Focused Ultrasound Mediated Blood-Brain Barrier Penetrance to Enable Precision Medicine by Facilitating Drug Delivery and Liquid Biopsy in Brain Tumors

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Abstract:

Precision medicine in oncology has advanced outcomes in many solid tumor diseases yet provided little benefit to brain tumor patients. Enablement of effective drug delivery and contemporary molecular profiling of tumor content are critical components to enabling precision medicine, but in brain tumors, precision medicine is inhibited by the blood-brain barrier (BBB). The use of focused ultrasound (FUS) devices to vibrate peripherally injected microbubbles (MB) is a promising technology to temporarily disrupt the BBB and enable precision medicine for patients with brain tumors.

Brain tumors, comprising of primary brain tumors or brain metastasis, represent a collection of highly lethal cancers for which the BBB inhibits 1) the delivery of oncology therapeutics at sufficient concentrations to the brain tumor and 2) the detection of circulating biomarkers for diagnosing, subtyping and guiding therapy selection. Current FUS + MB technologies have demonstrated impressive safety profiles enabling a temporary and targeted BBB disruption and facilitating the transfer of biomarkers and therapeutics across the BBB.

Cordance Medical has developed the **NeuroAccess™** platform, a next-generation, non-invasive, and community-based FUS + MB system designed to meet the needs of patients and physicians for deployment in the US and global healthcare markets. The system leverages a unique cap-based system that leverages a fiducial guidance system, low-frequency ultrasound for delivering ultrasound energy, and wide-band monitoring to ensure safety. The platform is designed to enable precision medicine in the community setting with minimal labor or patient interaction. Furthermore, initial safety and efficacy data from the NeuroAccess platform support clinical investigations confirming safety and efficacy and are commencing.

Key Points:

- FUS + MB technology has demonstrated safety and efficacy at opening the BBB.
- Cordance has developed the **NeuroAccess™** platform, a platform scalable for community use, which leverages a novel beamforming targeting system.
- The platform is designed to enable liquid biopsy and drug delivery applications in primary and metastatic brain tumors.

Introduction:

Malignant brain tumors are highly invasive and have devastating consequences. Outcomes for patients with glioblastoma (GBM), the most common primary brain tumor, have not significantly improved over the years, largely due to few new therapies approved to treat these patients.[1] In contrast, patients with metastatic non-small cell lung cancer and breast cancer have experienced significant improvement in clinical outcomes over the last decade.[2-5] Critical to brain tumor patients' poorer outcomes are 1) poor drug delivery inhibiting both small and large therapeutic molecules in reaching their tumor targets and 2) understanding the molecular underpinnings of these diseases due to lack of access to recurrent tissue combined with the low concentrations of circulating tumor DNA (ctDNA) analyte in cell-free DNA (cfDNA), thereby reducing the utility of liquid biopsy assays.[6,7] The resulting lack of knowledge on the molecular underpinnings of the diseases inhibits the ability to practice precision medicine for brain tumor patients. The utilization of ctDNA liquid biopsy tests in non-brain solid-tumor cancers demonstrates a strong correlation to tumor biology and provides a critical tool for enabling genome aberrations in the DNA of tumors to guide the use of targeted therapies.[8,9]

Importance of the Study:

The BBB limits brain access to systemic therapies as well as the detection of brain pathology through peripheral liquid biopsy. Initial FUS + MB devices to open the BBB have demonstrated safety and strong efficacy signals in pre-clinical and clinical settings. However, the devices as designed are invasive, expensive, and large while needing highly trained staff – limiting community access and global scalability. The Cordance device is designed to facilitate both biomarker analysis and drug delivery to patients in their community and across the globe.

The lack of effective drug delivery and liquid biopsy solutions is directly related to the presence of the blood-brain barrier (BBB). Vessels throughout the body that carry blood are lined with a layer of cells called endothelial cells. In the brain, this layer prevents the passage of almost all but essential substances, such as nutrients and waste products, between the blood and the tissue. As a result, the BBB inhibits most therapeutic molecules from accessing the diseased portion of the brain at effective concentrations. The BBB is estimated to prevent 98% of the small and 100% of the large molecule therapeutic agents from crossing from the blood into the tissue.[6] In oncology, numerous chemotherapies have demonstrated clinical efficacy for solid tumors, yet only one chemotherapy has shown an extension of life in primary brain cancers. This molecule, temozolomide (194 Da), has the smallest molecular mass of current clinically utilized cytotoxic chemotherapies. Other larger-sized chemotherapies which have demonstrated higher potency against glioma cells in vitro, such as taxanes (808 Da) and platins (371 Da), are too large to cross the BBB and thus not effective and not utilized clinically.[10-12] In metastatic brain tumors, many small therapeutics, such as tyrosine kinase inhibitors (TKIs) have had mixed efficacy in patients with brain metastasis, demonstrating that BBB penetration remains a critical limitation.[13] Larger therapeutics, such as chemotherapies, gene therapies, and monoclonal antibodies, are too large to penetrate the BBB and have led to multiple phase III trial failures.[14] Not unsurprisingly, and likely due to the difficulty of BBB penetrance, most pharma-sponsored therapeutic trials exclude patients with brain metastasis.[15] The lack of effective therapeutic options for brain metastasis patients has been exacerbated by the success of therapeutic opportunities for non-brain metastatic patients. The improvement in survival in non-brain metastatic patients has led to an increased incidence of brain metastasis. For example,

metastatic breast cancer to the brain has risen from an incidence of ~10% in 2000 to >30% in 2020.[16] Similarly, brain metastasis is estimated to be ~30% for metastatic non-small cell lung cancer and ~40% for metastatic melanoma.[17,18]

