COLUMBIA UNIVERSITY
IN THE CITY OF NEW YORK

DEPARTMENT OF BIOMEDICAL ENGINEERING

Neuronavigation-guided focused ultrasound-induced blood-brain barrier opening in Alzheimer's disease patients

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1 Introduction

1.1 Background

Central nervous system disorders are currently being symptomatically treated since the molecular events provoking their onset have not been yet identified. Drug delivery techniques have to overcome the almost impermeable blood-brain barrier (BBB) and can be grouped into two main categories: (i) invasive and targeted, and (ii) non-invasive and non-targeted methods. However, the technological advances of the past decades revealed the immense potential of focused ultrasound (FUS) in transcranial applications. In contrast to other techniques, FUS-mediated BBB opening is both non-invasive and targeted. The localized energy delivery coupled with the circulation of intravenously administered microbubbles initiates biological effects confined only to the vessel walls and contained only in the targeted region within the brain.

Microbubbles are gas-core, lipid-shelled particles with diameters between 1-10 μm. Their minute size allows them to circulate throughout the vasculature. Microbubbles are compressible, thus they respond to the alternating phases of an ultrasound wave by expanding and contracting. These volumetric oscillations exert stresses onto the surrounding vascular walls and temporarily open the tight junctions that formulate the BBB. The magnitude and distribution of this bioeffect can be controlled by adjusting the sonication parameters. BBB opening using FUS and microbubbles is fully non-invasive and has been proven to be safe and reversible. The integrity of the BBB is being restored within hours depending on the selected parameters. This technique has shown efficacy in delivering various compounds in the brain parenchyma such as contrast agents, sugars, antibodies, viruses, chemotherapeutics and neurotrophic factors in both benign and diseased brains. In the context of Alzheimer's disease (AD), FUS has been shown to effectively decrease amyloid plaque load in AD rodents. Moreover, rodents tested behaviorally showed significant memory improvement following FUS treatment [1-13].

1.2 Investigational Device

Device name: Focused Ultrasound by Sonic Concepts
Device description: A single-element, spherical-segment FUS transducer (H-231, Sonic Concepts, Bothell, WA) driven by a function generator (Agilent, Palo Alto, CA, USA) through a 50-dB power amplifier (E&I,

Rochester, NY, USA). The confocally mounted hydrophone (Y107, Sonic Concepts, WA, USA) is driven by a preamplifier (Olympus, Waltham, MA, USA).
Device Model/Version #: H-231

Device name: Neuronavigation System by Rogue Research
Device description: This device will be used for monitoring the procedure.
Device Model/Version #: Brainsight system 2.3.3

Drug name: Definity
Dose: 10 µl/kg
Route of administration: Intravenous
This drug is FDA-approved (NDA 21-064) but not being used in accordance with labeling.

Drug name: Dotarem
Dose: 0.2 ml/kg
Route of administration: Intravenous
This drug is FDA-approved (NDA 20-4781) but not being used in accordance with labeling.

Drug name: Florbetapir F^{18}
Dose: 370 MBq (10 mCi) – 10 ml bolus injection
Route of administration: Intravenous
This drug is FDA-approved (NDA 20-2008) and used in accordance with labeling.

Device name: Vantage
Device description: A programmable ultrasound research platform used for performing specialized and customizable ultrasound scans. (Verasonics, Inc, Kirkland, WA) will be connected to an array transducer (ATL Phillips, WA, USA) to achieve real-time passive acoustic monitoring.
Device Model/Version #: Verasonics Vantage 256

1.3 Preclinical Data

FUS has been extensively studied in safely opening the BBB of various animal species and different disease-mimicking models. The application of FUS in conjunction with pre-formed intravenously administered microbubbles produces bioeffects which are confined only within the targeted region. Our group and others have shown that this targeted drug delivery system can be successfully applied in preclinical studies on rodents, rabbits, pigs, and non-human primates (NHP). This technique has shown efficacy in delivering a wide range of drugs into the brain. Furthermore, FUS-mediated BBB opening has been shown to effectively decrease the amyloid plaque load and restore memory in AD rodents, even without the administration of an anti-AD drug [1-13].

