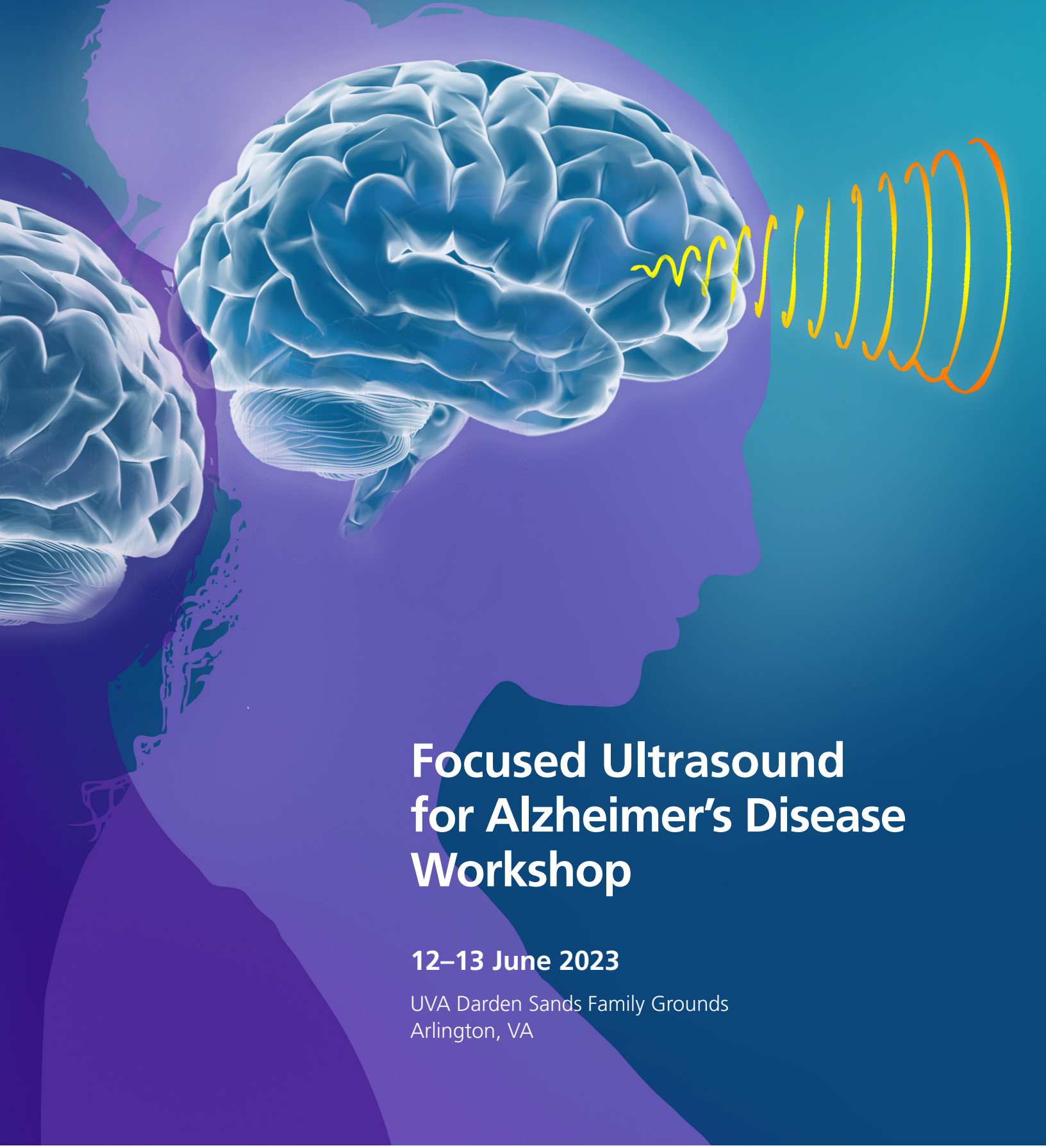

Focused Ultrasound for Alzheimer's Disease Workshop

12–13 June 2023

UVA Darden Sands Family Grounds
Arlington, VA

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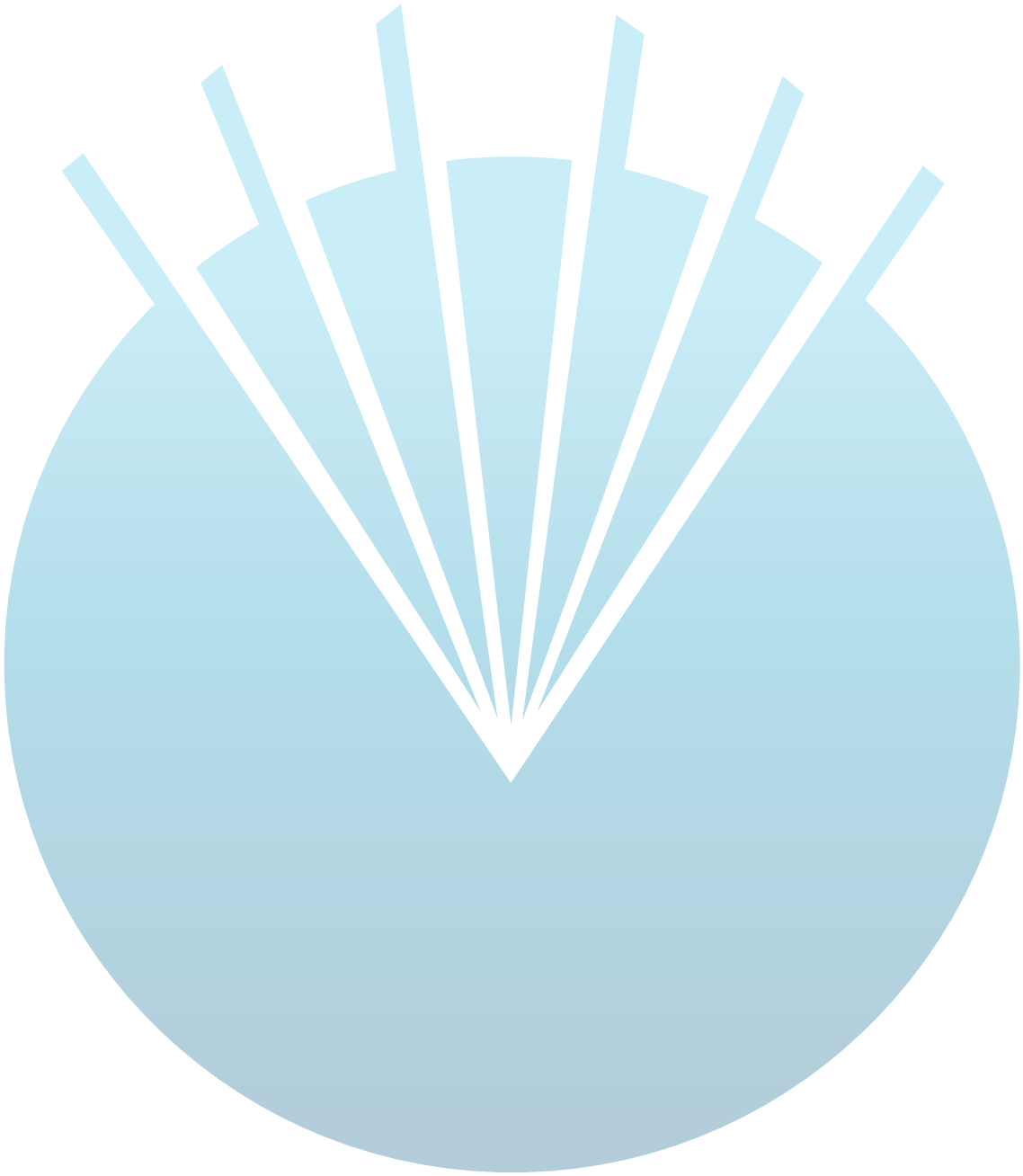
FOCUSED ULTRASOUND FOUNDATION



Contents

Executive Summary

This white paper summarizes a one and half day hybrid workshop, "Focused Ultrasound for Alzheimer's Disease (AD)," organized by the Focused Ultrasound Foundation. During the workshop, there were presentations on the clinical landscape for AD, the state of the field of Focused Ultrasound (FUS) for AD, safe and effective FUS parameters, and potential combinations of AD therapeutics and FUS blood-brain barrier opening (BBBO). Experts also led panel discussions on standardizing outcome measures, optimizing inclusion/exclusion criteria for upcoming clinical trials, and creating a roadmap to move the field forward.

Presentations on the state of field for AD focused on methods of BBBO, the lack of biomarkers for AD diagnosis and monitoring of disease progression, and therapeutics that are either currently FDA approved or are in clinical trials. Preclinical work supports the use of FUS and BBBO as a treatment for AD, but there are many unanswered basic research questions such as the underlying mechanisms of action. A first in human clinical trial utilizing BBBO with FUS to enhance the delivery of an anti-amyloid beta (Aβ) antibodies (Aducanumab) is currently enrolling patients.

Experts also discussed the FUS parameters for optimal BBBO in human patients. Current clinical trials have used FUS BBBO to treat up to a volume of 40 cc per session, but the optimal volume and number of FUS procedures necessary for AD treatment is unknown. Another topic that received attention was quantifying and monitoring BBBO for drug delivery. Additionally, FUS BBBO could be used for liquid biopsy, to retrieve analytes from the brain back into the peripheral blood for either AD diagnosis or monitoring. Standardized MRI imaging protocols could help standardize data across centers and clinical trials.