In addition to drug delivery, the BBB inhibits disease biomarkers from being assessed in peripheral blood draws. Circulating tumor DNA (ctDNA; ~146 bases or ~92 kDa) analysis, commonly accessed in solid tumors for therapy selection, prognosis, and treatment responses, is largely not present in primary brain tumor patients.[19,20] Recent studies have demonstrated as few as 27% of patients have accessible ctDNA through a blood draw analysis.[21,22] In a paired sample analysis, patients with a cerebrospinal fluid (CSF) sample have higher ctDNA than peripheral blood samples demonstrating the BBB as an inhibitor to ctDNA migration into the circulatory system.[23,24]

Tumor molecular profiling with a next-generation sequencing (NGS test has become standard practice for many oncologic malignancies, most commonly using tissue obtained from biopsy or surgical resection.[25] The combination of improved identification and understanding of molecular subtypes of tumors, combined with the rapid adoption of NGS tests, has led to the 2016 and 2021 WHO recommendations for molecular characterization of central nervous system (CNS) tumors. The WHO issued new guidance for the pathologic classification of CNS tumors that prioritizes molecular characterization of tumors over histopathological findings.[26,27] These changes reflect a major shift in the approach to diagnosing and treating primary brain tumors that relies on the availability of molecular information. Tissue obtained at the time of original diagnosis from resection or biopsy is reviewed for diagnostic purposes; however, some patients are not candidates for surgery due to tumor location or clinical factors and repeat sampling of tumor at the time of progression is not widely applied for various reasons including surgical risks (e.g., infection, neurologic deficits, hemorrhage), cost, and availability. Consequently, clinicians and patients are left to make treatment decisions without critical molecular and diagnostic information that may provide valuable guidance, especially when considering enrollment in clinical trials. Collectively, the difficulties of drug delivery and access to reliable molecular characterization of liquid biopsy samples are limiting outcomes for patients with brain cancers. Fundamentally, drug delivery and liquid biopsy solutions require an ability to unlock or bypass the BBB at the brain tumor location to be effective.

The concept of disrupting the BBB using FUS technology has been studied over the last 20 years with several different types of systems used in conjunction with FDA-approved MBs (approved for use as contrast agents), in numerous animal models and multiple human clinical trials. FUS oscillates the MBs causing the MBs to expand and contract multiple times a second. This vibration exerts mechanical pressure on the endothelial wall and causes inter-cellular channels to temporarily open (see Figure 1A-C). Many FUS + MB devices in development are primarily directed toward drug delivery, are invasive, need patient fixation devices, and require large capital-intensive devices.[28-30]

FUS + MB for opening the BBB has been studied extensively in animal models, where the BBB remains open for a half-life of ~4-5 hours and is entirely closed in ~24 hours.[31-34] Collectively, this research has been mainly focused on targeted drug delivery but has demonstrated that FUS + MB can successfully and safely open the BBB transiently and enable therapeutic agents to pass through the BBB from the peripheral blood into the tissue. In addition, to enabling therapeutic agents to pass through, the open BBB also allows the transfer of biomarkers from brain tissue into the blood.[35] As such, the proof of concept for enabling the transfer of biomarkers to the peripheral circulation from the brain tissue is supported.

Based on results from preclinical studies, various investigators have initiated first-in-human

Figure 1: Physics of FUS + MB

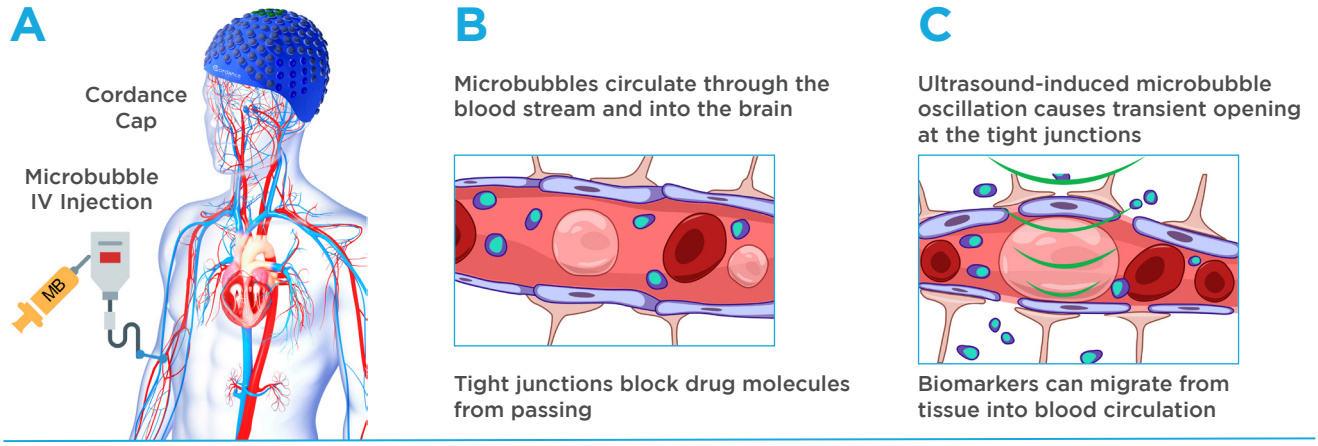


Figure 1A: While wearing the Cordance Cap, microbubbles (ultrasound contrast agent) are injected.
Figure 1B: Microbubbles distribute through the entire body including into the blood vessels in the brain.
Figure 1C: Insonify brain accerately with ultrasound which causes the microbubbles to vibrate in size, thus streching the BBB transiently and allowing biomarkers from the tissue to enter the blood-stream.

