforms with either MR-guidance or without MR-guidance.

*MR-guided FUS*The most widely investigated device of this type for PBTs currently is ExAblate Neuro Model 4000 Type 2/2.1 (Insightec Inc, Miami, FL and Dallas, TX), with 1024 individual transducers embedded in a hemispherical platform.[116] The device has an FDA approval for movement disorders but remains investigational for PBTs.[9,116] An MR-compatible stereotactic head frame is used for head fixation and anatomic localization,[117] although a dental mold assembly may be used instead for populations where frameless approaches are preferred (such as children). Early treatments required head shaving, but now shaveless treatments may be performed. Following head fixation and start of continuous MB infusion, the procedure is done in MRI suite. Following imaging and MR-registration, the operator (typically neurosurgeon) performs patient-specific target (grid) planning, followed by sonications in short bursts (our current approach for brain tumors being of 90 seconds at sonication dose of 1.0–2.0 units). Post-procedure, new enhancement on T1-weighted MRI with contrast indicates BBBO.

In a pilot study to evaluate MB-FUS BBBO to improve chemotherapy delivery for PBTs,[118] five high-grade glioma (HGG) patients underwent MR-guided MB-FUS treatments 1 hour after receiving a sub-therapeutic dose of chemotherapy. No serious significant ultrasound-related adverse effects were noted. All patients showed safe, transient, and successful BBB opening, as evidenced by contrast-enhancement in target regions, which resolved by 24 hours. One day after MB-FUS treatment, patients underwent tumor resection, and chemotherapeutic levels were quantified by liquid chromatography-mass spectrometry (LC-MS). Only two patients yielded sufficient volume of resectable tissue sample for LC-MS analysis, but both showed elevated concentrations of chemotherapeutic agents in tumor samples, confirming drug delivery.[118]

Another clinical trial (NCT03322813) assessed MR-guided MB-FUS in patients with WHO Grade II/III infiltrating gliomas. Here, real-time AEM-based CFL power control allowed target-specific dosing. Adaptive power cycling combined with image-guidance created a highly precise environment for FUS delivery. Operative, imaging, and acoustic findings were histopathogically correlated with the degree of change in FUS-treated tissue. Fluorescein accumulation increased by ~2-fold in FUS-treated non-enhancing tissues compared to untreated non-enhancing tissues.[49] Given that the power required to achieve the desired bioeffect varied significantly between targets, therefore, FUS targeting was learnt to ideally be dosed on an individual per-target basis with careful planning, contouring, and personalization. Recently, capacitive micromachined ultrasonic transducers (CMUTs) have been reported for both acoustic transmission and high-sensitivity detection.[119,120]

Repeated MB-FUS BBBO in HGG patients has been reported safe, as seen in South Korean trial (BT008K/NCT03712293) of $N = 6$ HGG patients post-resection. Here, patients received MB-FUS BBBO concurrently with monthly adjuvant TMZ treatment cycles, upto 6 total cycles. No severe adverse effects (AEs) were observed, while 92.4% of targeted sites showed evidence of BBBO.[121] One-year follow-up confirmed no severe AEs. Notably, all patients survived 1-year post-resection and only one had recurrence of tumor.[122] Results are awaiting publication for its counterpart North American multicenter trial of MR-guided MB-FUS BBBO combined with adjuvant TMZ for newly diagnosed HGG (BT008NA:NCT03551249 + NCT 03616860).

Results are also awaited from trials of MR-guided MB-FUS combined with doxorubicin [NCT02343991, and (BT016:NCT05630209 + NCT05615623)], and carboplatin (BT009: NCT04440358 + NCT04417088). A representative case of MB-FUS BBBO for glioma drug delivery is shown in **Figure 4**.

In addition to the required baseline MRI sequences, we recommend obtaining and comparing pre- and post-procedure T2*/GRE/SWI sequences (highly sensitive for detecting small microbleeds)—**Table 2** discusses a novel framework for T2*-based safety assessment. We also recommend performing MB-FUS BBBO with (1) real-time AEM, (2) real-time T2*, and (3) AEM-based active CFL power control.[123]

*Non-MR-guided FUS*This approach, by not requiring intraoperative MR-guidance, and therefore, head frame fixation, or access to an MRI suite, intends to reduce operative expense, decrease procedural time, and improve