Our group has published extensive work confirming the accuracy and safety of this technique performed both in rodents and, more recently, in NHP. We have shown that the BBB can be safely and reversibly opened using focused ultrasound and microbubbles, and that its integrity is restored within 2 days in rodents and between 2 and 4 days in NHPs. Recently, our group focused on developing a new clinical system. The new system consists of two parts: (i) ultrasound unit for sonication, real-time passive acoustic monitoring, and B-mode imaging and (ii) neuronavigator providing real-time guidance. In contrast to MR-guided FUS which requires sonication within an MRI magnet, our approach is based on an open-space, neuronavigator-controlled sonication. The real-time guidance is achieved by registration of the physical space to the preloaded MRI that is displayed on the screen. The registration is achieved by the infrared camera reflecting the position of the beads secured close to the brain. The transducer is attached on the neuronavigator arm and the position varies depending on the structure that will be sonicated. The transducer carries beads as

well so its relative position to the brain can be seen on the screen. Once the transducer is locked at the position of interest, the sonication starts. Real-time monitoring of the sonication by passive cavitation detection or passive acoustic mapping can be used to address the efficacy and safety of the technique. B-mode ultrasound imaging is also performed to acquire anatomical information. In B-mode imaging, a short pulse (<5 cycles) will be used and the mechanical index (MI) will be maintained below 0.1, well below the FDA limit of 1.9 MI. The sonication procedure coupled with the neuronavigation system has been tested on sitting, awake primates and the resulting BBB opening was similar to the results obtained from our previous equipment [14-20].

1.4 Clinical Data to Date

There are a few completed clinical studies while some are in progress. In France, researchers have implanted a transducer through the patients' skull and at each session they sonicate at low intensity to achieve a BBB opening to assist glioblastoma multiforme (GBM) chemotherapeutic treatment. The results have shown successful BBB opening along the ultrasound beam path and targeted drug delivery within the brain tumor and its adjacent tissue. However, this approach is restricted by invasive surgery resulting in the lack of flexibility and selection of the targeted area. Our study aims to overcome this limitation through neuronavigation guidance and using a noninvasive single-element transducer. In Canada, researchers have treated patients for the same purpose, i.e. BBB opening for chemotherapeutic drug delivery in GBM, without implanting the transducer but instead sonicating through the intact skull with MR guidance. Another trial from the Lipsman et al. focused on opening the BBB in patients diagnosed with early AD using the same MR guided ultrasound system. Published results from these trials have confirmed that a reversible BBB opening in humans is both feasible and safe.

1.5 Research Risks & Benefits

1.5.1 Risk of Investigational Device

Ultrasound and MRI are generally considered safe imaging modalities. However, there are potential risks related to their usage.

Although ultrasound exposure at high intensities may produce unintended biological effects in the body, such as hemorrhage and edema, our study will be limited to intensities far below the FDA-approved limits for imaging applications ($I_{SPPA} < 190$ W/cm^2, $I_{SPTA} < 720$ mW/cm^2 and MI < 0.8). FUS is expected to induce some intended mechanical effects in the brain, but there has been no damage reported in the literature during low-intensity sonication. Sonications at relatively high acoustic pressures have resulted in moderate hemorrhage at the targeted region, leading to instances of edema and inflammatory response. We will therefore operate close to the threshold for stimulation to avoid any unwanted effects and minimize the risk for the patient. It is possible that the patient will feel some heat from the ultrasound propagation through the skull. We will account for potential heating by using a cooling medium (i.e., cold water) between the transducer and the skull. All the necessary hardware and software precautions will be taken, in order to eliminate the possibility of transducer malfunction or protocol breach during the clinical sessions. For ultrasound B-mode imaging, the mechanical index (MI) will be maintained below 0.1, well below the FDA limit and there will be no heating or feeling by imaging.

Definity microbubbles are FDA-approved (NDA 21-064) and are routinely used in ultrasound imaging applications, such as echocardiography or renal imaging, for over 15 years. There have been reported side effects such as cardiopulmonary reactions, injection site reaction, back/renal pain, chest pain, dizziness, headache, nausea etc. Other possible risks during the procedure include bruising at the injection site, and hypersensitivity or allergic reaction to Definity microbubbles and/or gadolinium injections. All the potential

adverse reactions and their incidence rate can be found in the Definity and gadolinium safety datasheets which are attached to our protocol. In our study, we will use the clinically recommended dose of 10 μl/kg introduced by intravenous bolus injection followed by a 10 ml saline flush, according to the manufacturer's guidelines. Over the course of the study, the bolus injection may be replaced by a syringe-pump-controlled infusion within 30-60 seconds. We have shown that FUS treatment using the clinically recommended dose of Definity and the minimum ultrasound parameters (MI: 0.4) can safely open the BBB with 100% success rate, without compromising safety (appendix 6.1). We have also proved that the BBB is completely restored within 3 days after FUS treatment.

In the MRI the patient may experience anxiety or claustrophobia because of the small space he/she will have to spend an hour. However, this can be accounted for with proper preparation of the patient and/or providing music during the acquisition. Also, the strong electromagnetic fields used in MRI pose an underlying risk for patients. Therefore, any patients bearing metallic implants or pacemakers will be excluded from our study. PET tracer Amyvid (^{18}F- Florbetapir) may also cause allergic reactions to patients.

