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Ultrasound mediated blood-brain barrier opening increases brain tumor biomarkers: A review of preclinical and clinical trials

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ABSTRACT

The diagnosis of brain tumors typically relies on magnetic resonance imaging (MRI), computed tomography (CT), and invasive procedures like biopsies or surgical resection for confirmation and genetic profiling. However, these methods have limitations, especially in distinguishing treatment effects like pseudo-progression from actual tumor progression, and repeated biopsies pose risks. Liquid biopsy (LB) offers a non-invasive alternative, detecting tumor-derived biomarkers in blood and cerebrospinal fluid (CSF). Despite its potential, the low concentration of brain tumor biomarkers in blood due to the blood-brain barrier (BBB), limits the clinical utility of LB. MRI-guided focused ultrasound (MRgFUS) combined with microbubbles provides a novel solution by temporarily disrupting the BBB, facilitating the passage of therapeutic agents, and enabling tumor biomarker detection. This technique, termed "sonobiopsy," enables non-invasive biomarker collection for liquid biopsy, potentially improving brain tumor diagnosis and monitoring.

1. Introduction

The diagnosis of brain tumors relies on magnetic resonance imaging (MRI) and computer tomography (CT) followed by stereotactic biopsy or surgical resection to confirm histopathology and perform genetic profiling [1,2]. However, these diagnostic and prognostic techniques have limitations. The changes observed, such as pseudo-progression or radiation necrosis, are difficult to interpret using neuroimaging, especially after chemotherapy and/or radiotherapy [3]. Moreover, repeated tumor biopsies, essential for monitoring tumor evolution, treatment response, and recurrence, are often impractical and can carry risks such as bleeding and infections. Additionally, obtaining tissue samples can be difficult if tumors are in challenging locations or if patients are too sick to undergo invasive procedures [4]. The alternative way to diagnose and profile brain tumors involves non-invasive liquid biopsies from blood and cerebrospinal fluid (CSF) [1,2].

Blood-based liquid biopsy (LB) detects and analyzes tumor-derived biomarkers such as circulating tumor cells (CTCs), circulating tumor DNAs (ctDNA), RNAs (ctRNA), extracellular vesicles (EVs), and a series of tumor-related proteins in the bloodstream, eliminating the need for invasive procedures like open surgery or stereotactic biopsies [5,6]. It has the potential to serve as a comprehensive platform for diagnosis and prognosis, detail tumor heterogeneity, guide treatment selection, and assist in monitoring disease response to therapeutics [7–11]. However, it remains difficult to implement LB for brain tumors in clinical settings due to certain challenges [12–14]. One of the challenges is the lower concentration of tumor biomarkers due to the blood-brain barrier (BBB), which limits the passage of these biomarkers to blood [15,16], resulting in lower detection sensitivity [12,15]. Focused ultrasound has recently been utilized to address this challenge.

MRI-guided transcranial focused ultrasound (MRgFUS), in combination with injected microbubbles (MB), presents a promising method for achieving noninvasive, spatially targeted, and reversible BBB disruption. This innovative approach utilizes the real-time imaging capabilities of MRI to accurately direct focused ultrasound waves to specific areas of the brain [17]. Recent clinical studies have shown that focus ultrasound (FUS)-mediated opening of the blood-brain barrier is both feasible and safe for delivering drugs to the brain in patients with Alzheimer's disease [18], amyotrophic lateral sclerosis [19], brain tumors [20–23], and Parkinson's disease [24]. FUS-induced blood-brain barrier opening facilitates "two-way trafficking" between the brain and the bloodstream [25]. This process allows therapeutic agents from the bloodstream to enter the brain to treat neurological conditions while also enabling the release of brain tumor-derived biomarkers into the