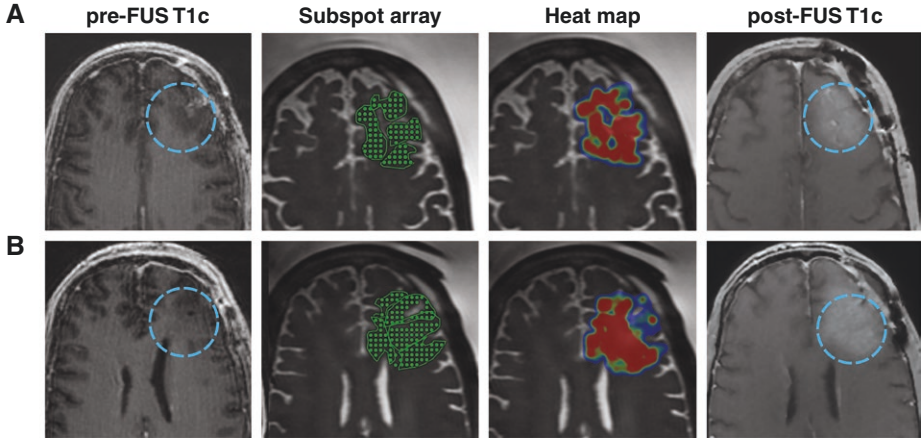


**Figure 4. Case of transcranial focused ultrasound (FUS) for blood–brain barrier (BBB) opening for a patient with a high-grade glioma.** This adult patient was treated with a 220 KHz FUS system at the University of Maryland after standard-of-care gross total resection for a suspected WHO grade 4 glioma, followed by 6 weeks of chemoradiation therapy. FUS was delivered using an Exablate Neuro Model 4000 Type 2 with perflutren lipid microspheres (DEFINITY, Lantheus, N. Billerica, MA), that is, microbubbles for BBB opening and temozolomide delivery. Shown here are pre-FUS T1-weighted MR images, followed by the treatment plan showing the subspot array (target grid) and post-FUS heat map (of acoustic dose). The last column shows the post-FUS T1-weighted constrast-enhanced imaging, showing new contrast enhancement (new T1c), indicating achieving of BBBO.

**Table 2.** The "Maryland Grading Scale" for Post-procedural Safety Assessment and Reporting for MB-FUS BBBO Treatments

| Grade | Finding on post-MB-FUS SWI/T2* MRI |
|---|---|
| 4 | Mass effect, intracranial bleed, or new edema |
| 3 | More than 15 foci of signal change |
| 2 | 6–15 foci of signal change |
| 1 | 1–5 foci of signal change |
| 0 | No signal change |

Sonications can result in susceptibility-weighted imaging changes, likely related to the extravasation of erythrocytes from the brain microvasculature. This can be detected as varying degrees of foci in the susceptibility signal on T2*-weighted imaging. Aligning with evolving development of research standards for MB-FUS, a semi-quantitative scale has been created and used in clinical MB-FUS investigations at the University of Maryland for post-procedural imaging-based safety assessment, classification, and reporting purposes. Grade is assigned in a two-step process: (1) locating the region with the highest density of T2* signal within a 1 cubic cm volume and (2) assigning grade to the post-operative T2* imaging based on the relative number of T2* foci within the selected 1 cubic cm volume. Note that this grading is meant for application on post-procedure T2* imaging, distinct from intra-procedural real-time T2* imaging.

patient access.[44] Frequently, non-MR-guided FUS utilizes a small, portable console coupled with neuronavigation, as exemplified by NaviFUS (Navifus Corp, Taiwan) and NeuroAccess (Cordance Medical, Mountain View, CA, USA).[14,44,45,124] Here, the patient is positioned on a treatment chair and the head and neck stabilized with semi-rigid support. Using neuronavigation, the neurosurgeon registers specific physical points on the head and face to "register" the patient's anatomy to preoperative contrast-enhanced T1-weighted MRI. Once the navigationally registered, the trajectory of the FUS transducer can be visually localized in relation to the previously acquired MRI scans in real-time. This information is used to manually position the ultrasound transducer to target the region of interest. This allows for a short total treatment, typically in an outpatient setting.

Early studies demonstrated similar technical efficacy (ie, BBBO) and precision (ie, targeting) to that stereotactic MR-guided procedures.[125–128] In non-human primates, neuronavigation-guided FUS paired with passive AEM[48] for MB activity monitoring showed targeting precision of 2 mm.[45]

Chen and colleagues in 2021 reported the first pilot clinical study confirming that neuronavigation-guided MB-FUS was feasible for achieving transient, targeted BBBO.[124] This has led to several ongoing trials (**Table 2**). Another phase 1 trial (albeit in Alzheimer's disease patients) showed the feasibility of a portable neuronavigation-guided MB-FUS system for achieving BBBO (of less than 1-by-1 cubic centimeters) in $N = 5/6$ patients. Results are awaited from a phase 2a, open-label, single-arm trial in Taiwan that combined neuronavigation-guided MB-FUS with 10 mg/

kg IV bevacizumab for recurrent GBM (NCT04446416). Two other ongoing trials are evaluating the safety and feasibility of combining neuronavigation-guided MB-FUS with oral panobinostat (NCT04804709) and oral etoposide (NCT05762419), respectively, based on promising preclinical data,[125] for children with progressive diffuse midline glioma (DMG), a pediatric-type HGG (**Figure 2**). FUS for pediatric neuro-oncology is further described elsewhere in this supplement.[129] Other ongoing trials are described in **Supplementary Table S2**.

### A New Paradigm of Drug Discovery through MB-FUS BBBO

MB-FUS BBBO can potentially contribute to reinvigorated efforts for the discovery of more effective therapeutic agents for HGGs by reshaping the current drug discovery framework. In the current paradigm, the hurdles that a lead agent must cross, amongst others, include demonstration of brain penetrance.[61,130] Thus, several drugs in the preclinical drug discovery pipeline have been eliminated from further consideration over the past decades due to their failure to meet this neuropharmacokinetic requirement, even though the bar to demonstrate success remains low, given the modest brain-penetrance of TMZ itself.[63]

Through BBBO, MB-FUS allows for a novel paradigm for brain tumor drug discovery where the question of brain penetrance may not require consideration. This potentially allows the study of both novel and existing drugs (many used in non-CNS cancers), which have anti-tumor activity but are not innately brain-penetrant. The incorporation of tools like genome-scale metabolic modeling[131] and machine learning[132] could further accelerate efforts in this paradigm. For instance, machine learning-based models utilize data (across different cancers and preclinical contexts) on anti-tumor activity, without accounting for brain penetrance (where data is much more limited). While in the classical neuro-oncology framework, these tools have (so far) seen modest success for drug discovery,[133] however, with BBBO, their utility could potentially become amplified.