1.5.2 Other Risks of Study Participation

Other possible risks during the procedure include bruising at the injection site.

1.5.3 Potential benefits

Subjects participating in research may not benefit directly from this study due to the absence of anti-AD drugs. There is evidence that BBB opening through FUS application removes amyloid-β plaques and restores memory in an AD mouse models, even without drug administration [13]. However, there is no data on plaque reduction in a NHP model. Nevertheless, the information collected from this study may help others in the future by establishing that this technique is safe and efficient in achieving reversible BBB opening. Feasibility of small BBB openings in the brain with safety confirmed on MRI will open avenues for future clinical trials with potent drugs that are currently shelved due to their BBB impermeability.

2 Study Objectives

2.1 Primary Objective

The purpose of this study is to assess the safety and feasibility of FUS-induced BBB opening in human subjects using a single-element transducer with neuronavigation guidance. We have built and tested a new system for clinical application, based on the system used in our NHP studies. The human module for the neuronavigator will be implemented and initial safety in a small cohort of patients with early AD will be tested. Given the promising results obtained in *ex vivo* human skulls and the safety profile established in NHP *in vivo* over the past five years, our primary objective is to demonstrate initial clinical feasibility in patients with AD.

In the clinical neurological practice, it is well known that over the past decade there has been a lull in the treatment of the disease without any of the pharmacological agents capable of slowing down the disease. Invasive alternatives such as deep brain stimulation (DBS) are currently in clinical trials. In this aim, initial feasibility and safety of the noninvasive FUS technique on patients *in vivo* will be performed. The information collected in this study may be used to design future clinical trials – to ultimately provide a viable alternative for treatment of AD in a safe and noninvasive manner.

2.2 Secondary Objective(s)

A secondary objective is to define the minimum ultrasound parameters required to safely and reversibly open the BBB in humans. These parameters will provide the threshold of FUS-induced BBB opening and will be used in future clinical studies aiming at targeted brain drug delivery using FUS. Based on our animal study report (appendix 6.1), treatment at MI of 0.4 produced BBB opening in all subjects. Therefore, the objective here is to define the success rate using these minimum parameters which are close to the BBB opening threshold.

3 Study Design

3.1 General Design

The clinical neuronavigator will be identical to the NHP study with the difference of human module (Brainsight) in the software used, as well as employing a separate set of navigation tools that are used for human subjects only. Similar to the NHP study, initial feasibility of the system targeting will be shown with the channel phantom using ex vivo human (parietal bone) skulls as in our preliminary studies.

The neurologist on the study, Dr. Lawrence Honig, MD at the Taub Institute of Alzheimer's Disease and Aging at Columbia, will be guiding this clinical application. The subjects will first be diagnosed at Columbia. Six (6) patients will be asked to participate in this initial safety and feasibility study. The subjects will have to meet at least the following criteria: 1) 50 years old or older, 2) have been diagnosed with early AD or mild cognitive impairment (MCI) at minimum and 3) ability to provide informed consent. Approvals and consent forms approved by the Clinical Trials Office (CTO) and the Institutional Review Board (IRB) of Columbia University will be obtained through an IRB approved study (CTO will guide the pre-IDE approval in the first year). All subjects agreeing to participate will undergo the following series of procedures: **1)** a high-resolution MRI (without gadolinium contrast agent) will be acquired prior to BBB opening on the GE SIGNA^{TM} Premier (3T) (same scanner as in the NHP study) for pre-treatment planning and screening purposes. Also, a PET/CT scan with Amyvid (^{18}F-Florbetapir) will be performed on the SIEMENS Biograph 64 to assess the baseline amyloid plaque load before the treatment. **2)** BBB opening will be attempted (in a designated facility in Radiology) using the clinical FUS system. First, a baseline signal will be acquired by applying ultrasound prior to microbubble injection. Then, an intravenous injection of Definity microbubbles under the FDA guidelines will be administered as a bolus or via a syringe pump and cavitation signal will be monitored and recorded during sonication at parameters under the FDA safety guidelines. **3)** A MRI (with and without gadolinium) will be acquired immediately after the sonication in order to assess the BBB opening and safety. **4)** A follow-up MRI (with and without gadolinium) will be conducted 3 days (±1 day) after the treatment to confirm BBB closing and/or safety. Also, two follow-up PET/CT scans using Amyvid (^{18}F-Florbetapir) will be conducted 3 weeks (±1 week) and 3 months (±2 weeks) post-FUS to assess amyloid plaque load.

MRI will be performed at the Neurological Institute of New York / CUMC.

3.2 Primary Study Endpoints

Feasibility of FUS-induced BBB opening in humans.

3.3 Secondary Study Endpoints

Optimization of FUS parameters for future studies.