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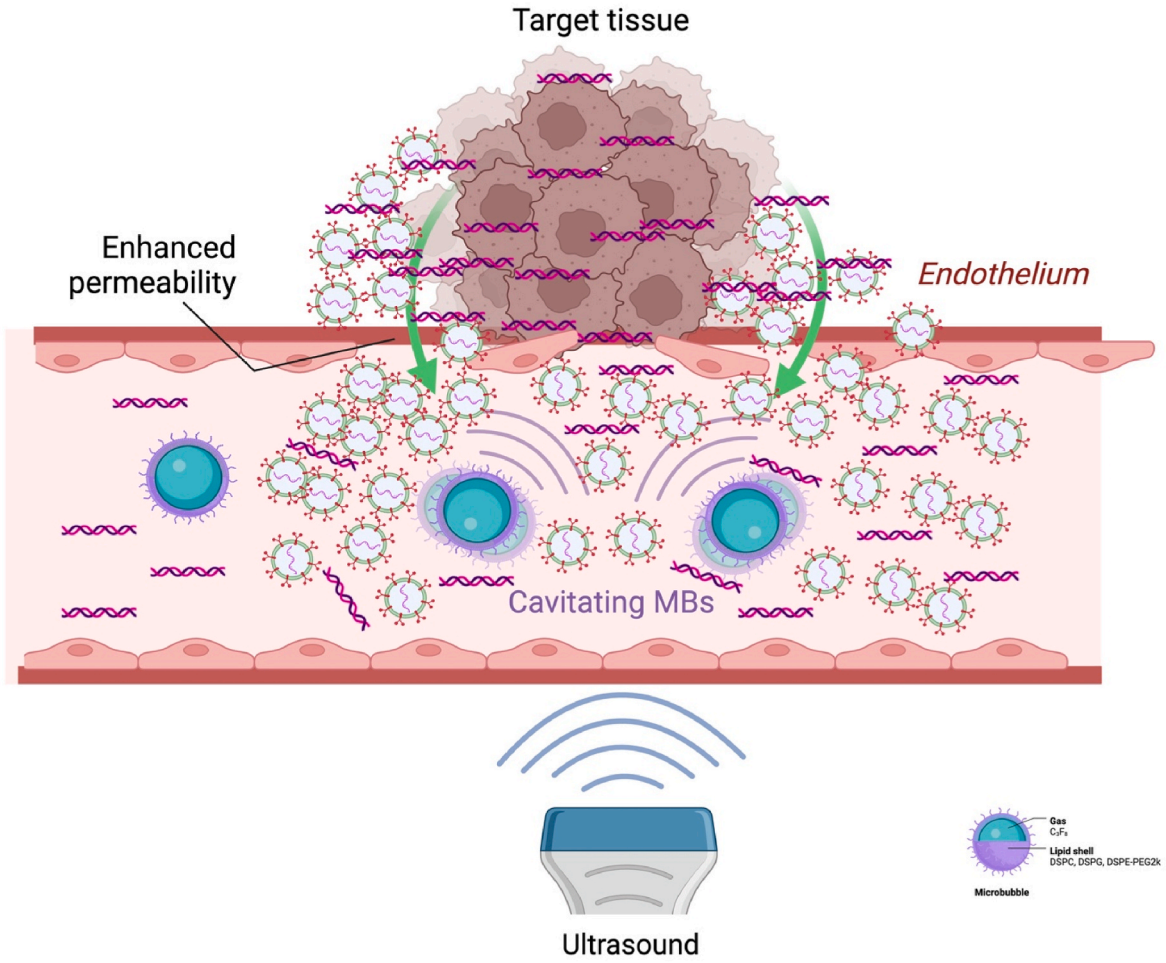

Fig. 1. Mechanism of FUS-mediated opening of BBB for tumor biomarker release. *As the MB travel through tumor micro-vessels in the FUS-targeted area, they undergo cavitation—expansion, contraction, and collapsing in the acoustic field. During expansion, the microbubbles stretch and separate the endothelial cell lining, while contraction causes the vascular lining to invaginate. This push-pull mechanism widens the tight junctions of the BBB, enhancing its permeability and release of tumor biomarkers.*

bloodstream for diagnostic purposes. This FUS-mediated release of biomarkers for blood-based liquid biopsies is termed "*sonobiopsy*" [2]. In this review paper, we will highlight the current literature on the role of MRgFUS in liquid biopsies for brain tumors.

2. Mechanism of MRgFUS-induced BBB opening

The BBB consists of endothelial cells, astrocyte end-feet, and pericytes, all interconnected by tight junctions that block the passage of most substances [26]. Typically, substances cross the BBB through passive diffusion of small, non-polar lipophilic molecules (under 400 Da) or through active transport mechanisms. This selective permeability significantly restricts the delivery and effectiveness of most drugs for brain tumors or small-molecule therapies and the release of tumor biomarkers [2,27,28]. FUS combined with MB has emerged as a noninvasive method for disrupting the BBB to enhance drug delivery [29]. MB, used as an ultrasound contrast agent in clinical imaging, is injected intravenously. An extracorporeal ultrasound transducer generates FUS, penetrating the skull and focusing energy on a specific brain region. As MB travel through this targeted area, the ultrasound waves cause them to undergo cavitation—expanding, contracting, and collapsing in the acoustic field [30]. During expansion, the MB stretch and separate the endothelial cell lining, while contraction causes the vascular lining to invaginate. This push-pull mechanism helps widen the tight junctions of the BBB, enhancing its permeability [31] (Fig. 1). This technique has shown success in both small and large animal models, including nonhuman primates [32,33], sheep [34], and pigs [35,36].

3. Preclinical trials: FUS enhances biomarker release across BBB

In a preclinical trial, Zhu et al. [37] studied mice with orthotopic implantation of enhanced green fluorescent protein (*eGFP*)-transfected murine glioblastoma (GBM) cells. They used MRgFUS at three different peak negative acoustic pressure (PNP) levels: 0.59 MPa, 1.29 MPa, and 1.58 MPa, with five mice (n = 5) in each group. *eGFP* mRNA was chosen as a representative biomarker because it was highly specific to their tumor model [37]. Additionally, mRNA is more abundant in plasma than the more commonly studied ctDNA [38] and easily detected using established PCR-based assay [39]. Two sets of primers were used to enhance the detection of qualitative polymerase chain reaction (qPCR). When comparing the MRgFUS-treated groups to the control group (no FUS and MB), all three treated groups showed a significant increase in *eGFP* mRNA levels. The group treated with 0.59 MPa demonstrated a 55-fold increase in *eGFP* mRNA with primer A and a 221-fold increase with primer B compared to control. Similarly, the groups treated with 1.29 MPa and 1.58 MPa showed a 2000-fold and 8000-fold average increase in *eGFP* mRNA level relative to the control, respectively. No significant difference in microhemorrhage density between the group treated with 0.59 MPa FUS and the control group was found. However, the microhemorrhages were significant in the 1.29 MPa and 1.58 MPa groups compared to the 0.59 MPa and control groups. In the control mice, microhemorrhages were predominantly scattered within the

Table 1
Highlights the details of all preclinical trials involving FUS mediated opening of BBB and release of biomarkers across it.