Separately, while initial and repetitive brain tissue sampling has been discussed as a critical future measure for effective glioma drug discovery,[87] this requirement could also be potentially bypassed, if the promise is sono-liquid biopsy is validated in larger trials, as discussed later.

### Other FUS Mechanisms for Drug Delivery

Interestingly, mild hyperthermia through FUS has been reported in a few studies for precision drug delivery (without requiring MBs). In rodents, a transcranial 1.7MHz MR-guided FUS system with temperature-based CFL has been reported for gentle hyperthermia.[134] Here, doxorubicin was delivered using lyso-thermosensitive liposomes (LTSLs) with FUS-triggered release, using target temperature of 41.5 ± 0.5 °C for ~10 min (ie, under threshold of intracranial damage). Beyond tumor-targeted doxorubicin release, thermal stress also corresponded to improved transvascular transport.[134] Large volume hyperthermia for clinical use has been reported.[54]

Clinically, the TARDOX trial (NCT02181075), confirmed the possibility of using LTSLs plus mild hyperthermia with FUS (ΔT <+4 °C) for drug delivery, albeit in liver tumors ($N = 10$).[135] Investigators have also begun looking at simultaneously harnessing both hyperthermia and MB-FUS BBBO.[136,137] These findings remain to be validated in brain tumor trials.

### Beyond Systemic Chemotherapy

MB-FUS BBBO may be utilized for delivering other therapeutics, including gene therapy, viral vectors, chimeric antigen receptor (CAR) T-cells,[138] and nanoparticles.[138–142] CRISPR/Cas9 plasmids targeting O6-methylguanine-DNA methyltransferase (MGMT) gene, which encodes for TMZ-resistance-mediating enzyme, have been successfully delivered in lipid-polymer hybrid nanoparticle formulation with MB-FUS BBBO.[142] After plasmid delivery in orthotopic GBM-bearing mice, nanoparticle accumulation in tumor region was seen, corresponding to increased TMZ efficacy, hindered neoplastic growth, and extended survival with reasonable safety.[142] Another work reported the use of MB-FUS for preclinical delivery of adenoviral-mediated Herpes Simplex Virus (HSV) Thymidine Kinase (HSV-TK), with effective over-expression of HSV-TK gene in neoplastic cells corresponding to longer survival times in mice.[140] FUS enhanced the delivery and persistence of CAR-T cells in a glioma model, leading to significantly extended survival.[143] Further clinical investigations are awaited on these approaches.

## FUS-Enabled Blood-Based Liquid Biopsy

Plasma cfDNA, which includes the tumor-derived ctDNA fraction, has become a cornerstone of LBx in most systemic cancers. While conventional LBx is hindered by limited levels of plasma CTCs and ctDNA in HGG patients, MB-FUS BBBO promotes the release of tumor-derived factors into circulation (analogous to a 2-way street) allowing for blood-based "sono-liquid biopsy" or SLB.[144] MB-FUS BBBO causes tumor biomarker enrichment in circulation, thus allowing clinicians to meet the long-recognized need for longitudinal molecular evaluation, which has unable to be met through repeated brain biopsies.[85,145] Diagnostics run on these tumor-derived plasma biomarkers can be utilized for personalized treatment planning and disease monitoring. SLB also enables spatial selectivity as FUS beams can be steered to target specific tumor regions, enabling selective enrichment of biomarkers from areas of interest, such as regions of progression or predicted progression in tumors.[146] Additionally, in patients where a minimal residual disease state has been achieved, SLB-based longitudinal evaluation also enables disease surveillance in HGGs (detecting plasma biomarker rise at early recurrence before brain radiographic evidence).

### Preclinical Evidence

Several studies in rodent and porcine models have demonstrated that MB-FUS leads to elevated brain tumor biomarkers in blood samples.[12,15,144,147,148] A study using eGFP-transduced glioma cells in mice showed a significant increase in circulating eGFP mRNA levels after FUS-BBBO.[15,144] FUS has been shown to increase the detectability of clinically relevant mutations like EGFRvIII and telomerase reverse transcriptase (TERT) C228T in ctDNA in systemic circulation in GBM-bearing rodents and pigs.[147]

### Clinical Investigations

Work from our and other groups has validated the feasibility of plasma cfDNA-based sono-liquid biopsy.[13,49] The first major clinical report of sono-liquid biopsy (albeit from an exploratory analysis of an early-phase MB-FUS trial) was published in 2021. Here, the Canadian site of BT008NA (NCT03616860, part of BT008NA), had blood samples collected before and after MR-guided MB-FUS BBBO from $N = 9$ GBM patients, with Alzheimer's disease patients used as external controls.[13] MB-FUS led to increased plasma levels of cfDNA, neuron-derived extracellular vesicles, and astrocyte-derived S100 calcium-binding protein-B. Post-MB-FUS samples showed enriched tumor-specific methylation signal compared to samples collected both pre-procedure as well as from pre-MB-FUS blood samples as well as samples collected from Alzheimer's patients.[13]

Yuan et al. in 2023 reported the first prospectively prespecified FUS for SLB trial for HGG patients (NCT05281731).[14] Here, a portable neuronavigation-guided MB-FUS approach (NeuroAccess, Cordance Medical) was utilized in 5 patients (4 with GBM and 1 with diffuse HGG) prior to surgical resection. MB-FUS increased plasma cfDNA, as well as levels of TERT promoter mutation and IDH wild-type, in plasma samples. Histopathological and transcriptomic analysis did not show any structural damage or inflammatory response, respectively, post-procedure. Overall, this work demonstrated the safety and feasibility of sono-liquid biopsy in HGG patients with spatial targeting and temporal control.[14] Further analysis in this trial reported changes in plasma cfDNA fragmentomic profiles, with much shorter median plasma cfDNA fragment size post-FUS.[149]