Participants also mentioned that the optimal window for treatment with FUS BBBO was earlier in the course of disease progression; yet more preclinical and clinical research is needed. Microbubble standardization is also key to moving the field forward. Participants highlighted the need for commercially available microbubbles optimized for use in FUS BBBO, and the need to standardize the reporting of FUS parameters, experimental procedures, and microbubble properties currently in use.

Welcome and Introduction

Suzanne LeBlang, MD, welcomed attendees. Attendees included clinicians, researchers, industry participants, pharmaceutical representatives, the Food and Drug Administration (FDA), and other nonprofits. Dr. LeBlang reminded the audience that the Focused Ultrasound Foundation previously held a workshop on FUS for AD in 2015 to help create a roadmap for the future. The outcomes of that meeting included developing clinical trials for FUS with microbubbles targeting the hippocampus in patients with AD, preclinical research to inform the optimum design of clinical trials, and preclinical research to understand the mechanisms responsible for transient blood-brain barrier (BBB) opening and Aβ clearance after FUS treatment.

To date, there have been 15 clinical trials of FUS for AD treatment completed or ongoing. Dr. LeBlang thanked **Paul Fishman**, **Ali Rezai**, **Sandra Black**, **Jürgen Götz**, and **Nir Lipsman** for serving on the workshop's steering committee.

Lauren Powlovich, MD, provided an overview of the workshop schedule. She also asked attendees to think about a few key questions facing the field that will be discussed during the meeting.

Presentations

Delivering Disease Modifying Therapy for Alzheimer's Disease

Paul Fishman, MD, PhD, stated that the idea of improving therapeutics for AD is arguably one of the most important scientific and public health clinical issues today. Despite a tremendous amount of understanding of the basic sciences for neurological diseases, there has been little progress made in developing disease-modifying therapies. There are several potential disease-modifying therapies for neurological diseases in development such as:

- Large proteins, including trophic factors, enzymes, and antibodies.
- Genetic material, antisense, viral vectors, nanoparticles, and gene editing.
- Stem cells for the treatment of Parkinson's disease, AD, amyotrophic lateral sclerosis, multiple sclerosis, Huntington's disease, and lysosomal storage diseases.