Author	Model/Cell line/ Number(n)	Biomarkers/ Target	Detection Technique	Results	Change in BBB permeability	Complications	FUS type	US set-up
Zhu et al. [37]	• NIH Swiss mice, Strain 550 • Mouse glioma GL261 cell lines (Washington University School of Medicine, St. Louis, MO, USA). • N = 20 (treated = 15, control = 5)	mRNA/*eGFP*	qPCR	At 0.59 MPa, there was a 55 to 221-fold increase in *eGFP* mRNA levels. At 1.29 MPa, the increase was 2000-fold, and at 1.58 MPa, it was 8000-fold.	Assessed by Contrast-enhanced T1-weighted turbo spin-echo MR images (TR, 500 ms; TE, 13 ms; acquisition matrix, 96 × 96; resolution, 0.2 mm × 0.2 mm × 0.5 mm)	Microhemorrhages were observed in all cases. peritumoral hemorrhages were observed in 1 out of 5 mice treated with 0.59 MPa FUS and in 4 out of 5 mice in the 1.29 MPa and 1.58 MPa FUS groups.	• MR-guided FUS system (Sonalleve V2, Profound Medical Inc., Mississauga, Canada) • clinical MRI scanner (Ingenia 1.5T, Philips Healthcare, Best, the Netherlands) with a 256-element phased-array FUS transducer.	FUS center frequency = 1.44 MHz, sonication duration = 240 s, pulse repetition frequency = 1 Hz, duty cycle = 1 %, and pulse length = 10 ms
Zhu et al. [40]	• NCl athymic NCr-nu/nu mice (Strain 553) injected with U87 human GBM cells. • NIH Swiss mice (Strain 550) implanted with GL261 murine GBM cells • N = 21 (6 control, 15 treated)	mRNA/*eGFP*	qPCR	mRNA level of *eGFP* were increased significantly in both cohorts after treatment with FUS	Assessed by contrast-enhanced MRI	On histological analysis, red blood cells (RBCs) extravasations were seen in all GL261 mouse models. More severe hemorrhages were found on H&E staining of brain slices obtained from mice treated with high acoustic pressures than those treated with low pressure.	• US imaging-guided FUS system (VIFU 2000; Alpinion US Inc., Bothell, WA, USA) for U87 models • Clinical MRgFUS system (Sonalleve V2, Profound Medical Inc., Mississauga, Canada) equipped with a dedicated small animal adapter (FUS Instruments Inc., Toronto, Ontario, Canada) for GL261 models	• *US imaging-guided FUS parameters:* frequency = 1.5 MHz, peak negative pressure = 3.82 MPa, pulse length = 10 ms, pulse repetition frequency = 1 Hz, duration = 30 s at each location, 4 locations for each tumor. • *MRgFUS parameters:* frequency = 1.44 MHz, peak negative pressure = 1.52, 2.74, and 3.53 MPa, pulse length = 10 ms, pulse repetition frequency = 1 Hz, duration = 2 min
Pacia et al. [16]	Pigs/normal brain/n = 16, Cohort 1 = 8 for BBBO study Cohort 2 = 8 for biomarker study	Brain-specific proteins/ *MBP* and *GFAP*	ELISA	FUS opened BBB in 7 out of 8 pigs in cohort 1 and increased *GFAP* and *MBP* on cohort 2	• Increased contrast enhancement T1-weighted MRI compared to contralateral side (p = 0.0156). • Increase in K^{trans} of the targeted brain site compared to the contralateral side (p = 0.0053).	No sign of hemorrhage or tissue damage was found on MRI, gross pathological assessment, and H&E histological analysis post-FUS.	• MRI-compatible FUS system (Image Guided Therapy, Pessac, France). • The FUS transducer (Imasonics, Voray sur l'Ognon, France)	MRgFUS parameters: frequency = 0.65 MHz, peak negative pressure = 1.5 MPa, pulse length = 10 ms, pulse repetition frequency = 1 Hz, duration = 3 min
Pacia et al. [41]	• Immunodeficient mice (strain: NCI Athymic NCr-nu/nu) and pigs (breed: Yorkshire white, Oak Hill Genetics) • U87-EGFRvIII^{+} cells carrying TERT	cfDNA/ *EGFR vIII* and *TERT C228T*	ddPCR	• Diagnostic sensitivity of *EGFR vIII* increased from 7.14 % to 64.71 % and 28.57 %–100 % post-FUS in mouse and	Contrast-enhanced T_1-weighted MRI	Microhemorrhages were in tumor region of interest without any off-target parenchymal microhemorrhages or damage.	• MRI-compatible FUS transducer (Imasonics, Voray sur l'Ognon, France). • MRI-guided FUS (Image Guided	• Frequency = 1.5 MHz, pressure = 1.0 MPa, pulse repetition frequency = 5 Hz, duty cycle = 3.35 %, pulse length

(*continued on next page*)

Table 1 (*continued*)