3.4 Primary Safety Endpoints

Safety of the technique will be assessed by MRI and neurocognitive tests, to guarantee that FUS-induced BBB opening at low frequency and pressure does not cause adverse effects in patients.

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

AD patients will be recruited in person and have to be over 50 years of age and able to give consent. Patients diagnosed with early stages of AD or with MCI will be included in our study. Other more severe symptomatic patients will be eligible to enroll, as long as they have the ability to consent for their participation. Screening is essential and will be performed by our collaborator and neurologist of the study, Dr. Lawrence Honig, MD at the Taub Institute of Alzheimer's Disease and Aging at Columbia. After the initial screening we will acquire MRI scans as part of the study and finalize enrollment or exclusion of the patient. Inclusion criteria thus include:

- Age greater than 50 years old.
- Diagnosis of early AD or MCI at minimum. All following criteria must be met:
    - Probable MCI or AD consistent with criteria outlined in (McKhann *et al* 2011, Petersen *et al* 2018).
    - MMSE score between 12 and 26.
    - Modified Hachinski Ischemia Scale (MHIS) score of <= 4
    - Short form Geriatric Depression Scale (GDS) score of <= 6.
    - PET scan confirming amyloid plaque load using Amyvid (^{18}F-Florbetapir).
- Ability to provide informed consent.

4.2 Exclusion Criteria

Exclusion criteria include surgeries and other pathologies not associated with AD, as outlined in the following list:

- Contraindication for MRI.
- Contra-indication history or hypersensitivity to MRI contrast agents (e.g., Dotarem) or microbubbles (e.g., Definity).
- Prior brain surgery, including deep brain stimulation.
- Metallic implants.
- Moderate or severe uncontrolled hypertension.
- Abnormal coagulation profile, e.g. hemophilia A or B.
- Coagulopathy or under anticoagulant therapy.
- History of stroke or cardiovascular disease.
- Active gingivitis, herpes simplex, hepatitis, tuberculosis, and minor skin or respiratory infections.
- History of seizure disorder.
- History of asthma or allergies to food or medication with significant symptoms in past 3 years.
- Severe brain atrophy.
- Inability to comply with the procedures of the protocol, including follow-up MRI scans.
- Pregnancy or lactation.
- Impaired renal function with estimated glomerular filtration rate (eGFR) <30 mL/min/1.73m^2 provided by a standard blood test 2-4 weeks prior to the ultrasound treatment.
- Active infection/inflammation.
- Acute or chronic hemorrhages, i.e. > 4 lobar microbleeds, and no siderosis or macrohemorrhages.
- Tumors or space occupying lesions.
- Meningeal enhancements.
- Intracranial hypotension.

4.3 Subject Recruitment and Screening

Subjects who have been diagnosed with early AD or MCI, will be identified and informed about our study by our neurologist, Dr. Lawrence Honig, or any other trained neurologist. Neurologists will follow the guidelines suggested by the American Academy of Neurology, assessing for functional impairment and behavioral/neuropsychiatric symptoms to establish MCI or AD (Petersen *et al* 2018, McKhann *et al* 2011) according to the following inclusion criteria:

- Probable MCI or AD consistent with criteria outlined in (McKhann *et al* 2011, Petersen *et al* 2018).
- Modified Hachinski Ischemia Scale (MHIS) score of <= 4.
- Mini Mental State Exam (MMSE) scores of 12-26.
- Short form Geriatric Depression Scale (GDS) score of <= 6.
- PET scan confirming amyloid plaque load.

Should the patient agree to participate, a written permission will be asked to allow the treating physician to forward their name and contact information to the research investigators. The patients may also be given the contact information for the researchers, so that they may contact them if they are interested in participating. Dr. Honig or any other participating neurologist will document in the subject's medical record that permission has been obtained for the study team to contact the subject about enrollment in this study. Dr. Honig will enroll patients with moderate AD (MMSE scores: 12-18) who are not subject to higher risk of adverse effects due to mental impairment. PET scans are routinely conducted to confirm AD and are not expected to affect the safety of the FUS treatment.

Patients will be screened using the iNYP database on a password-protected computer with a secure ethernet connection. Discussion with subjects regarding research participation will take place in a private area (i.e., a patient exam room). All procedures related to the research study will also be carried out in a private room. The data acquired during research procedures will be anonymized and encrypted, and immediately removed from the ultrasound systems and transferred to the secure endpoint using an encrypted portable hard drive. After the transfer is complete, the imaging data will also be removed from the portable drive. Focus groups will not be used for this study. Subject privacy will be protected as described in attached HIPAA form.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

Patients may withdraw at any time during the procedure by notifying the lead investigator and explaining the reason. Withdrawal at any time will result in partial withdrawal of the reimbursement/compensation, according to the compensation scheme per visit (see informed consent form).