The underlying question is why are these strategies failing? Is it due to an incorrect mechanism of action or are they too large to cross the BBB in sufficient concentration? Current routes of brain delivery include intracerebral injection, intraventricular infusion, intrathecal injection, BBB disruption (mannitol or pulsed FUS with microbubbles), transcytosis across the endothelia (trojan horse, nanoparticles, and viral vector), and intranasal delivery.

- Intracerebral injection is efficient and can achieve high local concentrations with low off-target effects. It is best for highly targeted therapy and used for gene therapy trials, but it is invasive with risk of bleeding. There is also limited spread of therapeutics from the injection site.
- Intraventricular infusion is designed for long term treatment with an indwelling catheter/reservoir and allows widespread brain treatment with less off-target effects and has shown promise in clinical trials for enzyme replacement therapy. This is also invasive with the risk of bleeding and infection, has limited penetration to deep-brain areas, and no brain specific targeting.
- Intrathecal injection is less invasive than intracerebral or intraventricular delivery with widespread treatment effects and less off-target effects. It has also shown promise in clinical trials for enzyme replacement therapy. It is invasive, requiring repeated lumbar puncture for chronic conditions with limited penetration to deep-brain areas and no brain specific targeting.
- Intra-arterial mannitol injection is less invasive than other methods and allows localized/targeted brain treatment and has widely been used

to deliver cancer chemotherapy. It is still invasive and requires arterial catheterization. There are known side effects of edema with a limited amount of tissue volume treated.

- Intranasal injection is the least invasive injection method with wide clinical experience for many agents and indications including AD. It has also already been combined with FUS to enhance distribution of therapeutics. This method only permits limited distribution to many areas of the brain and previous clinical trials have reported mixed results.
- Intravenous transcytosis is non-invasive and uses endothelial receptors for getting across the BBB neuronal receptors. It is already in use with clinical trials for enzyme replacement therapy. There are a wide array of carriers and targeting molecules (trojan horses) and the technique has widespread brain distribution and penetration. The limitations are limited efficiency of delivery, potential for non-target effects, and it is difficult to target specific areas within the brain.

Next, Dr. Fishman described the current state of the field regarding anti-Aβ antibodies for AD. Typically, only 0.1% of IV Aβ antibody reaches the brain.[1] It is unknown how much aducanumab gets into the brain. Lecanemab has a 0.4% cerebrospinal fluid (CSF)/serum ratio with repeated dosing. The combination of FUS plus BBBO may enhance antibody delivery 5 to 10 times greater than IV administration.[2] However, it remains unknown whether greater concentrations of brain antibody will yield greater Aβ removal and increased clinical benefit. The currently available anti-Aβ antibodies greatly reduce Aβ accumulation in the brain but have only modest clinical benefit compared with the amount of Aβ reductions.

Anti-Aβ antibodies are associated with amyloid-related imaging abnormalities (ARIA) in the brain, which is a concentration-dependent process. ARIA are likely the result of both vascular injury and brain inflammation. It is unknown if increasing brain levels of anti-Aβ antibodies will result in greater brain inflammation. Preclinical studies on FUS with aducanumab enhance Aβ clearance and cognition, but also showed increased microglial activation.[3]

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Clinical State of the Field for Alzheimer's Disease

Sandra Black, MD, provided an overview of AD. Autopsy data showed that people with probable AD had potential signaling alterations in their brains such as TDP-43 and synuclein as well as vascular disease. CSF has remained the gold standard for fluid biomarkers. Recently a CSF based assay was created to diagnose Parkinson's disease with a biomarker assay for α-synuclein. In the case of AD, potential biomarkers like TDP-43 occur in smaller amounts and are not as easy to detect in CSF. Blood tests for neurodegenerative diseases are in development and would be game changing, allowing for a quick and simple diagnosis.

Dr. Black stated that there is still a lot of uncertainty regarding the removal of Aβ with antibodies. While the antibodies help clear Aβ, the accumulation may have built up over decades. A study is enrolling patients with AD that are pre-symptomatic and will administer lecanemab in a randomized controlled trial (AHEAD study). The theory is that earlier treatment may help prevent disease progression. Dr. Black stressed the importance of early diagnosis in general for neurological disorders.

Another agent in development is donanemab that targets Aβ(pE3), a pyroglutamate form of Aβ that is aggregated in Aβ plaques. Preliminary results suggest that patients had no decline in cognitive measures at 1 year. Additionally, 52% and 72% of patients achieved plaque clearance at 1 year and 18 months, respectively and were subsequently taken off treatment.

Dr. Black listed several potential disease-modifying therapies in development:

- Anti-Aβ (Aβ-42).
- Anti-hyperphosphorylated tau.
- Anti-pan Tau (not targeting phospho-epitope)
- Other mechanisms repurposing agents such as glucagon-like peptide-1 (GLP-1) receptor agonists to treat neuroinflammation.
- Agents that target the gut microbiome.
- Healthy lifestyle choices and vascular risk factor management (including sleep apnea) remain fundamentally important.

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Panel Discussion

Panelists

Sandra Black and **Paul Fishman**

Q. Do anti-tau antibodies have the same risks as anti-Aβ antibodies?

- Results are not yet available on these trials and the relationship between tau and Aβ is not fully understood.

Q. What is the therapeutic window for AD?

- AD develops over decades. It is likely that preventative interventions could reduce disease development. Once the Aβ is deposited and pathways are destroyed, it might be too late.
- The therapeutic window also differs depending on whether you are targeting tau or amyloid.
- Vascular disease also likely plays a role in the pathogenesis of AD, and preventing vascular disease may also help prevent AD. Modifications are important, such as lowering lipids and blood pressure.
- Without lifestyle modifications, anti-Aβ antibodies may not have as much effect.

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Research Highlights

Preclinical Topics

Jürgen Götz, PhD, stressed that there continues to be a role for preclinical research in the AD field that has seen numerous drug failures.[4] The question facing the FUS community is to determine whether Aβ reduction is more important than improved cognition. The US FDA's accelerated approval of aducanumab established Aβ reduction as an appropriate surrogate endpoint, but the link between Aβ and cognition is not fully established, and ultimately, cognitive functions need to be restored.