Author	Model/Cell line/ Number(n)	Biomarkers/ Target	Detection Technique	Results	Change in BBB permeability	Complications	FUS type	US set-up
	C228T (provided by Dr. Frank Furnari from the University of California-San Diego).			porcine models respectively. • Similarly diagnostic sensitivity of *TERT C228T* increased from 14.29 % to 45.83 % and 42.86 %–71.43 % post-FUS in mouse and porcine models respectively			Therapy, Pessac, France)	= 6.7 ms, treatment duration = 3 min. • Frequency = 0.65 MHz, pressure = 3.0 MPa, pulse repetition frequency = 1 Hz, duty cycle = 1 %, pulse length = 10 ms, treatment duration = 3 min.
Zhang et al. [42]	Mouse GBM model/ C57/BL6 mice	cfDNA	• Qubit Fluorometer • ddPCR	• increasing acoustic power increases the cfDNA level. • At 0.4 MPa, the cfDNA was high at 60 min. • cfDNA also increases in a dose-dependent manner.	Fluorescent studies via Nikon AZ100 Epifluorescent microscope.	N/A	LIPU device (SonoCloud® technology manufactured by CarThera, Paris, France)	Frequency = 1 MHz, pulse length = 25 ms, pulse repetition frequency = 1 Hz, duration = 120 s, pressure = 0.3–0.4 MPa

tumor. In addition to scattered intratumoral microhemorrhages, peritumoral hemorrhages near the tumor-normal brain parenchyma interface were observed in 1 out of 5 mice treated with 0.59 MPa FUS and in 4 out of 5 mice in the 1.29 MPa and 1.58 MPa FUS groups. Moreover, no off-target damage in brain tissue was observed [37].

In another study [40], Zhu et al. used two different models, i.e., Orthotopic human glioma xenograft (U87) and orthotopic murine glioma xenograft models (GL261), to study the effect of FUS on biomarker release. In the U87 model cohort, the mRNA level of *eGFP* in plasma was significantly higher in the FUS-treated group than in the untreated control group ($p = 0.01$). Similarly, in the GL261 model cohort, circulating *eGFP* mRNA levels were significantly higher (1500–4800-fold, $p = 0.0045$) in the FUS-treated groups ($n = 9$) relative to the control group ($n = 3$). The *eGFP* mRNA levels of mice ($n = 3$) treated at the lowest pressure (1.52 MPa) were significantly higher than those of the other two groups ($n = 3$ each, 2.74 MPa, and 3.53 MPa, respectively). Red blood cell (RBC) extravasations were seen on histological analysis in all GL261 mouse models. More severe hemorrhages were found on H&E staining of brain slices obtained from mice treated with high acoustic pressures than those treated with low pressure.

Pacia et al. [16] used FUS and evidenced BBB disruption in 7 out of 8 pigs by measuring BBB opening (BBBO) volume using contrast-enhanced MRI and pharmacokinetic analysis of K^{trans}. The quantified BBBO volume in the FUS-treated area (1.21 ± 1.84 cm^3) was significantly larger ($p = 0.0156$) than that of the contralateral untreated area (0.013 ± 0.018 cm^3). Additionally, the BBB permeability, measured by Ktrans, was significantly higher in the targeted brain region ($9.9 \times 10^{-3} \pm 3.9 \times 10^{-3}$ min^{-1}) compared to the contralateral side ($1.4 \times 10^{-3} \pm 0.8 \times 10^{-3}$ min^{-1}, $p = 0.0053$). FUS significantly increased the plasma levels of two brain-specific biomarkers, glial fibrillary acidic protein (*GFAP*) and myelin basic protein (*MBP*). The *GFAP* concentration increased significantly ($p = 0.0074$) from 0.156 ± 0.068 ng/mL in pre-FUS blood samples to 0.353 ± 0.149 ng/mL post-FUS. Similarly, the *MBP* concentration significantly increased ($p = 0.0039$), rising from 0.091 ± 0.034 ng/mL to 0.364 ± 0.159 ng/mL post-FUS. No sign of hemorrhage or tissue damage was found on MRI, gross pathological assessment, and H&E histological analysis post-FUS.

Pacia et al. [41], in another study, injected human GBM cells (U87) overexpressing epidermal growth factor receptor vIII (*EGFR-vIII*) and harboring the telomerase reverse transcriptase C228T (*TERT C228T*) mutation to create a mouse model of GBM. This model was then used to compare the detection sensitivity *of EGFR-vIII* and *TERT C228T* mutations before and after FUS treatment. Approximately 10–12 days after intracranial implantation, the mice were assigned to either a control group (blood collection without FUS) or an FUS group (blood collection after FUS). The average tumor volumes between the control group ($n = 21$) and the FUS group ($n = 24$) showed no significant difference ($p = 0.78$). Terminal blood collection was performed via cardiac puncture 10 min after FUS sonication. The digital droplet PCR (ddPCR) analysis of plasma cell-free DNA (cfDNA) revealed that FUS enhanced cfDNA release compared to conventional liquid biopsy. The level of EGFR-vIII ctDNA in the FUS group was significantly higher, showing a 920-fold increase over the control group. Similarly, the *TERT C228T* ctDNA levels were 10 times greater in the FUS group compared to the control. Sonobiopsy improved the diagnostic sensitivity for detecting *EGFR-vIII* from 7.14 % to 64.71 % and *TERT C228T* from 14.29 % to 45.83 %. The non-significant microhemorrhages were found in the targeted tumor region with no off-target microhemorrhages or parenchymal damages. These findings were further validated in a porcine GBM model developed by implanting the U87 GBM cells into the cortex of pigs. The FUS sonication improved the diagnostic sensitivity from 28.57 % to 100 % for *EGFR-vIII* and 42.86 %–71.43 % for *TERT C228T* for porcine GBM models with a 95 % confidence interval. Microhemorrhages near the edge of the tumor were seen in some cases without off-target damage.