Currently, there is one major yet-to-be-reported trial on sono-liquid biopsy for brain tumor characterization, that has completed enrolment. LIBERATE (NCT05383872), via the North American ReFOCUSED consortium, investigates the use of MR-guided MB-FUS BBBO to demonstrate a ≥ 2-fold rise in plasma cfDNA post-MR-guided MB-FUS and to determine whether there is ≥ 70% molecular concordance between tumor tissue and plasma cfDNA in suspected GBM patients undergoing surgery.[150] A lesson from the conduct of this trial has been that, standardizing not only technical MB-FUS parameters across centres, but also pre-analytical blood processing approaches, will help optimize future SLB trials for brain diseases.[1] Joint efforts by FUS Foundation and Blood Profiling Atlas in Cancer (BloodPAC)[151] are ongoing to develop reporting standards for sono-liquid biopsy.

## FUS for Direct Destruction of Tumor Cells or Tissues

Here, three primary FUS-mediated mechanisms are at the forefront: thermoablation, histotripsy, and SDT. All of these also lead to anti-tumor responses through diverse mechanisms and thus facilitate FUS-enabled immunomodulation, as described later.[16,52]

### Thermal Ablation

HIFU-based thermoablation has seen long-standing investigation.[18,152,153] Given the high attenuation coefficient and high acoustic impedance of bone, early studies grappled with (i) inefficient energy transfer from transducer to skull, with (ii) concurrent skull heating.[29] Early solutions focused on creation of a skull window and deployment of external head cooling, In the clinical trial by Ram et al. (2006) three recurrent GBM patients were thermoablated post-craniectomy, which negated much of utility of thermoablation (over other surgical approaches).[154] While $N = 2/3$ patients had resolution of contrast-enhancement in thermoablated volume, one patient still had an adverse event from skull heating.[154] Large volume thermoablation also risks brain hemorrhage.

The development of multi-transducer hemispherical piezoelectrical component arrangement (as exemplified in Exablate platform) enabled transcranial thermoablation of limited-sized predefined areas with low collateral damage. HIFU is now being routinely utilized for brain ablation for functional neurological disorders (given the small target sizes), corresponding to its major successes in RCTs in patients with Parkinson's disease and essential tremor.[155–158] However, the results for patients with PBTs (much larger target sizes) have been less exciting. McDannold and colleagues attempted MR-guided thermoablation using ExAblate 3000 system (predecessor of current-generation ExAblate 4000 Type 1.0/1/1 system for HIFU) in $N = 3$ GBM patients. However, with the integrated real-time MR-thermometry, the maximum focal temperature achieved during a 20-second sonication remained at or below 51 °C, thus below the threshold for ablation, indicating suboptimal target heating.[39,46]

Notably, there is an ongoing trial—the BRAINFUL (NCT04940507) trial—being conducted at a single center in Canada, which is studying the time-dependent changes in ctDNA found in peripheral blood and CSF samples before and after MR-guided HIFU thermoablation using ExAblate Neuro Model 4000.

### Histotripsy

HIFU is also being worked upon in the pursuit of brain-directed histotripsy, a technology that has been seen more clinically translation for hepatic neoplasms.[25] Histotripsy

is a mechanical tissue ablation method that utilizes short, high-intensity ultrasonic pulses to generate bubble cavitation clouds (bubble activity that is sometimes referred to as "chaotic"), thereby fractionating tissues.[25,159–162]

Preclinical studies have demonstrated that histotripsy can be used to reliably create brain lesions (small), with multiple biological and mechanical effects.[24] In ex vivo bovine brains, hemispherical, transcranial, 256-element-histotripsy-transducer arrays operating at 250 and 500 kHz were shown to ablate deep brain tissues.[161] Porcine in vivo studies have demonstrated that histotripsy can generate specified, arbitrarily shaped lesions while sparing the perilesional tissue located >200 microns from histotripsy margin.[23] Recently, "boiling histotripsy' was first reported in ex vivo human brains, where it achieved reasonable treatment performance conducted with B-mode ultrasound guidance.[24,163] However, the risks with histotripsy rapidly rise with increasing ablation sizes. Thus, the potential to cause brain hemorrhages or cerebral edema owing to the mechanical tissue disruption has hindered histotripsy" clinical translation.[16,91]

Some promise remains from ongoing efforts at new device development and existing platform optimization. For instance, the Michigan Histotripsy Group (MHG) has reported a new stereotactic targeting system for preclinical investigations, as well as NaviTH, a neuronavigation-guided, transcranial, 750kHz, 360-element, hemispherical histotripsy platform, for clinical use.[20,164] MHG reported that administering fewer ultrasound pulses with transcranial histotripsy at certain number of foci led to both better brain tumor ablation and reduced bleeding across multiple preclinical models.[21] MHG also reported longitudinal characterization of downstream effects of histotripsy, with a library of MRI scans corresponding to histology for rodent brains, without and with brain tumors (~ 5 $mm^3$), that were subjected to an 8-element, 1 MHz histotripsy delivery platform.[21] A greater understanding of non-invasive radiological assessment can help facilitate the development of thresholds for stopping treatment delivery, as well as, advance imaging-based safety evaluation in humans for future histotripsy treatments. A new frontier of investigation is combining histotripsy with an injectable hydrogel, used post-resection. That attracted, captured, and encapsulated tumor cells in vitro,[165]—directed histotripsy was then utilized for a homogenous fractionation of hydrogel-encapsulated cells.[166] Recent developments in histotripsy offer promise, but further work is warranted to address demonstrate clinical feasibility.[16,23,25,160,161]