FUS can be used on its own, with microbubbles for BBBO, or with microbubbles plus drugs such as monoclonal antibodies. The amount of published literature regarding FUS and brain function continues to grow.[5,6] FUS research conducted in rodent models demonstrated altered neural cell composition, activation state, and neurotrophin levels.[6] FUS can also improve functional and cognitive outcomes in rodents.[6] Studies of FUS with microbubbles to open the BBB can result in microglial clearance of Aβ mediated by unidentified blood-borne factors.[7–9] Tau clearance may be mediated by activation of autophagy and/or microglia.[10,11] Some researchers show that BBBO is required for Aβ clearance, while others do not.[12,13] There is also mixed evidence for whether BBBO is required to improve cognition in animal models.[14,15] Research studying FUS BBBO combined with antibodies against Aβ (aducanumab), tau, or a novel agent targeting pGlu3-Aβ, report functional improvements, but so far there has been no uniform uptake of these therapeutics in the brain been reported. The efficacy of FUS with BBBO and gene delivery using adeno-associated viruses (AAV) depends on serotypes and differs for brain areas.

Tau is harder to target than Aβ, but cognition is linked more strongly to tau than Aβ. Preclinical animal work has shown the potential of FUS as a treatment option. However, many questions remain about choice of modality, primary objectives, the underlying mechanisms of action, variability in tissue responsiveness, and the FUS parameter space.

Clinical Topics

Ali Rezai, MD, briefly mentioned the three main types of FUS. These were high-intensity FUS, low-intensity FUS for BBBO, and low-intensity FUS for reversible neuromodulation.[16] A variety of preclinical research has shown safety and efficacy of FUS BBBO in AD animal models. To date, over 200 patients have been treated with more than 500 FUS BBBO treatment sessions for brain tumors, AD, and Parkinson's disease with no serious adverse events.

Initial experiences in human patients with AD showed safety and feasibility.[17-20] Dr. Rezai presented results from the AL002 trial of FUS BBBO in patients with AD. This was an open-label safety and feasibility study of FUS in patients with early-stage AD. The study

targeted the cognitive, attentional, memory and spatial orientation networks of the hippocampus, entorhinal cortex, frontal and parietal lobes positive for Aβ up to 40 cubic centimeters (cc) in volume. This was a multi-center study in the US and 22 patients have been enrolled to date and treated with the Insightec system. Initially the procedure required a stereotactic frame and head shaving. A dental mold assembly has since been created that is frameless and does not require head shaving. The Insightec system allows for cortical and sub-cortical targeting with precision (millimeter accuracy) and real-time closed loop acoustic feedback and modulation control of ultrasound energy. Pre-treatment Aβ positron emission tomography (PET) imaging identified target areas of peak Aβ deposition.

Ten patients underwent 30 separate successful FUS treatments that were safe with reversible BBBO with closure within 24 hours that was highly conformal and only in the FUS target region. T2* and fluid-attenuated inversion recovery (FLAIR) imaging showed signals that indicate BBBO that resolve within a few days. These signals are not associated with any adverse events (AEs). In these patients, an average of 82% of the targeted area had BBBO in the FUS treatment regions. There was no meaningful cognitive or behavioral worsening with FUS. The average reduction in Aβ plaque after 8 weeks was 5% SUVr and 14% centiloid units. The mechanisms of action are unknown at this time but may be related to localized immunological activation or modulation or enhanced clearance of substrates.[21] The AL002 study is ongoing. The next steps are to increase patient enrollment, treat larger brain volumes, carry out longer term clinical cognitive follow up, PET evaluation, and combine FUS BBBO with anti-Aβ antibodies and other targeted therapeutics.

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Open Discussion

Q. How compelling is the evidence that antibodies need to cross the BBB as opposed to acting as a sink in the circulation and pull Aβ out of the brain through passive diffusion?

- This theory seems unlikely. Tau is intraneuronal and not extracellular alone. It can also be enclosed in exosomes and is not accessible to antibodies. Additionally, activation of microglia may be necessary to clear Aβ.

Q. While tau accumulation seems to correlate with cognition, is there evidence that changing the levels of tau in the brain can affect cognition?

- Dr. Götz responded that Aβ exerts toxicity through tau, and the two molecules do communicate, and both need to be cleared for treatment effects. One reason that anti-Aβ treatments have not been successful could be due to the fact, in addition to timing and low doses in the brain, that tau is still present.

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What Focused Ultrasound Parameters Should be Studied?

Kullervo Hynynen, PhD, stated that his talk would focus on BBBO parameters, monitoring, volume of opening, and differences between devices. BBBO parameters include the following considerations:

- Frequency: as frequency increases, the focus gets smaller. Attenuation and distortion also increase along with frequency.22
- Burst length: BBB disruption magnitude and its threshold depend on burst length.[23]
- Pulse repetition frequency, changing this parameter did not have an effect on BBB disruption magnitude.[23]
- Peak negative pressure: as the pressure amplitude is increased, there is greater enhancement until a threshold is reached and the blood vessel cannot open further.[24] This can be normalized with the mechanical index, 0.4-0.5 mPa is the threshold for safe BBBO.[24]
    - Increased pressure can lead to T2* changes on MRI. 25 These signal changes may indicate blood vessel damage due to red blood cell extravasation. Repetitive exposure can cause sterile inflammation and damage, and noting these spots may indicate upper threshold limit of treatment parameters.
    - Healing time can vary. Longer BBBO for 24 hours or more corresponds to histological damage in animal models.[26]
- Bubble concentration: with higher concentrations of bubbles there may be more gadolinium enhancement but also a greater risk of inertial cavitation and tissue damage. There are a few commercial bubble options, Definity and Optison, and enhancement seems similar between the two.[23]
- Bubble size: there is a difference in gadolinium enhancement with bigger bubbles producing greater enhancement.[27]
    - Larger bubbles may also increase proinflammatory mediators and place greater force on the vessel wall. There is a lot of opportunity and ongoing research into bubble composition as distinct types of bubbles should be used for different FUS applications (e.g., BBBO versus thermal ablation).
    - Lipid-pluronic nanobubbles stay in the bloodstream much longer than Optison and Definity bubbles and produce BBBO.[28] The histological data on nanobubbles is missing and more research is needed.
    - Acoustic cluster therapy (ACT) is administered as free flowing clusters of negatively charged microbubbles and positively charged microdroplets, the clusters are activated with FUS.[29]