Zhang et al. [42] used a mouse GBM model to study the effects of different parameters of sonobiopsy. They demonstrated an increasing level of cfDNA in the plasma of mice when the acoustic power level was increased from 0.3 to 0.4 MPa while keeping the MB dose (Lumason, Bracco, 10 mg/kg) and all other pulsing parameters constant. At 0.4 MPa, cfDNA was increased in the blood 30 min post-FUS compared to the MB-only control (36.09 vs. 9.252 ng/mL, $P = 0.0086$) and lower acoustic power (0.3 MPa) group (36.09 vs 13.63 ng/mL, $P = 0.0039$), suggesting that higher acoustic powers were associated with increased biomarker release. Next, they determined the optimal time for blood collection post-FUS while maintaining the acoustic power at 0.4 MPa and keeping other parameters constant. Peripheral blood samples were

Table 2
Highlights the details of completed or partially completed clinical trials involving FUS mediated opening of BBB and release of biomarkers with published results.

Clinical Trial No.	Status	No. of Patients	Type of Tumors	Biomarkers/ mutation	Detection strategy	BBB opening assessment	Results	Complications/Adverse events	FUS setup	Principal investigator
NCT03739905 and **NCT03616860**	completed	N = 9	*IDH*-driven GBMs	• cfDNA, *IDH1* • ndEV marked by *NCAM* and *L1CAM* • *S100b*	• Qubit 3.0 Fluorometer with Qubit dsDNA HS Assay Kit, Agilent 2100 Bioanalyzer • nanoscale flow cytometer • ddPCR • ELISA	T1-weighted MRI	• 2.6 ± 1.2-fold increase in cfDNA • 3.2 ± 1.9-fold increase ($P < 0.01$) in double-positive NCAM and L1CAM particles • 1.4 ± 0.2-fold increase in S100b levels	**Intra-procedure** Headache, Presyncope, Nausea/vomiting, Agitation, Hypotension. **Post-procedure, transient** Pin site tenderness or edema and T2 GRE hypointensity.	FUS system (ExAblate 4000, 230 kHz; InSightec, Haifa, Israel). For each spot, 2 msec on, 28 msec off pulses were repeated for 300 msec, pulse repetition frequency = 2 msec/30 msec × 0.3 s/2.7 s = 0.74 %, Total sonication time = 50 s, Acoustic power levels = 3–20 W. **MB** (Definity®) = (4 μl/kg)	Nir Lipsman [5]
NCT05281731	Incomplete and recruiting	N = 5	4 GBMs, and 1 diffuse high-grade glioma	cfDNA/*IDH1* wild-type, *TERT* promoter.	• Illumina NovaSeq-6000. • ddPCR • qPCR	N/A	• The cfDNA concentration was significantly higher in the post-FUS samples of 4/5 patients. • Sonobiopsy enhanced the detection of 50 patient-specific ctDNA in the plasma of 3/5 patients. • the ddPCR analysis showed an increase in *TERT* mutation level in post-FUS blood samples of *TERT* + patients.	No adverse events or microhemorrhages were observed during and after FUS sonication.	FUS +15 concentric individual ring transducers with a center frequency of 650 kHz (Imasonics, Voray-sur- l'Ognon, France). The FUS parameters were: center frequency = 650 kHz (f_0); pulse repetition frequency = 1 Hz; pulse duration = 10 ms; treatment duration = 3 min. **MB** = (Definity) 10 μL/kg body weight.	Albert Kim, M.D[2]

IDH = Isocitrate Dehydrogenase, ***NCAM*** = Neural Cell Adhesion Molecule, ***L1CAM*** = Cell Adhesion Molecule L1, **FUS** = Focused Ultrasound, **MB** = Microbubbles.