### Sonodynamic Therapy (SDT)

SDT combines LIFU with a sonosensitizer, a compound that becomes cytotoxic when activated by ultrasound. Sonosensitizing agents, many of which are also used in photodynamic therapy, are delivered to the tumor microenvironment and activated in the acoustic fields, resulting in multimodal therapeutic effects.[116,167] Further, ultrasound also activates protoporphyrin-IX (PpIX), which generates reactive oxygen species (ROS), leading to tumor cell death.[168,169] Compared to other tumor cell killing methods like thermoablation and histotripsy, SDT carries less risk of cavitary lesions and hemorrhagic complications, which could be advantageous for HGGs (with altered microvasculature and higher risk of bleeding).

SDT allows for precision molecular targeting with minimized off-target toxicity, as recognized from extensive preclinical work.[27,167,170–172] SDT with MR-guided FUS and 5-ALA leads to tumor cell apoptosis and suppressed proliferation in murine models.[27,28,172] SDT with TMZ has also been preclinically investigated using a liposomal delivery approach to induce immunogenic cell death.[170] Other preclinically investigated sonosensitizers include sinoporphyrin sodium, hematoporphyrin monomethyl ether, and Rose Bengal, as described in a recent systematic review.[173] Recently, a portable benchtop SDT system for preclinical studies has been reported.[174]

Sonosensitizers currently being clinically investigated include 5-ALA, fluorescein, and TMZ. In a Chinese open-label, phase 1 trial of SDT for $N = 9$ patients with recurrent GBM patients published in 2023, no major AEs were reported (ChiCTR2200065992). $N = 1/9$ patients had stable disease, while $N = 8/9$ had further disease progression, and no survival benefit was observable.[175] The same group of investigators in 2025 has reported findings from combining SDT plus radiation from $N = 11$ patients with brainstem gliomas, potentially the first such phase 1 trial (part of ChiCTR2200065992). Patients received hematoporphyrin as sonosensitizer, followed by SDT + radiation, with MRI-based monitoring. No grade ≥ 3 AEs and no dose-limiting toxicities (DLTs) were reported. $N = 8/11$ had stable disease while $N = 2/11$ had partial response, with median PFS being quite promising at 9.2 months (95% CI 6.2–12.2).[176] Final results are awaited from the SONALA-001 trials, where dose-escalation of IV 5-ALA dose and MR-guided FUS acoustic energy levels was done in patients with (i) diffuse intrinsic pontine glioma (DIPG) or DMG (SDT-201/NCT05123534) and (ii) recurrent GBM (SDT-202/NCT05370508). Interim results presented at SNO 2024 annual meeting indicated reasonable safety in SDT-201 trial with no grade 3 or higher adverse events.[177] $N = 10/12$ of recruited DIPG patients were alive 11 months post-diagnosis.[177] In SDT-202, SONALA-001 was administered 6–9 hours prior to surgery, with acceptable safety.[178] Several SDT trials, where 5-ALA is administered 1–3 weeks prior to surgery in HGG patients, also remain ongoing across both the newly diagnosed (NCT04845919) and recurrent setting (NCT06039709, NCT04559685, NCT05362409)—See **Supplementary Table S2**.

While not FUS, low-intensity diffuse transcranial sonication has seen interest. Final publication is awaited from a phase 1 trial of diffuse sonication using CV01 device (Alpheus Medical, North Oakdale, MN, USA) combined with 5-ALA for SDT for patients with recurrent HGG, which has completed enrolment (NCT05362409). No dose-limiting toxicities or serious AEs were reported.[179]

## FUS-mediated Immunomodulation

Given the cold TME in HGGs,[66] the heterogeneous molecular signature of HGGs,[180] the immunosuppressive effect of several SOC therapies for HGGS such as temozolomide

and corticosteroids,[64,65] and the historic failure of immune checkpoint inhibitors (ICIs) to improve OS in major RCTs of HGGs,[67–69] interest in immunomodulation for these patients has been long-standing, now being tackled with FUS as well.

## Mechanisms and Preclinical Evidence

FUS has been recognized to modulate the immune system through diverse and often complementary mechanisms,[181] as summarized below and discussed in detail in another review in this supplement.[182]

*Enhanced and controlled delivery of immunotherapies*FUS-mediated BBB opening can facilitate the delivery of immunotherapeutic agents, such as mAbs (like ICIs), interleukins (like IL-12), and chimeric antigen receptor (CAR)-T cells, into the brain.[183–185] Preclinically, improved uptake and efficacy of anti-PD-L1 and anti-CD47 mAbs in glioma models has been noted after FUS treatment.[107,186] BBB opening has been preclinically demonstrated to synergistically enhance the efficacy of anti-PD-1 mAbs and CAR T-cells.[138,187] Here, improved antitumor immune response and extended survival has also been reported.[138,187] Importantly, mAb penetration has been noted in non-contrast-enhancing regions,[105] a critical aspect given the residual tumor cells post-resection.