assessments of FUS for temporary BBB opening in patients with brain tumors to allow the delivery of therapeutic agents to the tumor or enhance measurements of biomarkers such as cfDNA in the peripheral circulation. Supplementary Table 1 summarizes the safety of FUS-mediated BBB disruption in human clinical studies. A review of the safety of FUS-mediated opening of BBB clinical studies by Paun et al. examined collective experience across a total of 45 human subjects.[36] From these studies, FUS-mediated BBB was considered safe, "with very low complication rates". The use of this technology for temporary BBB opening is in human clinical trials, and initial results assure safety. Additionally, numerous human trials are underway examining FUS + MB for BBB opening in primary brain tumors, brain metastasis, Alzheimer's, Parkinson's, and ALS, see Supplementary Table 2.

The efficacy of FUS-mediated BBB disruption has been examined for both drug delivery and liquid biopsy applications. Numerous preclinical and clinical studies have demonstrated a robust increase in concentrations from small to large molecules, a subset of these studies is highlighted in Table 1.[37-40] In the application of liquid biopsy, the study by Meng et al. at Sunnybrook, Toronto, investigated liquid biopsy results pre- and post-BBB opening.[41] The investigators examined cfDNA concentrations in the blood samples of GBM patients. The opening of the BBB was associated with increased cfDNA concentrations by ~2.6X in pre- vs. post-BBB opening blood samples, and this finding was validated in animal studies with similar conclusions, demonstrating improvements in the detectability of tumor mutations.[42] Initial published experience of FUS-mediated BBB opening appears safe and technically feasible within tertiary cancer centers in improving drug delivery and liquid biopsy applications. To further improve patient access and improve the deployment of FUS-mediated BBB opening, Cordance has developed the NeuroAccess™ platform, a non-invasive device conducive to being used in space-constrained environments, such as infusion treatment centers or community clinics, where ~85% of cancer patients in the United States receive their care.[43]

We seek to evaluate the safety and efficacy of the NeuroAccess platform as a non-invasive and community-based device to temporarily open up the BBB for patients with malignant brain tumors.

Table 1: Summary of Clinical Efficacy Data with FUS+MB

Disease	Drug Size	Animal Model Drug Penetration	Human Patient Drug Penetration	
GBM	Rx CARBOPLATIN 370 Da	**3x** in Rats **3x** in **NHP**	**5.9x** **+107%**	5.9x increase (PET-imaged); 3.1 months median survival (vs. 1.5 months for Carboplatin alone)[37]
GBM	Abraxane® 850 Da	**5x** in Mice	**3.7x**	3.7x increase (PET-imaged)[38]
GBM	AVASTIN® 149 Da	**5.7x** in Mice	**+140%**	9.5 months median progression-free survival (vs. 3.9 months for Avastin alone)[39]
Brain Mets (Breast)	Herceptin® trastuzumab 145 Da	**3x** in Mice	**4x** **+30%**	Up to 4x PET Imaged drug penetration, Single-dose 30% average reduction in tumor volume[40]

37. Sonabend, et al. in preparation, presented at ISTU 2022 & FUSF 2022 - NCT03744026
38. Sonabend, et al. in preparation, presented at ASCO 2022 & ISTU 2022 - NCT04528680
39. Chen, et al. in preparation, presented at ISTU 2022 & FUSF 2022 - NCT04446416
40. Meng, Ying, et al. "MR-guided focused ultrasound enhances delivery of trastuzumab to Her2-positive brain metastases."

Methods & Materials

The NeuroAccess™ platform utilizes a transcranial dynamically focused ultrasound (TcDFUS) approach to enable BBB opening in physician-selected regions of the brain. The FUS energy vibrates the MBs, which puts mechanical pressure on the BBB (see Figure 1). Utilizing the TcDFUS approach, the BBB can be reversibly opened, enabling either 1) improved drug delivery or 2) the passage of biomarkers such as tumor-derived cell-free DNA (cfDNA) into the peripheral circulation which FDA-approved next-generation sequencing oncology panels can analyze (e.g., Guardant 360CDx, FoundationOne Liquid CDx, etc.). The operation of the Cordance device is illustrated in Figure 2 and begins with the receipt of standard-of-care diagnostic CT and MR images.

Treatment planning occurs when a physician leverages contouring software, like software currently being used for radiation therapy planning, to contour the region or regions of interest where the physician would like the BBB opened. The contoured MR images are processed using Cordance proprietary software to develop a personalized treatment plan. The treatment planning software leverages time-reversal beamforming methodology to determine transmission parameters (which transducers to utilize, timing sequence, amplitude, and phase of the electrical input) to beamform onto the pre-selected region of interest. The software will return a proposed treatment plan to the physician for acceptance.

The main device of the platform is intended to reside within healthcare settings such as infusion treatment centers where the patient

will be seated in an infusion treatment chair. The ultrasound transducer assembly (the "Cordance Cap") on the patient's head (Supplementary Figure 1), inclusive of the use of the Cordance proprietary water jacket, which is designed to 1) enable the one-size cap to comfortably conform to the patient's head and 2) to create an acoustic coupling between the head and the Cordance cap.