Dr. Hynynen presented other FUS considerations. Scanning ultrasound (SUS) moves from spot to spot in scanning mode with a 6 second sonication time per spot and has been used in animal models.[30] The effect of age in preclinical models has also been investigated and older mice or an AD mouse model had greater BBBO than younger mice with identical FUS parameters.[31] Research with bolus versus infusion injection of microbubbles suggests that bolus injection results in a much higher concentration of bubbles. Real-time modulation of treatment pressures can be monitored using acoustic emissions from the exposed microbubbles.[24,32] In preclinical models, 3D subharmonic imaging can be used to calibrate exposure levels for safe FUS-induced volumetric BBBO.[33] The BBBO volume can be controlled by multi-point sonications.[33] Healing time is relational to BBBO volume, larger volumes take longer to close.

For clinical treatments, the following questions need to be answered:

- What parameters enable a large volume of treatment in 60 minutes?
- How do we control enhancement magnitude?
- How do sonication parameters impact different sizes of molecules?

Next, Dr. Hynynen also discussed sonication parameters for various FUS BBBO devices. These included the Insightec Exablate 4000, SonoCloud-9, NaviFUS, NS-US100, as well as others still in development.

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Panel Discussion

Panelists

Nir Lipsman, **Nathan McDannold**, **Jürgen Götz**, **Elisa Konofagou**, **Jin Woo Chang**, and **Kullervo Hynynen**

Dr. McDannold commented on the cause of T2* spots and theorized that the spots are likely petechia caused by inertial cavitation.

Dr. Chang stated that he had experience with 4 clinical trials with the Insightec Exablate system. The head shaving requirement made it difficult to recruit patients. An ongoing clinical trial of BBBO for AD has a treatment interval of 3 months. Early analysis suggested some improvements in memory. This study is also administering anti-Aβ antibodies in combination with BBBO and data is expected next year. The spots appearing with T2* imaging are important to note. The significance of the spots is unknown, and they seem to resolve with time. It is important to understand what these spots are and the mechanisms causing them.

Dr. Konofagou described some preliminary research with patients with AD using a new system with a single element transducer with one-time BBBO. It is important to use optimized parameters. Mechanical index 0.4 is the threshold for BBBO. It is important to estimate attenuation through the skull using simulations based on CT scans. There was one case of edema and subarachnoid hemorrhage when skull attenuation was not properly estimated. In subsequent patients, there was no evidence of damage or spots on T2*.

Dr. Lipsman compared other brain interventions like deep-brain stimulation (DBS) with FUS. He shared his surprise about the concerns of the safety and mechanisms of FUS and how risks such as more intense bleeding along DBS tract is much greater than with T2* changes with FUS. He also addressed T2* spots and mentioned that these spots were an indication of BBBO and usually resolved within 24 hours. There is some concern that too much power could overstimulate blood vessels and cause them to spasm without letting medications across. The FUS procedure is rapidly changing and can now be done without a stereotactic frame and head shaving.

Dr. Götz commented that they have moved from preclinical research in mice to sheep. A clinical trial was started this year using FUS as a neuromodulatory modality in up to 12 patients with a target in the precuneus. FUS alone without BBBO also has effects on brain tissue. 15 There is little data from humans on the effect of different FUS frequencies. Preclinical research in mice with AD showed differences between frequencies, but the same pressure demonstrated that higher frequencies resulted in better cognitive effects.[9]

Q. Is there an additive effect of thermal dose for BBBO?

- Dr. Hynynen stated that exposures at high energies are additive but does not seem to be additive for the lower energies used with FUS BBBO.
- Dr. Konofagou responded that there is no large temperature elevation during BBBO, and it is a purely mechanical effect. However, reopening the BBB prior to complete closure could cause damage.
- Dr. McDannold answered that longer sonications can produce greater BBBO, and there is some cumulative effect. However, there is a pressure amplitude where there is a saturation point. It would be interesting to study the occurrence of a small BBBO and repeat the process to see whether there is a cumulative effect. Several panelists agreed that this should be investigated.

Q. Is there a standardized method to measure BBBO particularly for clinical trials?

- Dr. Chang agreed that this kind of measurement is needed. The optimal interval for BBBO for patients with AD is unknown. In animal trials, 1 to 2 weeks has been shown to be effective, but this has not been repeated in humans. Another unknown is the appropriate number of times the BBB should be opened in human patients.
- Dr. Lipsman answered that contrast enhancement on MRI with gadolinium is increasingly used. Other markers should be investigated, such as radiolabeling antibodies and evaluating CSF and plasma for biomarkers that could signify BBBO.

Q. There was a comment on the effects of BBBO and allowing substances, both good and bad, into the brain.

- Dr. Konofagou replied that there is a lack of basic science research in general. No one really knows what happens when the BBB opens. It also tends to open during REM sleep and exercise, as the tight junctions relax and become more permeable.

Q. There was a comment on the difference between BBB leakiness caused by disease and BBBO.

- Dr. Konofagou mentioned that this should be studied to note the differences between BBBO caused by disease and that caused by FUS.

Q. Is there a way to compare the different FUS devices for BBBO and will this be important for human patients?

- Dr. McDannold responded that determining how to gain FDA approval and wide acceptance of FUS treatments may be more important than comparing devices. Liquid biopsy may be an easier path than therapy for a given treatment. The field needs to ensure that there is enough BBBO in the target area before comparing devices.
- Dr. Konofagou commented that FUS has not been widely accepted, particularly by pharmaceutical companies. The field needs to demonstrate safety. Monitoring and reporting parameters are essential to demonstrating safety and reproducibility.