A study from Taiwan reported no significant alteration in T-cell populations in normal brain, post-FUS, while neoplastic brains had increased tumor-infiltrating lymphocytes (TILs), especially CD3 + CD8 + T-cells. Combining FUS with intraperitoneally delivered IL-12 led to substantial increase in (i) brain IL-12 concentration, (ii) TILs, particularly CD3 + CD8 + T-cells, and (iii) ratio of CD3 + CD8 + T-cells to CD4 + CD25 + T-cells.[185]

FUS, through multiple approaches, allows for controlled boost to the utility of CAR T-cells for HGGs.[143,188,189] MB-FUS BBBO preclinically enhances delivery and persistence of CAR-T cells in glioma, extending survival.[143] Given the sobering history of off-target adverse CNS effects seen with CAR T-cells in prior (non-FUS) HGG trials,[188,190,191] "acoustogenetic control" is a promising new approach for controlled FUS-based activation of engineered T-cells.[143] Here, biocompatible heat-shock proteins that modulate protein expression, can be activated by short-pulsed stimulation at specific spatial regions, enabling control of off-target immune responses.[143] Reversible acoustogenetic control of CAR T-cells (with inducible CAR) was reported in 2021 for treating rodents with subcutaneous tumors with minimal off-target effects with MR-guided FUS-based activation.[143] Recently, using ultrasensitive heat-shock-based promoter, sonogenetic EchoBack-CAR T-cells have been developed, which showed greater cytotoxicity and less exhaustability than conventional CAR T-cells in GBM models.[189]

*Improved antigen presentation*Given that HGG patients have hindered trafficking of tumor antigens or genetic material into the systemic circulation due to the BBB,[64] MB-FUS BBB opening can enhance the release of (erstwhile sequestered) tumor-associated molecules into the systemic circulation,[52] the same principle underlying sonoliquid biopsy. This potentially improves antigen presentation to immune cells in lymph nodes, increasing systemic immune activation.

*Localized immune activation*FUS leads to a mild and localized sterile inflammatory response.[192] This neuroinflammation can convert the cold TME to an hot TME. MB-FUS upregulates both innate and adaptive immunity within TME, with potential dose-dependence on sonication power and MB concentration, as well as promotes the infiltration of immune cells like NK cells. The latter also enables engineered T-cell infiltration, underlying the rational combination of FUS and CAR T-cell therapies. As part of FUS-induced immunogenic tumor cell death, damage-associated molecular patterns (DAMPs) also get released, which can further stimulate the immune system by enhancing dendritic cell maturation and T cell activation. In mice glioma models, FUS leads to increased dendritic cell recruitment,[183] while FUS hyperthermia leads to increased release of extracellular vesicles, with corresponding upregulation of IL-12p70 in dendritic cells, a pro-inflammatory signal.[193] Meanwhile, SDT can lead to reduction in myeloid-derived suppressor cells, which are a crucial immunosuppressive component in HGGs.[167,193] All of these lead to bolstered antitumor responses.

Recently, MB-FUS has been preclinically reported to lead to differential gene expression in BBB cells, given the presence of inflammatory pathways that are susceptible to microvascular wall shear stress. This could be utilized to pursue both molecular and spatial therapeutic windows.[194]

## Clinical Investigations

While some clinical investigations have been carried out in the context of implantable ultrasound devices,[195,196] more embedding of immune profiling, both local and systemic, in transcranial FUS trials is needed. Immunomodulation has been explored in a clinical trial of neuronavigation-guided MB-FUS for BBB opening ($N = 6$, NCT03626896).[124] The trialists saw no major clinically relevant changes in immune profile in all patients enrolled at the applied FUS dose, and therefore, preclinically explored the impact of higher sonication, which did lead to greater immunostimulation in rats.[124] Another RCT was previously investigating (now listed as "withdrawn") whether FUS combined with neoadjuvant pembrolizumab may be clinically superior to neoadjuvant pembrolizumab alone for GBM (NCT05879120).

Further clinical translation of FUS-mediated immunomodulation will be enabled by a greater understanding of the interaction between adjustable FUS settings and immune responses.[195,196] Work is needed to determine the optimal specific sonication parameters matched, most importantly, with specific immunotherapy type,[194] as well as disease state, immune status, and

molecular profile. Combinatorial investigations with other immunotherapies (like cancer vaccines) could also be of value. Further insights are described in another review in this supplement.[182]

## FUS-Mediated Radiosensitization

Radiosensitization can be pursued through both HIFU and LIFU, albeit our current understanding remains limited both of the intermediary mechanisms and of the contributory effect. Hyperthermia induced by FUS can make tumor cells more susceptible to various RT modalities.[168,197,198] FUS, by inducing local hyperthermia below the threshold for coagulative necrosis, can augment tissue oxygenation and perfusion,[199] mechanisms which are known to enhance radio-sensitivity.[168,197,198]

### Preclinical Evidence

Preclinically, FUS hyperthermia in combination with RT has been shown to significantly reduce tumor growth compared to RT and FUS alone, with DNA damage as potential intermediary mechanism.[200] In a mice GBM study, FUS + RT at 5Gy led to greater survival and control than RT at 2Gy alone, but not compared to RT at 5Gy. Another study combined PARP1 inhibitor Olaparib, with MB-FUS + RT, to investigate for enhanced radiosensitivity in vitro and in vivo through a patient-derived xenograft DMG murine model. Here, prolonged, low-dose olaparib exposure led to slower tumor growth compared to high-dose, short-duration PARP1 inhibition. However, no survival benefit was seen.[201]

Beyond hyperthermia, the safety and feasibility of combining MB-FUS-enabled BBBO with RT has been reported in healthy and glioma rat models.[202] The safety of combining MB-FUS (0.5 MHz transducer) plus RT (39 Gy/13 fractions) has recently been demonstrated in syngeneic brainstem murine DMG model.[203]