The NeuroAccess™ platform is illustrated below in Figure 2; the Cordance Cap includes multiple transducers of three different types: Type 1 high-frequency (HF) for imaging, Type 2 low-frequency (LF) for therapy, and Type 3 wide-band (WB) for safety monitoring. The adult human skull is generally ~8mm thick, however, the skull is thin (~4mm) in the temporal lobe region.[44,45] Physicians use this thin region of the skull to obtain ultrasound images of the brain, particularly ultrasound color Doppler images of the Circle-of-Willis for diagnostic purposes. As seen in Figure 2, Cordance has designed the system to use the Type 1 high-frequency imaging transducers on the side of the cap such that when it is placed on the patient, these imaging transducers will be adjacent to the temporal lobe region and operate at a center frequency of approximately 2MHz. The Type 1 transducers are used to calculate the position and orientation of the cap in relation to the patient's internal anatomy by aligning to the patient's circle of Willis. The Type 1 transducer enables the calculation performed by aligning the ultrasound color Doppler image of the circle of Willis as a fiducial marker to the image of the same structure within the diagnostic MRI of the same patient.

After internal registration, the patient will receive a bolus or steady infusion of commercially available MBs. The MBs are made of an inert gas core encapsulated in a protein or a lipid shell and are approximately 1 - 4um in diameter. The half-life in the bloodstream of these microbubbles is less than 10 mins.[46] The microbubbles distribute within the entire body, including within the blood vessels in the brain, within minutes. Leveraging Cordance's patented technology, the Type 2 low-frequency ultrasound energy transiently opens the BBB by

Figure 2: The Cordance Device & Process for Opening the BBB

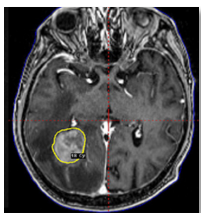
The Cordance cap contains three types of transducers:

Type 1: High-frequency (2MHz) for imaging though the temporal lobe (used for non-invasive alignment with diagnostic MRI)
Type 2: Low-frequency (220KHz) for providing therapy (i.e. opening the BBB)
Type 3: Wide-band monitoring transducers.

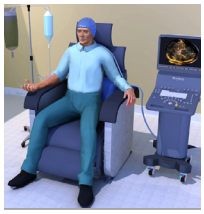
Low-frequency 220KHz therapy transducers interspersed with wide-band monitoring transducers

High-frequency 2MHz imaging transducers

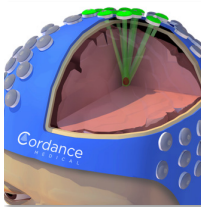
Diagnostic Input
MRI/CT imaging



Treatment Planning
Region of Interest Selection



Intravenous Administration
Microbubbles
(inert ~1-3um gas bubbles)



Focused Ultrasound Energy
Oscillates bubbles
(up to 7um)

exciting a subset of the these transducers. The low frequency used will be 250 kHz. This lower frequency enables a lower loss of acoustic energy than standard 2MHz transducers (approximately 4dB vs. 30dB), ensuring deeper penetration of the ultrasound energy into the brain.

At the focal region of the ultrasound energy, the bubbles interact with ultrasound and can exhibit one of two predominant behaviors depending on the incident acoustic pressure of the ultrasonic energy. Below a certain threshold of acoustic pressure (approximately 0.3MPa at 250 kHz), the bubbles oscillate stably in position and size.[47-49] These oscillations exert mechanical pressure on the endothelial cells that form the BBB, particularly on the bonds between the proteins in the junctions between the cells. These proteins, called tight junction proteins, in their normal state and, more specifically, the bonds between the proteins, are primarily responsible for the inability of larger molecules to cross the BBB. When mechanical pressure is applied with the expansion and contraction and oscillation of the microbubbles, the bonds "loosen", causing a transient decrease or loss of the barrier functions of the tight junctions.[50] The loss of barrier function lasts for a half-life of ~ four hours, during which the transport of therapeutic molecules that normally cannot cross the BBB can now pass.[50] The mode of microbubble vibration where such effects are observed is called "stable cavitation". If the incident acoustic pressure is above a certain threshold, the bubbles exhibit an unstable vibration mode called the "inertial cavitation" vibrating mode. In this mode, the bubbles can burst, potentially leading to microvascular damage, hemorrhaging, or both.[51,52] Fortunately, real-time feedback mechanisms can provide information about the vibration status of the bubble.[53] These real-time mechanisms are discussed in detail below and can be used in a feedback loop to control acoustic power transmitted into the body.

The real-time mechanism associated with bubble behavior can be understood by reviewing the underlying physics of bubble oscillations. Briefly, as stated above, the bubbles tend to vibrate in a predominantly orderly manner in the stable cavitation mode. However, in the inertial cavitation mode, the bubbles vibrate or behave chaotically. These bubbles also tend to reflect incident energy (the reason why they were developed in the first place as a contrast agent). The ultrasound reflections from these vibrating bubbles have characteristics or signatures associated with the vibration status. If the microbubbles vibrate in the stable cavitation mode, the reflections will have well-defined frequency components around the fundamental, harmonics, and ultra-harmonics of the fundamental signal (the frequency of the transmitted or incident signal). If the microbubbles are vibrating chaotically due to the incident of acoustic pressure being over a threshold, the reflections will have a less well-defined frequency spectrum – more akin to what may be expected from a very noisy source. The overall noise floor will be higher, and the frequency peaks may be less well-defined, obliterated, or both. To complete the description of the real-time mechanism, we can listen or receive the signals reflecting from microbubbles, analyze the frequency components in real-time and use the frequency information to modulate the amount of input power applied to the electronics that generate the ultrasonic energy thus modulating the acoustic pressure that is experienced in the focal region within the brain. The Type 3 wide-band ultrasound energy transducers are utilized for monitoring to provide safe operation implemented to monitor the harmonics and ultra-harmonics received from the MB vibration to ensure the acute safety of the procedure.