Q. From a clinician perspective, it is important to have a signal for BBBO. Another key need is to know that the targeting is accurate. Has Aβ been measured outside the areas of targeting?

- Dr. Lipsman responded that Aβ accumulation does not change outside of targeted areas. It is also unknown if FUS is being used early enough in the disease process.

Q. There was a comment that inertial cavitation is needed for BBBO, but at very low levels.

- Dr. Hynynen said that whenever they detected inertial cavitation, there was also tissue damage.
- Dr. Konofagou mentioned that inertial cavitation could be detected even without BBBO, such as with skull reflections, etc. It might be a promising idea to better define inertial cavitation.

Q. The FDA has not been concerned about the accuracy of targeting, but there was a question on whether this should be a future concern.

- Dr. McDannold commented that in most cases, a broad area is being treated, and the accuracy of targeting for BBBO is not a great concern.
- Several panelists agreed that the Insightec Exablate device is extremely precise and has accurate targeting.
- For AD, the Aβ is throughout the brain, so treating a slightly different area may not be a concern for this indication.
- The amount of BBBO is also measurable with MRI after the procedure.

Q. The panelists were asked to comment on the volume and target of BBBO in clinical trials.

- Dr. Götz indicated that this depends on how you look at the disease. Accumulation of Aβ happens throughout the brain. There may be progressive stages and the area of treatment may depend on the stage of disease. He referred to the Braak stages for tau which are more stereotypical than the Thal stages for Aβ. The earlier the treatment, the smaller the area to treat, but possibly the entire brain needs to be treated.
- Dr. Konofagou stated that 40 cc seems to be the current treatment volume based on information provided by several investigators at the meeting. The larger the BBBO, the larger the Aβ reduction and the longer the duration of treatment. Ideally, they would like to treat multiple areas and also perform multiple FUS treatments. The aim of this treatment is not only a reduction in Aβ or tau, but also improvement of cognitive measures.
- Dr. Chen was concerned that opening an area bigger than 40 cc would increase the risk of complications. He suggested that different areas of the brain could be targeted in separate treatment sessions.
- There was a brief discussion on preclinical evidence that BBBO in aged mice both with and without AD, improved cognitive function.

What Therapeutics Should be Combined with FUS BBBO for Alzheimer's Disease?

Aducanumab and Other Anti-aβ Antibodies

Marc Haut, PhD, presented WVU's clinical experience with FUS-mediated BBBO combined with Aβ antibody infusion in patients with AD. There are a number of anti-Aβ antibodies in clinical development for patients with AD, including aducanumab, lecanemab, donanemab, gantenerumab, and solanezumab.[34] Aducanumab was granted FDA accelerated approval in 2021, lecanemab was granted FDA accelerated approval in 2023 with full approval expected later in the year.[1] Donanemab was denied FDA accelerated approval based on small sample size in a phase 2 trial; phase 3 readout and submission for FDA traditional approval expected in 2023/2024. Gantenerumab did not meet the phase 3 endpoint for reducing cognitive decline or reduction of Aβ.

In general, anti-Aβ antibody infusions have demonstrated reduction in Aβ and delayed clinical progression. These antibodies require higher dosing/frequency and a long treatment duration of 18 months or longer of once or twice per month infusion with additional maintenance thereafter. Aducanumab, donanemab, and lecanemab are all associated with ARIA on MRI, most cases are asymptomatic and resolve, but fatalities cannot be ignored. At this time, these agents are also costly for the patient as the Centers for Medicare & Medicaid Services (CMS) does not cover them, although this is most likely changing given the recent FDA approval of lecanemab.

The BBB does not allow particles larger than 400 to 500 Da to readily cross. The average size of active central nervous system drugs is 357 Da. More than 98% of small molecule drugs do not cross the BBB. By combining anti-Aβ with BBBO, there may be an opportunity for accelerated reduction of Aβ by increasing the penetration of monoclonal antibodies. It could improve the safety profile by reducing the dose, frequency, and duration of treatment. BBBO could be combined with FDA-approved therapies as well as emerging therapies that did not meet safety and efficacy end points. Preclinical research showed that FUS-mediated BBBO significantly increased the delivery of aducanumab.[3]

West Virginia University is conducting an FDA-IDE pilot safety and feasibility study of aducanumab administered with BBBO. Patients enrolled have early-stage or mild AD with positive Aβ plaque, enrollment began in August 2022. Aducanumab is administered according to the FDA-approved label on a monthly IV infusion schedule. For the first 6 months, patients receive standard monthly IV infusion up to 6 mg/kg and FUS BBBO. FUS BBBO is performed 2 hours after the aducanumab infusion in one brain hemisphere and compared with the opposite (control) non-FUS treated location. There is frequent monitoring with MRI (to check for ARIA and BBBO/closing), PET (to check for Aβ changes), and there is safety oversight. Patients are excluded if they have double APoE4 or the FUS treatment location is located in a frontal non-dominant area. The treated brain volume area was escalated from 10 cc to 40 cc in the first three patients. With each dose escalation of aducanumab, the DSMB reviews the data to confirm safety. Patients are admitted for 24 to 48 hours after treatment for observation.

Three patients have been treated with no clinical safety issues over 6 months with a total of 18 FUS BBBO with IV infusion of the drug. There were no procedure-related AEs, no serious adverse events or study stopping criteria, no imaging safety issues, and no ARIA. This is an ongoing study and additional patients will be treated with continued clinical and safety monitoring.

Questions

Q. There was a question on if T2* spots were observed.

- Dr. Rezai responded that this was observed, but these areas were not re-treated for safety reasons.

Q. A participant asked if there were any changes in cognition or memory?

- Dr. Rezai responded that no changes in cognition or memory have been observed so far. This is a safety and feasibility study, so changes in cognition are not expected.