### Clinical Investigations

The earliest trial of FUS + RT was reported in 1991, where $N = 15$ patients were treated with FUS delivered post-craniectomy (given technological limitations of the time) with a target temperature of 42.5°C at lesion boundary, along with external beam RT.[51]

An ongoing single-arm, pilot trial in Taiwan is evaluating the safety and preliminary efficacy of FUS + RT for recurrent HGG patients, where FUS is delivered using the NaviFUS system 2 hours before RT and re-irradiation is considered for disease control (NCT04988750). In an interim analysis of $N = 6$ patients, 50% had stable disease while 50% had rapid progression post-RT + FUS, with or without salvage therapy. Twenty-four treatment cycles of RT + FUS had been given. No FUS-related AEs but one re-irradiation-associated radionecrosis (grade 3) was observed, which warrants further evaluation. With planned final enrolment of $N = 8$, the primary endpoints are radiation dosimetry and FUS energy distribution.[204,205]

## Optimizing Future Investigations of FUS for PBTs

Beyond aspects discussed in each specific FUS application, below we discuss additional and broad considerations for optimizing future investigations of FUS, especially for HGGs. First, optimized preclinical models that accurately reflect human PBT characteristics, especially their behaviors with clinically utilized FUS modes remain urgently needed, to allow for high-accuracy predictive testing of combinatorial FUS strategies.[206] Further, while preclinical and clinical studies have demonstrated safety and tolerability of FUS, especially MB-FUS BBBO, for brain tumor patients, its effectiveness in improving SOC management needs multicenter randomized/quasi-randomized trials. In neuro-oncology, therapeutic efficacy seen in single-arm, uncontrolled trials has not proven to be predictive of success in controlled investigations,[1,85,190,207] This is illustrated by the failure to improve survival, after highly promising early studies, seen in trials of systemic immunotherapies,[67–69] EGFR inhibitors,[73,208] and anti-VEGF agents.[81–84] Success in large trials, many being ongoing (**Supplementary Table S2**), will require coordinated multicenter efforts, thoughtful trial design that is optimized for both clinical success and planned extraction of translational data (enabled by harmonized brain tumor tissue processing[209]), standardization of FUS treatment protocols and development of practical implementation approaches enabling greater site participation.[206,210–212] These efforts will be aided by support from professional biophysics and neuro-oncology societies, ongoing development of FUS delivery treatment platforms, and dedicated FUS research consortia (such as ReFOCUSED).[206,213,214]

Key predictors of success in FUS studies remain to be determined.[206] Input parameters often differ from one center to another, with significant variability in procedural protocols.[213] This delays standardization and optimization for multicenter trials, and also affects precise, reproducible control over BBBO. For better treatment planning for planned SOC integration, work is needed to quantify specific impact of treatment duration, BBBO delivery dynamics, and co-administered therapeutics, on clinical efficacy.[206] These FUS-related aspects can vary based on, in addition to FUS platform, patient, disease-state, and therapeutic approach.[49,206,215–217] Standardization of these aspects, will not only increase technical and clinical efficacy of FUS in future trials, but also facilitate future evidence syntheses.

The technology, regardless of mode, still has substantial room for optimization, where multi-disciplinary, diverse expertise will remain crucial for advancement, as the history of FUS has shown.[9] A major multi-institutional study using MB-FUS BBBO for HGGs has recently reported advanced technical capabilities for transcranial, volumetrically contoured, repeated BBB opening treatments.[123] Here, AEM-based CFL-controlled power cycling at subsonication-level helped improve dose uniformity,