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

Data will be collected from the researchers involved in this protocol and stored in an encrypted computer. Follow-up for withdrawn subjects will be subjected to the time point they withdrew.

5 Study Device

5.1 Description

The system consists of two parts: (i) ultrasound unit for sonication and real-time passive acoustic monitoring (Sonic Concepts and Verasonics) and (ii) neuronavigator (Rogue Research) providing real-time

guidance. The guidance is achieved by registration of the physical space to the preloaded MRI that is displayed on the screen. The registration is achieved by the infrared camera reflecting the position of the subject tracker secured close to the brain. The transducer with a passive cavitation detector or an array transducer embedded is attached on the mechanical arm and the position varies depending on the brain structure to be sonicated. The transducer carries a tool tracker so its relative position to the brain can be seen on the screen. Once the transducer is locked at the position of interest the sonication begins. Real time monitoring of microbubble activity during sonication will be achieved via passive acoustic detection or passive acoustic mapping. B-mode ultrasound image will be acquired using the Vantage system for anatomical information and the mechanical index (MI) will be maintained below 0.1, well below the FDA limit of 1.9 MI. This investigative device will be operated in conjunction with microbubbles (Lantheus Definity) administered intravenously.

5.2 Treatment Regimen

The investigational device will be operated at acoustic parameters demonstrating safe and effective BBB opening in NHP followed by microbubble injection. The Definity microbubble dosage with an FDA guideline is 10 µl/kg. The acoustic parameters for BBB opening are based on our NHP studies [17]-[21] and correspond to an I_{SPPA} of 2.81 W/cm^2, I_{SPTA} of 56.3 mW/cm^2 and a mechanical index (MI) of 0.4. These parameters produce safe and reproducible BBB opening in NHPs, as shown in our animal study report (appendix 6.1). These values are well below the limits set by the FDA for ultrasound imaging applications (I_{SPPA} < 190 W/cm^2, I_{SPTA} < 720 mW/cm^2 and MI < 1.9, respectively). To minimize the risk to subjects, we will use multiple simulation packages (e.g., kWave and SimSonic) in the treatment planning stage, in order to estimate the minimum attenuation coefficient through the subject's skull. We will use location-specific attenuation maps and perform a range of simulations (n = 10/package) to get a range of estimations. We will then choose the minimum attenuation estimation, which would lead to the minimum delivered dose possible.

5.3 Allocation to Treatment

This is a single arm, non-random trial.

5.4 Subject Compliance Monitoring

All the procedure will be performed in the hospital and patient compliance will be advised and monitored by researchers and/or healthcare providers.

5.5 Prior and Concomitant Therapy

Although BBB opening is reversible and transient, it may lead to unintended diffusion of pharmaceutical molecules into the brain parenchyma. To avoid such potential effects, non-critical medications will be withheld prior to treatment and until closure of the BBB is confirmed (at least 24 hours before and after the procedure). For critical medications, medication withholding will be judged based on the subject's diagnosis, condition, and medication type.

5.6 Packaging

The ultrasound transducer and the associated electrical components, i.e. function generator, power amplifier, and hydrophone, will be gathered in a mobile cart. The Vantage system with a linear array probe also will be in another mobile cart. All components will be connected prior to the patient's arrival and will be checked by two investigators to ensure smooth operation and safety.

The neuronavigation system along with the accompanying equipment, i.e. infrared camera, reflecting beads, holders etc., will be packed separately and gathered in a mobile cart. The parts will be assembled following the patient's arrival and the imaging beads will be attached to the patient's head and the transducer to allow for real-time neuronavigation.

New packages of Definity microbubbles will be shipped from Lantheus Medical Imaging Inc and will be used in conjunction with the ultrasound transducer to temporarily open the BBB. Definity microbubbles will be freshly activated before sonication and will be withdrawn using a 1-ml syringe to reach the clinical dose (10 μl/kg) right before the IV injection.

New packages of Dotarem will be shipped from Guerbet LLC. Dotarem will be used as an MRI contrast agent to detect areas in which the BBB has opened. It will be administered according to manufacturer's guidelines at the recommended dose (0.2 ml/kg).

All study drugs will be provided free of charge to research participants.

5.7 Blinding of Study

There is no blinding, and the safety and the effectiveness will be compared with the contralateral treatment site of the patient.

5.8 Receiving, Storage, Dispensing and Return

5.8.1 Receipt of Study Device

The device will be delivered to the operating room from the storage room in the investigative site by the researchers prior to the procedure.

5.8.2 Storage

The ultrasound system including the focused transducer and the Vantage system will be stored in a room equipped with a locker and will be kept under in temperature till usage.