Guidance of the FUS energy through the skull leverages a time-reversal methodology (Figure 3), which propagates sound back from the target to transducers in a modeled environment to find the drive(s), amplitude, and phase required to beamform while reducing standing waves. The focusing of ultrasound onto a focused area will yield areas of the brain which reach a peak negative pressure (PNP) for which

the MB has sufficient acoustic pressure to open the BBB and a transition zone where pressures may but are unlikely to open the BBB which determines the spot size of the focused region.

The concept of opening the BBB using FUS technology has been studied with several different types of systems or systems used in conjunction with microbubbles in numerous animal models. As previously noted, several investigator-initiated clinical studies have used various systems that are generally invasive and/or difficult to open the BBB in cancer patients. Each system leverages a similar mechanism of action by opening the BBB through the deployment of focused ultrasound onto circulating MBs. As noted, the key differences between the systems are in the interaction with the patient, largely driven by the technique to guide the ultrasound energy (see Table 2). NaviFUS leverages optical navigation, Carthera is manually placed and implanted via a surgical craniotomy, and INSIGHTEC leverages live MR guidance. The mode of guidance and form facture further informs the invasiveness of the device: the requirement of the utilization of a hospital setting.

This research has been mainly focused on targeted drug delivery but has demonstrated that FUS + MB can successfully and safely open the BBB transiently and enable therapeutic agents to pass through the BBB from the peripheral blood into the tissue. In addition, the open BBB also allows the transfer of biomarkers from brain tissue into the blood.[41,54]

To evaluate the NeuroAccess™ platform, we examine the ability (in-silico) to guide ultrasound non-invasively & non-intrusively to five locations of the brain where brain tumors may exist, including the parietal lobe, temporal lobe right, temporal lobe left, frontal lobe, and cerebellum, this was analyzed on a large male and small female human brain to correcting for patient-specific acoustic and physical properties.