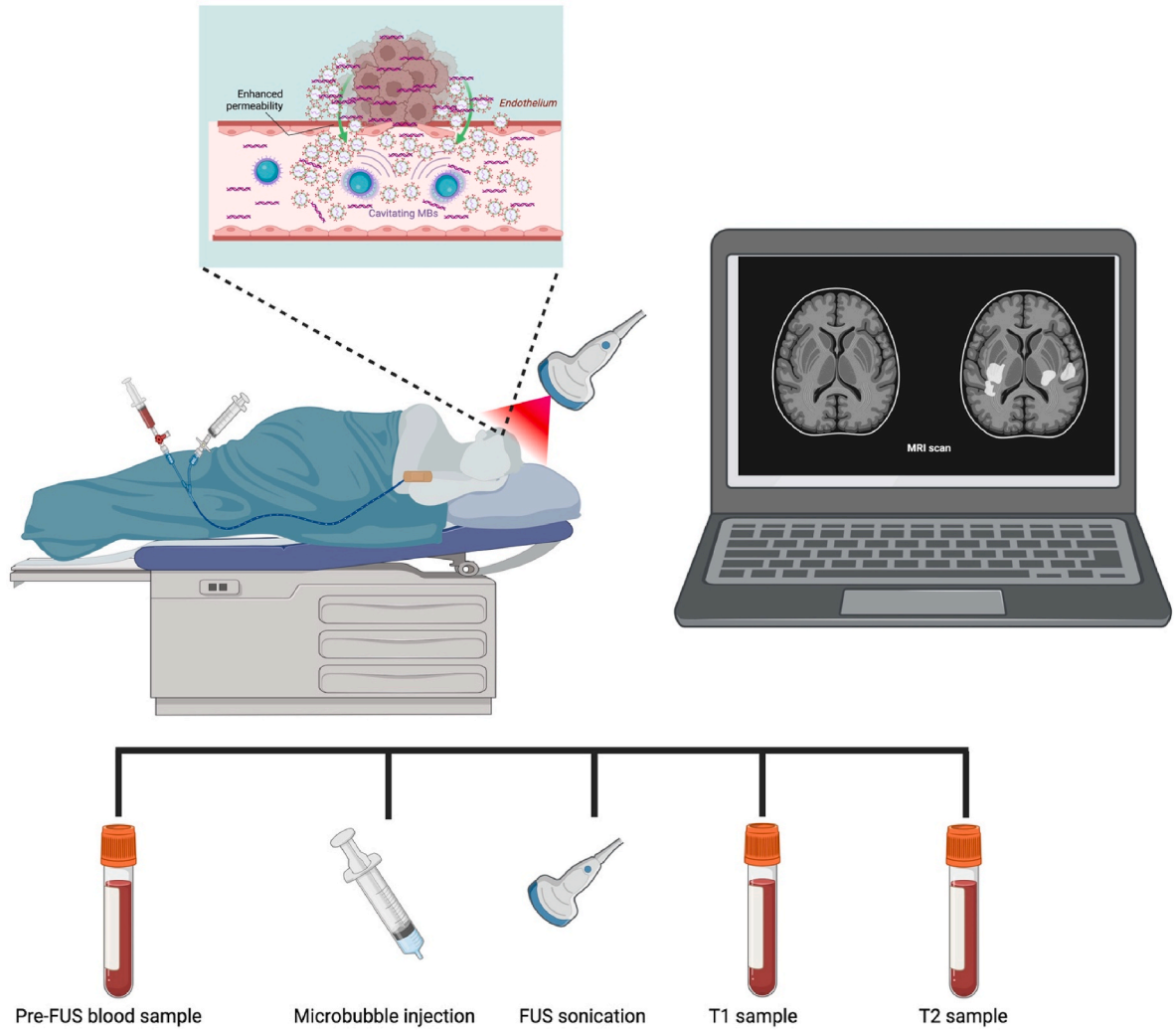

Fig. 2. Clinical workflow. *This figure demonstrates the clinical workflow of FUS-mediated liquid biopsy. Initially, a blood sample is collected before microbubble injection. Next, MBs are injected into the bloodstream, and the area of interest in the brain is targeted through FUS. The ultrasound waves cause the MBs to cavitate, which opens the BBB by disrupting endothelial cell junctions. After sonication, an MRI scan is performed to assess the extent of BBB opening. Finally, post-FUS blood samples are collected for liquid biopsy to analyze circulating biomarkers.*

collected at 2, 15, 30, 45, and 60 min after FUS. Significant increases in cfDNA concentrations were observed at 15 min compared to the non-sonicated control group. The highest average cfDNA concentrations were found in the group with blood collected 60 min post-FUS, showing significantly higher levels compared to the FUS-only group (no MB, 46.54 vs 13.01 ng/mL, $P = 0.0027$) and the MB-only controls (no FUS, 46.54 vs 8.48 ng/mL, $P = 0.0149$). They further discovered that mean cfDNA concentrations increased in a dose-dependent manner. They compared cfDNA levels in C57/BL6 mice that received either a single sonication (SS) or two sequential sonication (DS) treatments. Mice that underwent DS treatment showed significantly higher cfDNA levels compared to the MB control group (64.32 vs 7.907 ng/mL, $P = 0.0034$) and the SS group (64.32 vs 22.54 ng/mL, $P = 0.0166$). Further details of the pre-clinical trials are given in Table 1. These studies underscore the potential of FUS to enhance biomarker release across the BBB while varying in safety and efficacy across models and conditions.

4. Clinical trials for sonobiopsy

However, the pre-clinical studies show that FUS enhances the release of biomarkers from brain/brain tumors across the BBB. Similarly, two clinical trials were conducted recently to demonstrate the increased release of biomarkers from brain tumors and to evaluate the feasibility and safety of sonobiopsy. In a prospective single-arm, open-label trial (**NCT03739905**) [5], nine patients with GBM underwent transcranial MRgFUS, and blood samples were collected before and on average 34 min following the last sonication. Plasma cfDNA concentration was sharply increased after FUS sonication, rising by 2.6 ± 1.2-fold (from 7.0 ± 3.3 ng/mL to 16.3 ± 5.2 ng/mL of plasma, $P < 0.01$). An increase was also observed in the brain-derived biomarkers, specifically neuron-derived extracellular vesicles (ndEV) characterized by the surface proteins i.e. neural cell adhesion molecule (*NCAM*) and cell adhesion molecule L1 (*L1CAM*), as well as S100 calcium-binding protein B (*S100b*). From a randomly selected cycle for each patient, nanoscale flow cytometry revealed a 3.2 ± 1.9-fold increase ($P < 0.01$) in double-positive *NCAM* and *L1CAM* particles, along with a 1.4 ± 0.2-fold increase ($P < 0.01$) in S100b levels measured by enzyme-linked immunosorbent assay (ELISA). Some adverse events were reported during the FUS sonication, as given in Table 2.