The neuronavigator system will be stored in the same locked room with the ultrasound system, and will also be kept in room temperature till usage.

Definity microbubble vials will be stored in a hospital grade fridge under 4 °C till usage. Microbubbles will be removed from fridge before the patient's arrival, to allow them acquire room temperature.

Dotarem contrast agents will be stored in a locked drawer in the MRI unit and will be kept in room temperature till usage.

5.8.3 Dispensing of Study Device

The physician performing the procedure will dispense the microbubbles and MRI/PET contrast agents to the patient, and the other researchers will deliver the ultrasound device from the storage room to the procedure room.

5.8.4 Return or Destruction of Study Device

The device should be returned to the following address if damaged: 630 West 168th Street, Physicians & Surgeons 19-418, New York, NY, USA 10032.

6 Study Procedures

After patient screening based on the selection criteria, the eligible patients will sign the consent form and fill the information form for the study, be scanned in the MRI for treatment planning, and then the sonication procedure. Dr. Lawrence Honig, MD at the Taub Institute of Alzheimer's Disease and Aging at Columbia, will be guiding this clinical application. The subjects will first be diagnosed and referred to the Presbyterian Hospital at Columbia University Medical Center. Six (6) patients will be asked to participate in this initial feasibility and safety study. The subjects will have to meet the following criteria: 1) 50 years old or older,

2) diagnosis of early AD or MCI at minimum (assessing criteria described in section 4.1), and 3) ability to provide informed consent. Approvals and consent forms approved by the Clinical Trials Office (CTO) and the Institutional Review Board (IRB) of Columbia University will be obtained through an IRB approved study.

6.1 Visit 1

All subjects agreeing to participate will undergo the following series of procedures in 4 or 5 visits. In the first visit, a high-resolution MRI scan (without gadolinium contrast agents) will be acquired prior to BBB opening on the 3T MRI for pre-treatment planning and screening purposes. Also, a blood draw and a PET/CT scan with Amyvid (^{18}F-Florbetapir) will be performed to assess the baseline biomarkers and amyloid plaque load and before the treatment.

6.2 Visit 2

In the second visit, BBB opening will be attempted in a designated facility in the hospital using the described FUS system. First, a baseline cavitation signal will be obtained prior to microbubble injection. Then, an intravenous injection of Definity microbubbles under the FDA guidelines will be administered as a bolus or via a syringe pump and the cavitation signal will be monitored and recorded during sonication at acoustic parameters well below the FDA safety limit (MI = 0.4). In this EFS, we will target the prefrontal cortex (PFC). PFC has been shown to be affected by AD pathology, leading to deficits in attention, spatial memory and planned movements. Although we do not aim to treat AD in this EFS, PFC was selected as the targeted structure due to its relevance to this condition, its size and ease of targeting with our single-element transducer. A follow-up MRI (with and without contrast) will be acquired the same day after BBB opening to ensure treatment safety and efficacy. Finally, two blood samples will be collected before and after treatment through an intravenous catheter or a finger prick. Blood analysis will be performed to test whether there are acute changes in the blood test results due to the FUS application. Based on our animal studies, there is an acute increase in reticulocytes, platelets and eosinophils after FUS treatment, possibly due to the BBB restoration process. Blood samples will be used to test whether these changes in blood chemistry occur also in humans. Blood samples will be stored and analyzed to detect potential changes in Alzheimer's Disease biomarkers and other compounds that may exit the brain parenchyma because of the blood-brain barrier opening (e.g. exosomes). All time points will be compared to baseline acquired during screening.

6.3 Visit 3

A follow-up MRI (with and without contrast) will be performed three days (±1 day) after treatment to confirm BBB closure and to assess safety. A blood sample will be collected through an intravenous catheter or a finger prick, to test for short-term changes in blood chemistry and cell count.

6.4 Visit 4/5

Follow-up visits will be required three weeks (±1 week) and three months (±2 weeks) after treatment to assess long-term safety, amyloid plaque load and cognitive function through behavioral tests. Two follow-up PET/CT scan using Amyvid (^{18}F-Florbetapir) will be performed; one 3 weeks (±1 week) post-FUS and one 3 months (±2 weeks) post-FUS, to test for potential reduction in the amyloid plaque load. A blood sample will be also collected through an intravenous catheter or a finger prick, to test for long-term changes in blood chemistry and cell count. One follow-up visit following a single FUS treatment was deemed sufficient by the attending neurologist.