IV Immunoglobulin (IVIg)

Nir Lipsman, MD, PhD, discussed preclinical work with BBBO and IVig and planned work in clinical trials. Dr. Lipsman recently published a study of FUS BBBO in patients with mild-to-moderate AD that provided safety data for BBBO in multiple brain regions with a larger volume.[35] IVig is pooled antibodies from healthy donors; it is a common, safe, and readily available treatment option. IVig contains naturally occurring antibodies against Aβ. Several small trials suggested that biweekly (or every 4 weeks) infusion over 6 months was linked to increase Aβ in plasma and decreased Aβ in CSF, without cognitive deterioration.[36] Only a small proportion of IVig crosses the BBB in preclinical models. Preclinical work showed that FUS BBBO could deliver IVig to the brain and promote hippocampal neurogenesis.[37]

The rationale for an early phase trial of BBBO with IVig was that it remained an unanswered question whether the failure of IVig was due to low brain penetration across the BBB. The treatment plan for the clinical trial is to replicate the prior FUS and AD clinical trial but administer IVig (0.8 g/kg) prior to BBBO. This will be a prospective, escalating dose, open-label, single arm, non-randomized, phase IIa trial to evaluate safety and feasibility of FUS BBBO enhanced delivery of IVig immunotherapy. The aim is to enroll 20 patients with mild-to-moderate AD that will undergo 3 treatments, every 2 weeks, targeting up to 10 regions of the default mode network including the hippocampus and entorhinal cortex. The estimated start date is fall 2023.

Other agents also have the potential to treat AD when combined with FUS BBBO, such as tropomyosin receptor kinase A (TrkA) agonists, which is a selective nerve growth factor receptor. Nerve growth factor is critical for neuronal growth, resilience, and protection.

A trkA agonist has been developed to selectively stimulate the TrkA receptor and avoids p75 activation. Preclinical research showed memory enhancement and Aβ reduction in a mouse model of AD.[38]

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Preclinical Strategies

Jürgen Götz, PhD, described potential therapeutics to consider for combination with FUS BBBO. Combination therapy is partially determined by whether there is an assumption that AD is cell centric or network-centric.[4,36] Disease progression partially determines the choice of combination therapy. If one targets Aβ or tau, the stage of disease is critical as the pattern of progression of the two pathologies differs and there isnot necessarily a lot of overlap. Direct targeting prevents any of the steps involved in the generation, posttranslational modification and aggregation of Aβ/tau.[39] Indirect targeting could block downstream signaling; e.g. block excitotoxicity, improve synaptic functions, facilitate mitochondrial functions, and activate autophagy.[39] Many of the anti-tau antibodies in clinical development target hyperphosphorylated tau, others non-phosphorylated epitopes, and those tested in clinical trials so far did not lead to the intended positive outcomes. A way forward in a clinical setting may be to combine anti-tau and anti-Aβ antibodies to improve outcomes. The expression vectors delivered via AAV can be genetically engineered to add targeting motifs, such as proteasome-targeting sequences, in order to facilitate clearance of proteins such as tau. The problem with AAV vectors is that they can only accommodate a limited size of DNA (<4.8 kb) and AAV delivery is also not a routine procedure in the clinic. The efficacy of gene delivery to the brain using AAV and FUS further depends on serotypes and brain areas of interest.

Besides directly targeting tau and Aβ there are indirect targets in pathocascades affected by tau and Aβ such as down-stream mediators including the kinases Fyn, ERK and S6. A strategy may be to combat Aβ-mediated and tau-facilitated excitotoxicity with the anti-epileptic drug levetiracetam, or oxidative stress with antioxidants.

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Panel Discussion

Panelists

Sandra Black, **Jürgen Götz**, **Lawrence Honig**, **Nir Lipsman**, and **Ali Rezai**

Q. What kind of therapeutics in the AD pipeline should be combined with BBBO and FUS?

- If tau is being targeted for therapy, a discussion is about the relative role of intracellular and extracellular tau in the brain as this affects the strategy.
- There are 3 antibodies with enough data to show efficacy, and the panel agreed that these might be the ideal antibodies to start researching for combination with FUS and BBBO.

- It might also be useful to combine BBBO with antibodies that have not worked in clinical trials, or that have not progressed too much through the clinical trial pipeline.
- FUS BBBO might also allow for lower doses of antibodies in general and may even accelerate the reduction of Aβ.
- Future trials should quantify the amount of antibody that enters the brain following BBBO.

Q. There was a question on whether neuroinflammation should be investigated further.

- The difficulty with this kind of research is that it is unclear what outcomes would show improvement. The panelists were unsure if this could lead to reductions in cognitive decline over time in patients with AD.
- Biomarkers that could help measure endpoints would be useful in the future.

What Outcome Measures Should be Standardized?

Sonobiopsy

Hong Chen, PhD, presented work on sonobiopsy for AD diagnosis. Sonobiopsy is a shortened term for focused ultrasound enabled liquid biopsy. The BBB not only blocks drugs from getting into the brain, but also blocks the release of potential biomarkers into the bloodstream. The concept is to replace tissue biopsy with non-invasive methods. A proof-of-concept study demonstrated safety and feasibility in mice and healthy pigs, and a pig model of glioblastoma.[40–43]

Sonobiopsy enriched circulating tumor DNA in a mouse model of glioblastoma.[42] Markers that were specific to the glioblastoma cells used to create the mouse model (EGFRvIII and TERT C228T) were detected in the blood and sonobiopsy improved detection compared with a regular blood draw. The pig glioblastoma model was created with human glioblastoma cells, and sonobiopsy was found to increase circulating tumor DNA. A retrospective analysis of samples from human patients with glioblastoma, which underwent BBBO, confirmed that FUS enriches circulating biomarkers.[44]

Prior research on sonobiopsy used the Insightec Exablate, which requires MRI. Their team at WashU is now transitioning to a hand-held sonobiopsy device that uses neuronavigation. Data from the first 3 patients undergoing sonobiopsy reported improved detection of known patient-specific tumor variants, and these blood samples were obtained 30 minutes after BBBO.