In a prospective clinical trial (**NCT05281731**) [2] of sonobiopsy in high-grade glioma patients, the cfDNA concentration was significantly higher in the post-FUS samples of 4 out of 5 patients, with a fold increase ranging from 1.1 to 1.6 folds compared to pre-FUS samples. Furthermore, sequencing allowed the identification and selection of up to 50

Table 3
Highlights the details of all ongoing clinical trials involving FUS mediated BBB opening and biomarker study without published results.

Clinical Trial No.	Status	Purpose of the trial	Condition	Biomarkers	Intervention/ Treatment/FUS device	Location of the study	Principle Investigator(s) or Primary Contact
NCT04667715	Suspended	• To evaluate the safety and effectiveness of exablate model 4000 using MB resonators to temporarily mediate blood-brain barrier disruption (BBBD) in subjects with suspected infiltrating glioma. • To evaluate circulating tumor biomarkers (e.g. ctDNA)	suspected Grade II, III or IV infiltrating glioma	No specific	FUS: Exablate Type 2 system (Exablate Model 4000)	USA	Graeme Woodworth, MD
NCT05293197	Recruiting	• To study the safety, feasibility, and efficacy of SonoCloud for BBBD in pediatric patients treated with carboplatin chemotherapy for a recurrent supra-tentorial malignant brain tumor. • To assess ctDNA concentrations at diagnosis and during repeated opening of the BBB.	Recurrent supra-tentorial malignant brain tumors	ctDNA	FUS: SonoCloud® (9 transducers)	France	Kevin BECCARIA, MD, PhD
NCT04528680	Recruiting	• BBBO with an implantable ultrasound device, SonoCloud-9 and treatment With Albumin-bound Paclitaxel and Carboplatin in patients with recurrent GBM. • Measurement circulating biomarkers before and after sonication.	Recurrent GBM	ctDNA	FUS: Implantable SonoCloud-9	USA	Adam M Sonabend, MD
NCT05383872	Recruiting	• To study the safety and feasibility of FUS. • To demonstrate that there is increase in ctDNA following BBBD	GBM	ctDNA	FUS: Exablate Model 4000 Type 2.0/2.1	USA, Canada	• Richard Everson, MD • John de Groot, MD • Justin Hilliard, MD • Manmeet Ahluwalia, MD • Yarema Bezchlibnyk, MD • Michael Vogelbaum, MD • Graeme Woodworth, MD • Jordina Rincon Torroella • Terence Burns, MD • Alon Mogilner • Vibhor Krishna, MD • Gerald Grant, MD • Bhavya Shah, MD • Jeffrey Weinberg, MD • Christopher Cifarelli, MD • Nir Lipsman
NCT04940507	Recruiting	• To assess the utility of MRgFUS in enhancing the abundance of brain tumor ctDNA • To evaluate the utility of MRgFUS in enhancing the non-invasive detection of brain tumor methylation signatures • to improve ctDNA abundance using MRgFUS to allow for non-invasive detection of clinically relevant genomic alterations such as *IDH1/2, TERT promoter, CDKN2A/B, PTEN, EGFR, TP53, BRAF,* and *PDGFRA* mutations in GBM patients. • The optimal time-point of liquid biopsy acquisition. • Safety (procedure-related complications)	• New MRI-diagnosed intracranial lesions that are suitable to biopsy surgically • Essential tremor	ctDNA	• MRgFUS tumor ablation and liquid biopsy acquisition • MRgFUS Thalamotomy • ExAblate Neuro 4000 Device (InSightec Ltd, Tirat Carmel, Israel)	Canada	• Andres M. Lozano, MD, PhD • Gelareh Zadeh, MD, PhD

CDKN2A/B = Cyclin Dependent Kinase Inhibitor 2A/B, ***PTEN*** = Phosphatase and Tensin Homolog, ***TP53*** = Tumor Protein P53, ***BRAF*** = B-Raf Proto-Oncogene, ***PDGFRA*** = Platelet Derived Growth Factor Receptor Alpha.

tumor variant DNAs (ctDNA) present in the tumors but absent in the corresponding normal tissues. These selected ctDNA were then used to design a patient-specific panel for detecting ctDNA in plasma samples. The absolute levels of patient-specific ctDNA (copies/ml of plasma) were compared in samples collected before and after FUS treatment. The findings revealed that sonobiopsy enhanced the detection of patient-specific ctDNA in the plasma of 3 out of 5 patients. Moreover, the ddPCR analysis showed an increase in *TERT* mutation level in post-FUS blood samples. During FUS sonication, no adverse event was observed. Following FUS sonication, no hemorrhage or damage was observed on gross pathological assessment and H&E histological analysis. The clinical workflow is shown in Fig. 2.

Similarly, some other clinical trials are being conducted to study the feasibility and safety of sonobiopsy and show an increase in biomarker release from tumors across BBB after sonication. The details of all ongoing clinical trials are given in the table below (Table 3).