7 Statistical Plan

7.1 Sample Size Determination

Successful methodology will be concluded if the BBB opening is safely opened in the PFC region in at least 75% of the cases as evidenced on MRI. The scan with the BBB opening will be compared to the scan before BBB opening for each patient. The data will be statistically analyzed with parametric paired Student's t-test. Power analysis shows that for a detectable difference of 200 mm^3 (after BBB opening - before BBB opening) and a standard deviation of 50 mm^3, at least 6 subjects are needed to report a result at the level of α=0.05 and Power=0.95.

7.2 Statistical Methods

Standard statistical methods will be used in this study. Parametric analysis will most likely be applied to compare the gadolinium enhancement in the patient's brain before and after BBB opening through paired Student's t-test. However, the statistical analysis will be subject to the results obtained and other methods may be used as well, such as non-parametric statistical tests.

7.3 Subject Population(s) for Analysis

Protocol-compliant population: Any subject who was randomized and received the study device and complied with all protocol required processing

8 Safety and Adverse Events

8.1 Definitions

Unanticipated adverse device effect (UADE): Any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or IDE application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

Associated with the investigational device: There is a reasonable possibility that the adverse effect may have been caused by the investigational device.

Life-threatening adverse effect: Any adverse effect that places the subject, in the view of either the investigator or the sponsor, at immediate risk of death from the effect **as it occurred**. It does not include a reaction that, had it occurred in a more severe form, might have caused death.

Serious adverse effect: An adverse effect is considered "serious" if, in the view of either the investigator or the sponsor, it results in any of the following outcomes:

- death
- a life-threatening AE
- inpatient hospitalization or prolongation of existing hospitalization
- a persistent or significant disability/incapacity
- a congenital anomaly/birth defect.

Unanticipated adverse effect: Any adverse effect, the nature, specificity, severity, or frequency of which is not consistent with the risk information in the clinical study protocol or elsewhere in the current IDE application.

8.2 Recording of Adverse Device Effects

All observed or volunteered adverse effects (serious or non-serious) and abnormal test findings, regardless of treatment group, if applicable, or suspected causal relationship to the investigational device or, if

applicable, other study treatment or diagnostic product(s) will be recorded in the subjects' case histories. For all adverse effects, sufficient information will be pursued and/or obtained so as to permit 1) an adequate determination of the outcome of the effect (i.e., whether the effect should be classified as a serious adverse effect) and; 2) an assessment of the causal relationship between the adverse effect and the investigational device or, if applicable, the other study treatment or diagnostic product(s).

Adverse effects or abnormal test findings felt to be associated with the investigational device or, if applicable, other study treatment or diagnostic product(s) will be followed until the effect (or its sequelae) or the abnormal test finding resolves or stabilizes at a level acceptable to the sponsor-investigator.

8.3 Reporting of Adverse Device Effects and Unanticipated Problems

8.3.1 Investigator reporting: *Notifying the study sponsor*

N/A

8.3.2 Investigator reporting: *Notifying the IRB*

ALL unanticipated adverse device events will be reported **immediately and no later than within 2 business days** to the IRB committee, **regardless of seriousness or severity**. Unanticipated problems involving risks to subjects and others will be recorded and reported to IRB within 5 **business days**. Expected or unrelated adverse effects and potential protocol violations will be recorded and reported in our annual progress report.

Any suspension or terminations of IRB approval will be reported to NIMH no later than within **3 business days of receipt**. Unanticipated adverse effects or serious adverse events will be reported to NIMH no later than within **10 business days** of the study team becoming aware of the event.

8.4 Unblinding Procedures

N/A

8.5 Stopping Rules

Redundant safety features have been added to the hardware of the device, as grounding for the transducer, safety switch for the amplifier and function generator. Additionally, we will incorporate safety valves in our software. For any signs showing safety risk including high cavitation dose in the acoustic monitoring or unstable vital signs, the device can be stopped from the computer user interface and/or an emergency stop button. Treatment will be stopped in the case of any reaction of the subject to the ultrasound exposure or microbubble infusion. Pulse oximetry will be used during MRI scans to mitigate the risk of anaphylactic reaction to MRI contrast agents. Potential adverse effects have been grouped according to their severity in the following table:

Severity	**Adverse effect**			
Severe	Local hemorrhage	Edema	Intracranial infection	Status epilepticus
Moderate	Emesis	Dizziness	Allergic reaction	Nausea
Mild	Skull heating	Injection site bruising	Headache	

The investigation will be paused and the relevant authorities will be notified in case of any death, life threatening event, or any other serious adverse event described in the table above (including disability or clinically significant manifestation of local hemorrhage or edema) due to the procedure. An episode of status epilepticus during treatment will terminate the session immediately.

If the selected ultrasound settings (i.e., MI of 0.4) do not successfully open the BBB in 3 subjects, FDA will be contacted via a submission of an IDE supplement, to discuss revising the protocol's focused ultrasound parameters or increase the number of subjects.