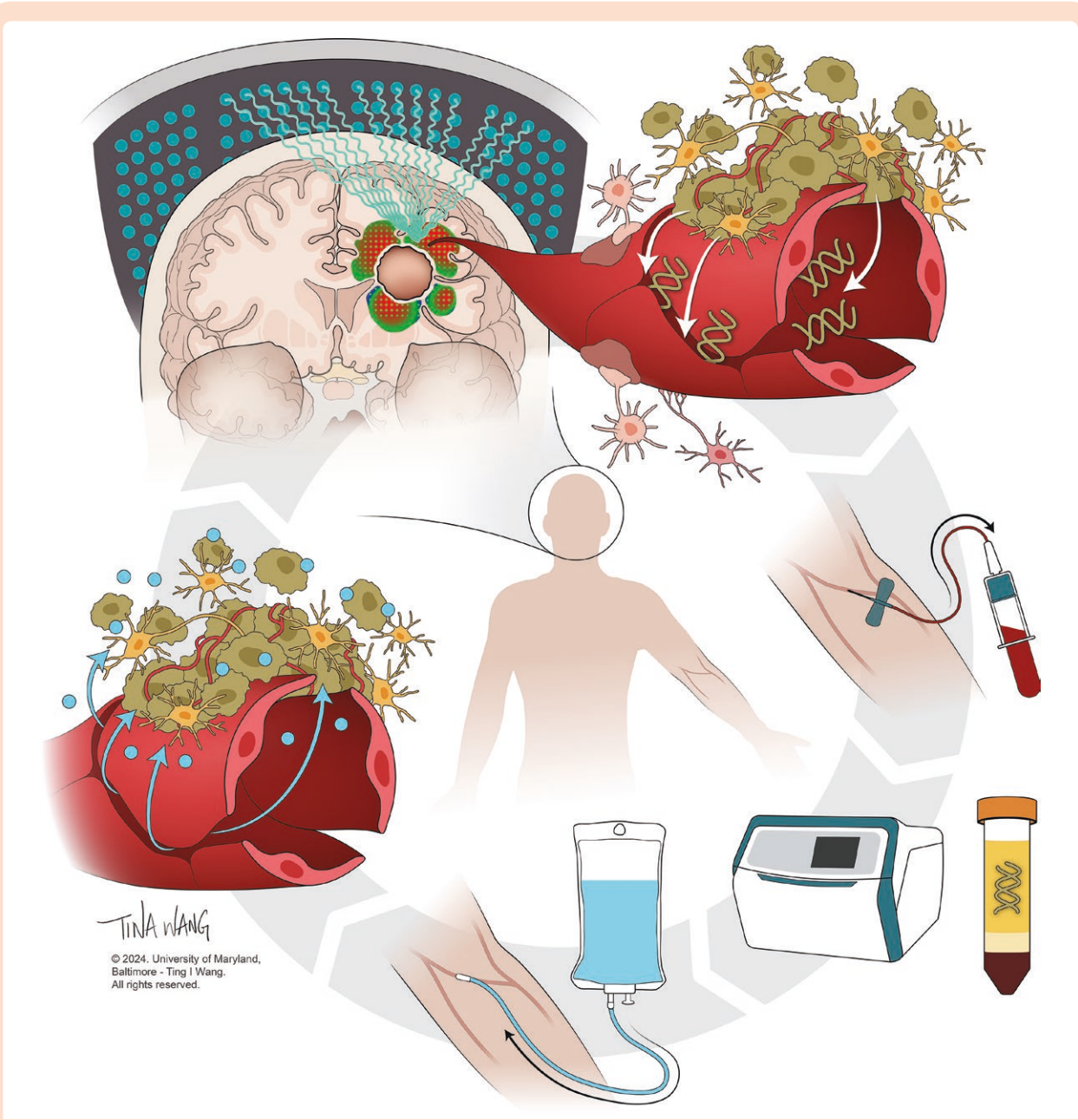


**Figure 5. MB-FUS BBBO-enabled adaptive theragnostic paradigm for high-grade gliomas.** Initial MB-FUS-enabled BBB opening facilitates sono-liquid biopsy of the tumor, hence allowing for longitudinal assessment of tumor molecular features non-invasively. This allows for bespoke treatment regimen, temporally personalized to the tumor characteristics. Following sono-liquid biopsy, MB-FUS is used for the enhanced delivery of neurotherapeutics though BBB opening. Longitudinal disease surveillance and liquid biopsy at disease progression are both enabled by MB-FUS, allowing for iterative changes to the personalized treatment paradigm.

lower power input, and enhance BBBO, thereby improving the overall safety and robustness of FUS.[123] This work has been amongst the first to describe a dynamic dose range for the relationship between AEM-based dosing and BBBO (per dose unit). Given that an optimal therapeutic window for MB-FUS remains to be fully delineated, such investigations are needed to facilitate prescription of most-optimized treatments within a specific safe range across iterative treatment cycles.

Consensus is also lacking on the surrogate indicators, and monitoring methods in tumor tissue and systemic circulation.[218] Reliable non-invasive monitoring being a major regulatory concern.[206] As FUS technologies evolve, with larger treatment volumes and higher sonication powers being used in clinical investigations, developing FUS-specific radiographic frameworks for detecting, ensuring, and/or reporting AEs will be essential, such as the novel grading scale for MB-FUS BBBO described in **Table 2**.

## Adaptive Theragnostics: Integrating FUS into SOC for Brain Tumors

An "adaptive theragnostic paradigm" may be envisioned as how FUS may integrate fully into SOC management of brain tumors (**Figure 5**). As part of a precision oncology paradigm, image-guided FUS gets utilized in adaptive fashion through BBBO-enabled plasma biomarker enrichment, allowing genomic, epigenomic, and other multi-omic profiling of tumor.[13] A bespoke systemic therapy strategy is created based on tumor molecular findings. The selected systemic therapy gets combined with MB-FUS BBBO[49] enabling localized, efficacious delivery. Interval LBx allows for prognostication and response assessment, without invasive procedures throughout the patient's disease course (thus maintaining quality of life). Serial LBx also allows for capture of dynamic disease evolution and modification of systemic therapy. This cycle get iteratively repeated across disease course, allowing "adaptive theragnostic" management. For patients achieving minimal residual disease, longitudinal disease surveillance could be carried out through SLB (along with MRI), allowing early detection and intervention.

## Conclusions

FUS offers tremendous promise for patients with PBTs, especially GBM. MB-FUS BBBO-enabled brain drug delivery and sono-liquid biopsy possess the most substantial clinical evidence and translational readiness of all FUS applications currently. These applications open a combinatorial path forward, targeting the root causes of failure to cure HGGs. Nevertheless, the success of FUS in RCTs will require standardization of sonication delivery protocols, coordinated global efforts, thoughtful trial design, and development of practical implementation approaches enabling greater site participation. FUS advancement will be enabled by multi-stakeholder collaboration, support from professional societies, translational research nested into clinical studies, and dedicated FUS research consortia (like ReFOCUSED). FUS-enabled adaptive theragnostic management paradigm may lead to curative approaches for HGGs.

## Supplementary Material

Supplementary material is available online at *Neuro-Oncology Advances* (https://academic.oup.com/noa).

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Conceptualization: AO, GFW; Supervision: GW; Project administration: AO; Visualization: AO, BM, PA, DG, GFW; Writing – original draft: AO, BM; Writing – review & editing: AO, BM, PA, DG, CB, PSM, JH, MSA, GFW; Writing – manuscript revision: AO.

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