5. Safety of sonobiopsy

Given the fragility of the nervous system, ensuring the safety of brain tumor diagnostics is of utmost importance; it is crucial to minimize or even eliminate any potential damage caused by the diagnostic process. Multiple studies have demonstrated that FUS-induced BBBO is reversible [19,24], with complete closure occurring within 6–24 h [43–45]. Additionally, FUS-mediated BBBO is non-invasive, and previous research has shown that BBBO, which facilitates drug permeation, does not result in significant acute damage to endothelial or neuronal cells [46].

In preclinical models, sonobiopsy significantly enhanced the release of brain-specific biomarkers but did not cause significant off-target brain tissue damage, as confirmed by histological analysis, gross pathological assessment, and MRI [16,37,40–42]. However, H&E-stained brain sections revealed microhemorrhages in targeted areas, with the density of the microhemorrhages correlating to the intensity of the applied ultrasound pressure [37,40–42]. In the study conducted by Pacia et al. [16], no microhemorrhages were observed in sonicated pig models at a PNP of 1.5 MPa. In animal models, higher acoustic pressures were more likely to cause microhemorrhages compared to lower acoustic pressures [37, 40]. However, in the clinical trial [2], no significant microhemorrhages and off-target brain tissue damage were observed. Some adverse events, such as headache, presyncope, nausea/vomiting, agitation, and hypotension during sonication, as well as pin site tenderness, edema, and T2 GRE hypointensity after sonication, were reported in one of the clinical trials [5], as detailed in Table 2.

Furthermore, previous studies have shown that FUS with MB can induce sterile inflammation in healthy mouse brains [47–49]. However, in the prospective clinical trial (NCT05281731) [2], RNA sequencing analysis of sonicated and non-sonicated tumor tissues from 3 out of 5 patients, collected within 1.7 $\pm$ 0.4 h post-sonication, indicated that sonobiopsy did not trigger a significant immune or inflammatory response.

6. Limitations and challenges

While advancements in sonobiopsy for the molecular diagnosis of brain tumors represent significant milestones, several limitations and challenges must be addressed to enhance its clinical applicability. Most studies have predominantly utilized animal models, indicating a pressing need for further clinical investigations to evaluate the sensitivity and specificity of sonobiopsies compared to traditional liquid biopsies [2]. Additionally, the optimal timing for blood collection following focused ultrasound (FUS) sonication remains undetermined; this timing is critical as it may be influenced by the dynamics of BBBO and the kinetics of specific biomarkers. Furthermore, many trials have not thoroughly investigated specific tumor-released biomarkers, which limits the understanding of their relevance in this context [43]. Determining optimal sonication parameters—such as acoustic pressure, pulse repetition frequency, pulse length, and duration—and MB characteristics (size and dosage) for effective biomarker release in human brain tumors is still unresolved while ensuring patient safety. Although preliminary clinical trial data show promise regarding the feasibility and safety of sonobiopsy, the relatively small sample sizes in existing studies hinder broader conclusions [2]. Moreover, spatial shifts in brain anatomy during surgical dissection can introduce localization errors for FUS-sonicated tumor regions [5]. The influence of prior treatments like radiation and chemotherapy on the feasibility and effectiveness of sonobiopsy also requires further investigation [2]. Variability among tumor types, densities, anatomical locations, depths, skull physiology, vascular structures, and tumor-specific biomarkers may necessitate tailored parameters for optimal sonobiopsy application [50]. Additionally, the exact mechanisms by which FUS facilitates biomarker release remain unclear, and inconsistencies in biomarker release under similar conditions pose challenges for reproducibility. Finally, while transient BBBO may allow beneficial biomarkers to enter circulation, it could also permit harmful substances to infiltrate the brain, potentially causing central nervous system damage [43]. Addressing these limitations is essential for advancing sonobiopsy as a reliable diagnostic tool for brain tumors.

7. Conclusion and future directions

In conclusion, preclinical and clinical studies have demonstrated the feasibility and safety of FUS-mediated release of brain tumor-specific biomarkers into the bloodstream. Sonobiopsy shows great promise for revolutionizing brain tumor diagnosis and monitoring, with recent advancements in liquid biopsies and MRgFUS paving the way for this non-invasive approach. Key challenges remain, such as optimizing FUS settings to maximize biomarker release based on tumor pathology and location. Ongoing research and clinical trials aim to address these issues. With continued development, MRgFUS-enabled liquid biopsy could transform the management of brain tumors, offering a safer, minimally invasive alternative to traditional biopsies and enabling more timely, personalized treatments.

In the context of future direction, to improve the efficacy and safety of sonobiopsy, future research must focus on several key areas. Expanding clinical trials with larger sample sizes is essential to validate the sensitivity and specificity of liquid-based sonobiopsies compared to traditional tissue biopsies. Optimizing blood collection intervals and refining sonication and MB parameters will enhance biomarker detection while ensuring patient safety. Studies must also investigate tumor-specific biomarkers in greater detail and tailor sonobiopsy parameters to tumor characteristics, such as type, density, and location. Addressing variability in biomarker release and exploring the mechanism behind FUS-enabled biomarker extraction will improve reproducibility. Moreover, evaluating the effects of radiation and chemotherapy, mitigating spatial shifts during surgery, and assessing the long-term safety of BBBO will be critical in integrating sonobiopsy into clinical practice.

8. Declaration of generative AI in scientific writing

The chat-GPT and Grammarly AI are used to improve grammar and readability. No AI-generated manuscripts or ideas are used in the review paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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