Figure 3: Process for Focused Ultrasound Beamforming

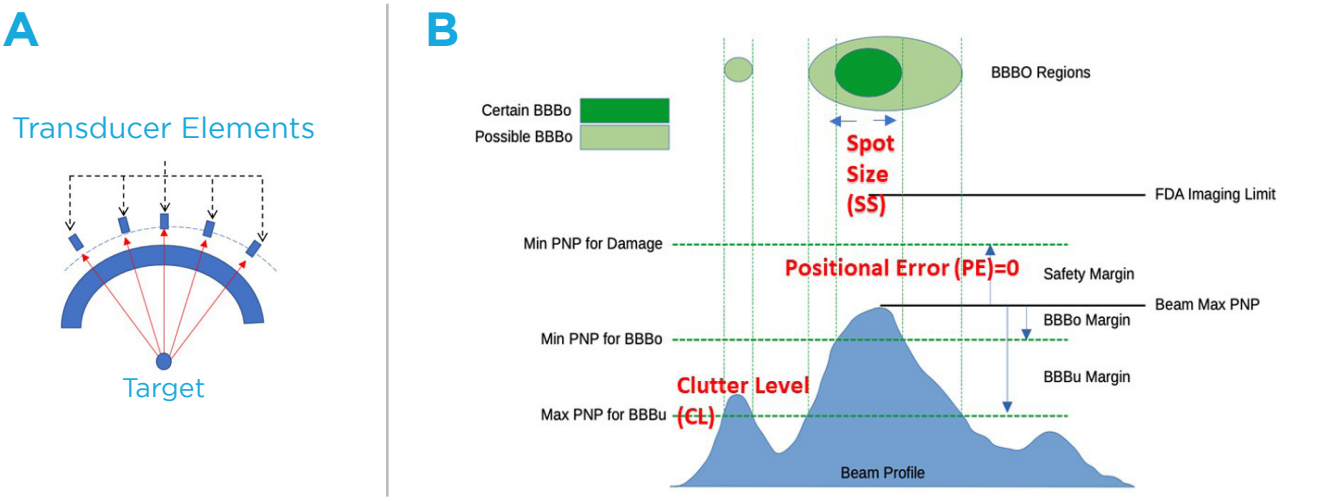


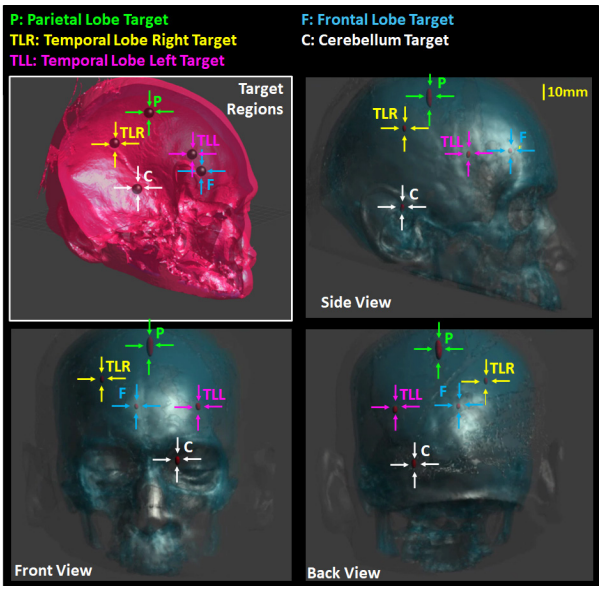
Figure 3A: Time-reversal method- Propagate sound back from target to transducers in a modeled environment to find the drive that should be applied.
Figure 3B: Normal Beam Ultrasound Profile at Focal Location- Highlights the peak negative pressure (PNP) for BBB opening and damage. Focus regions (dark green) where the BBB is expected to open, a transition zone (light green) where BBB may open.

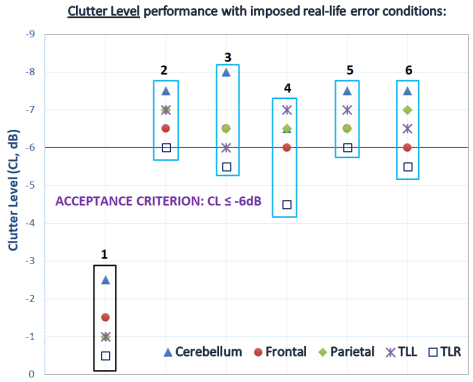
Results

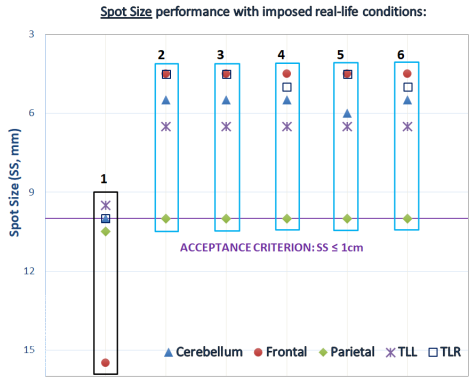
The NeuroAccess™ platform, leveraging time reversal focusing, was able to target regions in all lobes of the brain (Figure 4). Comparison of conventional ultrasound focusing to the Cordance time-reversal focusing demonstrated statistically improved clutter level (6dB), improvements in spot size (5mm), and more accurate targeting with a lower positioning error (3mm). Visualizations of the BBB region and the impact of ultrasound accuracy by both techniques compared to theoretical perfect focusing demonstrate improvements in all features (Supplementary Figure 2).

The clutter level near the skull in the temporal lobe region of the brain represented the most challenging focus region to address. In these regions, larger transition zones of potential BBB are expected.

Figure 4: Accuracy of Beam Forming







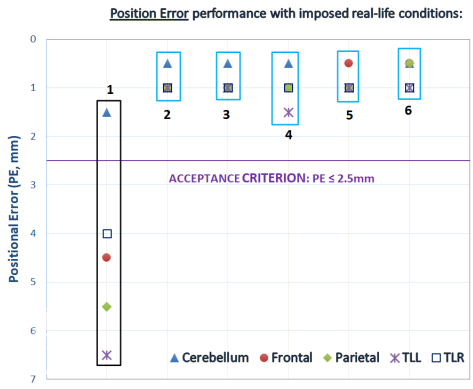


Figure 4A: Red skull (top left) represents the desired targets within the brain, Green skull images represent actual **BBB** focusing within the brain.

Figure 4B-D: Comparison of clutter level (CL), spot size (SS) and position error (PE) against Conventional beam forming (1) vs. Cordance time reversal focusing (2) along with low (3) and high (4) error due to brain property variations and low (5) and high (6) error due to cap positioning.

Table 2: Features of FUS Devices

Features	NeuroAccess™ Platform (Ultrasound	MR-Guidance	Implantable	Optical-Navigation
Non-Invasive	**Yes**	**No** Needs a patient fixation device, typically screwed on to the patient's skull.	**No** The transducer is implanted surgically underneath the skull.	**Yes**
Length of procedure and multiple regions of BBB opening	**~30 minutes**	**~4hrs+** Multiple openings over a large area can add significantly to the time as mechanical repositioning of helmet is required.	Surgery: **~2hrs** FUS+MB: **~2hrs**	**~60 mins+** Multiple openings over a large area cannot be accommodated conveniently as a manual repositioning of the transducer is required for openings not addressed within initial transducer placement.
Patient immobilization	**Not required**	**Restricted** Patient head is in a frame. No published evidence that ultrasound parameters can be calculated quickly.	**Most likely** Patient is most likely immobilized (through an esthesia) during craniotomy.	**Yes** Patient is put in a "semi-rigid" restraint during ultrasound
Increased risk of microbubble for multiple sites	**Low** Our technology can steer and focus the beam seamlessly, shortening the procedure time and thereby shortening the need for additional microbubble bolus or infusion.	**Higher Risk** The device requires a mechanical repositioning of the helmet adding to the time of procedure. This implies that additional microbubble doses are needed, or infusion has to be continued for a longer time.	**N/A** Multiple regions cannot be addressed.	**Higher risk** The device requires a mechanical repositioning or the transducer adding to the time of procedure. This implies that additional microbubble doses are needed, or infusion has to be continued for a longer time.
Use of anesthia	**Not required**	**Likely** Local anesthetic may be required while installing the stereotaxic frame.	**Yes** General or local is required for craniotomy.	**No** Patient must remain in rigid position for at least 30 minutes.