8.6 Medical Monitoring

Medical monitoring will be performed by the neurologist of this study, Dr. Lawrence Honig, MD at the Taub Institute of Alzheimer's Disease and Aging at Columbia. Dr. Honig or any other trained radiologist will be present during the trial and will assess the subject's clinical status during therapy. In the case of an allergic or any other reaction (e.g. status epilepticus) to the therapy, the study will be immediately discontinued. Resuscitation equipment will be readily available in the study room, in case of a serious adverse event.

Subjects will be monitored with follow-up MRI scans to confirm BBB opening and closing and PET scans to estimate amyloid plaque load. Should the MRI or PET brain scans of this study have incidental findings (e.g. brain tumor, subarachnoid hemorrhage, or structural abnormality) the subject will be immediately withdrawn from the study and directed for clinical care. MRI scans will be examined by Angella Lignelli-Dipple, MD, who is a certified in Diagnostic Radiology and is the Section Chief of Neuroradiology. MRI scan examination by Dr. Lignelli-Dipple will occur as soon as possible but no later than two weeks following receipt of the image.

If there are any incidental findings deemed by the clinical-investigator to be "life-threatening" or "important but not severe", both the subject and the subject's personal primary care physician will be notified. Communication may be initially verbal, followed by a formal written communication. The significance of the incidental finding will be determined by the clinical-investigator and communicated to the subject accordingly. Incidental findings of clinical significance and the management of such findings should be documented in the research records of this study.

At the time of a continuing review, if incidental findings were noted during the previous approval period, the principal investigator of this study will provide the IRB with the following information: the number of required review images, a list of the subject study numbers, the type of scan, the date of the scan, a description of the incidental finding of clinical significance, the date of communication with the subject and the outcome, if known.

If the participants expressed severe depression or suicidal ideation in the course of their visit, the subject will be urgently assessed by the study clinician and appropriate referrals will be made. In addition, we will exclude the participant from the study.

8.6.1 Data Monitoring Committee (if applicable)

The safety of the BBB opening using ultrasound and microbubbles has been confirmed by previous non-human primates studies, which revealed no adverse effects, permanent damage, or behavioral change following repeated treatments using low-intensity ultrasound and clinically-relevant microbubble doses. Ongoing clinical trials using this technique have not reported any acute adverse effect in either of the participants. However, long-term effects in the human brain have not been investigated to date.

To ensure the long-term safety in our study, the investigators will monitor and collect the data for processing and analysis while Dr. Honig will be present to assess the safety of the patient during FUS application. Prof. Honig will be present during the trial and will assess the subject's clinical status during therapy. In the case of an allergic or any other reaction to the therapy, the study will be immediately discontinued. Resuscitation equipment will be readily available in the study room, in case of a serious adverse event.

Subjects will be monitored with follow-up MRI scans to confirm BBB opening and closing. BBB opening will be confirmed with an MRI scan immediately after the ultrasound treatment, while BBB closing will be confirmed three days later. Additional clinical evaluation will be performed by Prof. Honig or any other trained neurologist 3 months after the ultrasound treatment. Should the MRI brain scans of this study have incidental findings (e.g. brain tumor or potential structural abnormality) the subject will be immediately withdrawn from the study and directed for clinical care. Incidental findings will be communicated with both the subject and the subject's personal primary care physician.

Cognitive function will be assessed by Dr. Honig using appropriate tests before, during, and after the treatment.

9 Data Handling and Record Keeping

9.1 Confidentiality

The data will be stored on a designated secure computer, which will be kept in the Vanderbilt Clinic, 12th Floor, Room 232 behind two locked doors. The hard drives on this computer will be encrypted using Bitlocker drive encryption, and all data files containing patient-sensitive information will be encrypted and Password protected.

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why.
- Who will use or disclose that information.
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Records Retention

The sponsor-investigator will maintain records in accordance with 21 CFR 812, Subpart G.

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

This study will be monitored in accordance with the monitoring plan to comply with the IDE holder responsibilities. The principal investigator and the clinician of the study will monitor the preparation, compliance, and successful completion of the study. The investigator will allocate adequate time for such monitoring activities on a bi-weekly basis, to ensure that the study is being conducted in accordance with the protocol at all times. The investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit. Finally, the IDE holder will be responsible for submitting IDE amendments, IDE safety reports and IDE annual reports to the FDA and the IRB, as needed.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the EC/IRB, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

11 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice, applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of EC/IRB members and their affiliate to the sponsor.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. See appendix 5 for a copy of the Subject Informed Consent Form. This consent form will be submitted with the protocol for review and approval by the EC/IRB for the study. The formal consent of a subject, using the EC/IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject and the investigator-designated research professional obtaining the consent.

12 References

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