Discussion

Overcoming the BBB to enable precision medicine by facilitating liquid biopsy and drug delivery to brain tumors is essential to improve outcomes for brain tumor patients. Additionally, BBB disruption will enable improved clinical trial designs, a better understanding of the molecular underpinnings of the disease, and a broader class of therapeutics that may be deployed. Temporary and regional BBB disruption through FUS + MB has been shown to have a good safety profile with multiple FUS devices.[55-58]

While existing FUS + MB devices have demonstrated promising safety and efficacy data, significant challenges remain for these devices, including invasiveness, high costs, location constraints, high labor costs, and inability to deploy in community settings, creating a significant challenge to enable precision medicine in real-world settings. Here we demonstrate the NeuroAccess platform and ability to safely focus the ultrasound to all lobes of the brain volume while retaining high accuracy and with minimal non-targeted openings, which are common with conventional ultrasound focusing techniques. The platform appears to deliver a non-invasive, community-based device compared with current approaches, which are more invasive and logistically constrained FUS platforms.

The NeuroAccess™ platform was designed to address accessibility, patient comfort, and labor requirements in the community setting. The device builds off existing FUS systems to enable a non-invasive medical approach designed for community oncology clinics to facilitate both liquid biopsy and drug delivery applications. The product development and clinical development challenge to prove the safety and efficacy of the device are forthcoming and will determine the clinical utility and attractiveness of the device. Despite these challenges, the promise of a community-based solution to enable precision medicine for brain tumors will be a central focus of investigators, pharmaceutical companies, diagnostics companies, and medical device manufacturers.

The opportunity to utilize FUS + MB to increase therapeutic concentrations and improve drug delivery provides a promising opportunity to improve patient outcomes. Additionally, increases in circulating biomarkers, such as cfDNA required for liquid biopsy molecular profiling to guide therapy and enable precision medicine, support the further exploration of the NeuroAccess platform in human clinical studies.

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Supplementary Table 1: Summary of Clinical Safety Data with FUS + MB

Clinical Studies Opening BBB with FUS

Study	FUS+ MB Product Used	Patients (Treatments)	Neurologic Side Effects	Summary of Findings
Clinical trial of blood-brain barrier disruption by pulsed ultrasound (Carpentier et al., 2016blood-brain barrier disruption by pulsed ultrasound (Carpentier et al., 2016)	Carthera SonoCloud	15 (41)	No evidence of acute hemorrhage, ischemia, or edema. No clinical symptoms related to BBB penetrance ischemia, or edema. No clinical symptoms related to BBB penetrance	"We report the minimally invasive, safe, reproducible, and transient BBB disruption by pulsed ultrasound in patients with recurrent GBM." ischemia, or edema. No clinical symptoms related to BBB penetrance
Blood-Brain Barrier Opening in Primary Brain Tumors with Non-invasive MR-Guided Focused Ultrasound: A Clinical Safety and Feasibility Study (Mainprize et al., 2019)	Insightec ExAblate	5 (5)	No clinically significant ultrasound related clinical or radiologic adverse events	"In this study, we demonstrated transient BBB opening in tumor and peritumor tissue using non-invasive low-intensity MRgFUS with systemically administered chemotherapy was safe and feasible."
Safety and Feasibility of Repeated and Transient Blood–Brain Barrier Disruption by Pulsed Ultrasound in Patients with Recurrent Glioblastoma (Idbaih et al., 2019)	Carthera SonoCloud	19 (65)	Toxicities in line with use of carboplatin chemotherapy. No severe neurologic AEs during or after sonications.	"These results further confirm the safety of BBB disruption using Low-Intensity Pulsed Ultrasound prior to carboplatin infusion."
Non-invasive hippocampal blood brain barrier opening in Alzheimer's disease with focused ultrasound (Rezai et al., 2020)	Insightec ExAblate Neuro	6 (17)	There were no treatment-related adverse effects or neurological changes (up to 15 mo post-FUS)	"We demonstrate that FUS can safely, noninvasively, transiently, reproducibly, and focally mediate BBB opening in the hippocampus/EC in humans."
MR-guided focused ultrasound enhances delivery of trastuzumab to Her2-positive brain metastases (Meng et al., 2021c)	Insightec ExAblate Neuro	4 (20)	3 Grade 1 transient AE's (Pin site tenderness, back discomfort, headache)	"The procedures were well tolerated with no serious AEs, and two cases of grade 1 and transient treatment-related AEs, with all patients, discharged on the same day of their procedure."
MR-guided focused ultrasound liquid biopsy enriches circulating biomarkers in patients with brain tumors (Meng et al, 2021)	Insightec ExAblate Neuro	9 (27)	None reported	"We show for the first time in human patients that transcranial low-frequency MRgFUS can enrich the signal of circulating brain-derived biomarkers, specifically proteins, cfDNA, and EVs... We also saw an increased signal in clinically actionable
Putaminal Recombinant Glucocerebrosidase Delivery with Magnetic Resonance–Guided Focused Ultrasound in Parkinson's Disease: A Phase I Study (Meng et al, 2022)	Insightec ExAblate Neuro	4 (12)	Dyskinesia (related to Parkinsons symptons), headache	"No SAE was detected, consistent with previous studies...The BBB was reconstituted in all patients within 24 hours by qualitative analysis and within 1 week by DCE analysis, demonstrating that MRgFUS did not affect long-term BBB integrity in PD" signal in clinically actionable plasma IDH1-R132H-mutant copies."

Supplementary Table 2: FUS + MB Clinical Trials for Opening the BBB

Clinical Studies Opening BBB with FUS

Study	Identifier
Non-Invasive Focused Ultrasound (FUS) With Oral Panobinostat in Children With Progressive Diffuse Midline Glioma (DMG)	NCT04804709
Assessment of Safety and Feasibility of ExAblate Blood-Brain Barrier (BBB) Disruption	NCT03551249
Assessment of Safety and Feasibility of ExAblate Blood-Brain Barrier (BBB) Disruption for Treatment of Glioma	NCT03616860
Efficacy and Safety of NaviFUS System add-on Bevacizumab (BEV) in Recurrent GBM Patients	NCT04446416
Sonodynamic Therapy With ExAblate System in Glioblastoma Patients	NCT04845919
Exablate Blood-Brain Barrier Disruption With Carboplatin for the Treatment of rGBM	NCT04440358
Exablate Blood-Brain Barrier Disruption for the Treatment of rGBM in Subjects Undergoing Carboplatin Monotherapy	NCT04417088
Exablate Blood-Brain Barrier Disruption for Glioblastoma in Patients Undergoing Standard Chemotherapy	NCT03712293
Ultrasound-based Blood-brain Barrier Opening and Albumin-bound Paclitaxel for Recurrent Glioblastoma (SC9-ABX)	NCT04528680

Supplementary Figure 1: Coupling the Cordance Cap to the Head

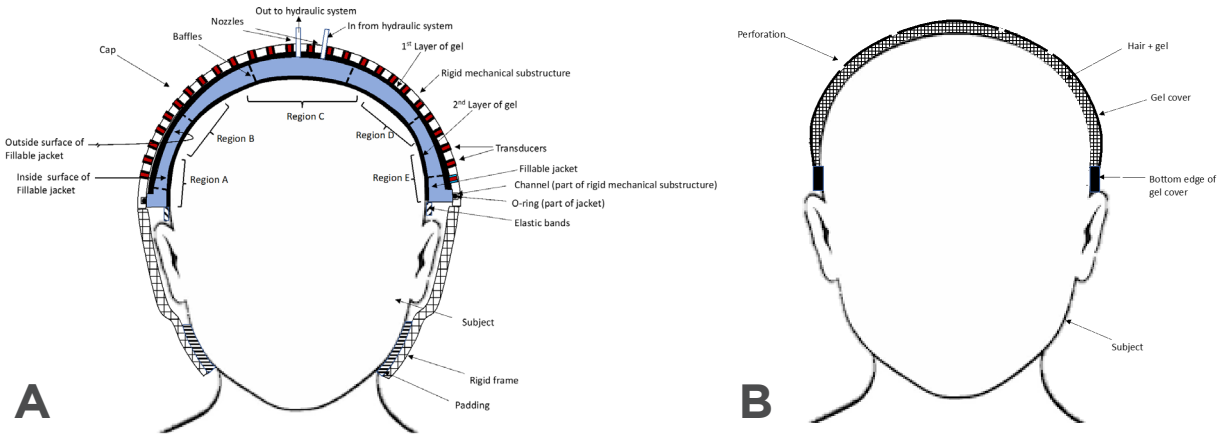
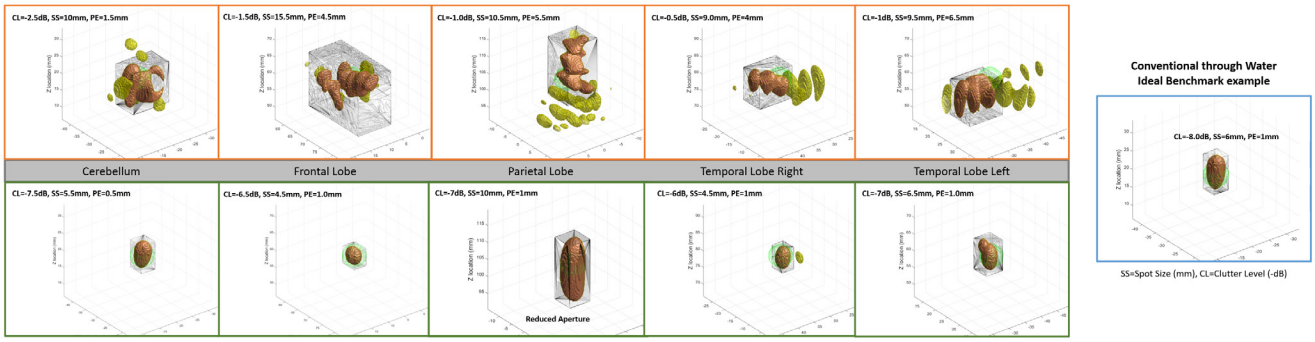


Figure S1A: Coupling Mechanism
Figure S1B: Gel cover for hair

Supplementary Figure 2: Comparison of Conventional Focusing and Cordance Time Reversal Focusing



Top Row: Conventional Focus Technique: Impact of standing waves cause off target BBB opening and sub-optimal BBB opening at desired location

Bottom Row: Cordance Time Reversal Focusing: Optimization of appropriate ultrasound focusing at different locations in the brain.