Sonobiopsy can be expanded beyond brain tumor diagnosis and used to release neurodegenerative disease biomarkers. In a study using tauopathy mouse models, findings reported the feasibility of sonobiopsy to release phosphorylated tau species and neurofilament light chain into the blood circulation and may potentially facilitate diagnosis of neurodegenerative disorders.[45] Sonobiopsy can fit into the existing clinical workflow without a hospital stay and allows location identification in the brain—targeted to a small brain region without the need for large volumes of BBB. Sonobiopsy may also be used at multiple timepoints for a single patient.

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Current Status of Imaging for Alzheimer's Disease

Prashanthi Vemuri, PhD, discussed some of the imaging surrogate biomarkers for AD including Aβ PET and Tau PET. Staging of AD and tracking disease progression can be done with MRI. There is a 21.1% detection rate with Aβ in the general population. Some patients have Aβ and never develop dementia, particularly those that do not have tau deposition. The centiloid is a 100-point scale that has an average value of zero in "high certainty" Aβ negative participants and an average of 100 in typical patients with AD.

There are several radiolabeled tau imaging tracers. Patients with elevated levels of tau in the medial temporal lobe are at elevated risk of progression and PET imaging could be

used to identify these patients. The use of tau as a biomarker has been used in clinical trials to exclude patients with disease progression. There is a project underway to standardize the measurement of tau similar to the centiloid for Aβ.

Ventricular volume was previously thought to be a measure of progression. However, a recent meta-analysis showed anti-Aβ antibodies accelerated brain volume loss and ventricular volume increase was correlated with an increased risk for ARIA.[46] The field is now trying to determine whether the volume loss reflects neurodegeneration or pseudoatrophy, and patients will be followed for several years to investigate this issue.

Standardized contrast MRI protocols are also in development so that results can be compared across study locations as well as improve efficiency and shorten scanning time. This could also be used to track disease progression.

Dr. Vemuri briefly mentioned that AD and cerebrovascular disease commonly occur at the same time and also cause cognitive decline. Screening for cardiovascular disease (CVD) biomarkers could also help to identify progression and safety markers.

Panel Discussion

Panelists
Sandra Black, **Hong Chen**, **Paul Fishman**, **Lawrence Honig**, and **Prashanthi Vemuri**

Q. There was a question on the location of blood samples for sonobiopsy.

- Dr. Chen responded that blood was collected from an IV line in the arm, and there were no other options available for blood sampling. An audience member suggested that the jugular vein might be a better source of biomarkers as the concentrations would be higher.
- Plasma is probably fine for blood sampling as biomarkers likely circulate very quickly in the body and the BBBO may last for a few hours.

Q. A participant asked whether different time points had been looked at for some of the biomarkers, particularly considering the short half-lives for some of the biomarkers.

- Dr. Chen mentioned that they are looking at optimizing the blood collection times for each biomarker of interest.

Q. There was a question on how artificial intelligence (AI) and other methods of automation might impact MRI protocols.

- So far, when AI has been applied to MRI analysis, there has been identification of many false positives. AI might be useful for looking at patterns in the entire health record, not just MRI or cognitive testing alone.

Q. A question was asked on what stage of disease should be treated with the combination of BBBO and an anti-Aβ antibody, should there be a requirement that patients have progressed to tau accumulation.

- Patients with cognitive impairment likely have Aβ accumulation, including patients with other neurodegenerative diseases such as Parkinson's disease or Lewy body dementia. It is likely better to treat patients with dementia and Aβ accumulation, even in the absence of tau accumulation.
- Dr. Fishman commented that the appropriate neurological testing capable of detecting changes in cognition with focal treatment is unknown.

Q. Could MRI fingerprinting be useful for BBBO?.

- The difficulty with MR fingerprinting in the clinical trial setting is the challenge with quality control and data cleaning. This is much easier to do in a single-center trial. Each scanner has to have the right software updates and it has to work seamlessly between any scanner used in the trial.

Q. For a patient with early cognitive impairment with borderline Aβ accumulation with or without tau, what brain area should be targeted for a sonobiopsy?

- For a patient with cognitive impairment and Aβ the precuneus would be the area of interest. For tau accumulation, the entorhinal cortex should be targeted.

Q. Magnetic resonance spectroscopy could be useful in AD, what metabolites should be looked at?

- This concept needs more basic research before it could be used in clinical trials.
- There was a comment that the Aβ tau ratio could potentially be different across various ethnic groups.

Q. There is a question of whether BBBO could stimulate neurogenesis in human patients and how this could be measured.

- Pattern separation in a MRI while measuring blood flow shows activation in the dentate gyrus. While this cannot be confirmed in human patients, neurogenesis was confirmed with preclinical work.
- A comment was made that perhaps measuring neurotrophins, such as BDNF, could serve as surrogate markers of neurogenesis.

Q. How can the FUS Foundation help the field?

- In coordination with the FDA, there is a need to determine the key parameters that should always be reported in both preclinical work and clinical trials.
    - There was a recommendation to look at Gail ter Haar's published recommendations for reporting.[47]

- Determine how often the BBB can be opened with FUS.
    - Attendees mentioned that BBBO trials are wide ranging with once per month, every 2 weeks, and three times per week.
    - There are also fundamental differences between devices that can influence the frequency of BBBO.
    - Blood samples can be used to look at Aβ clearance as well as markers of damage.
- The combination of BBBO with anti-Aβ antibodies might accelerate the removal of Aβ to levels that cannot be detected with imaging. Aβ accumulation could be monitored through blood tests to indicate the need for retreatment.
- It would be helpful for the FDA if researchers reported on the success of procedures with custom microbubbles. Need to detail the characteristics such as the materials used in the shell and the gas.
    - There was some debate on the use of bubbles approved by the FDA for cardiovascular imaging versus treating brain tissue. It would be great to have a commercially available bubble made specifically for BBBO with FUS.
    - It is difficult to compare studies without a standardized bubble.
    - Preparation and administration sites are also important.
- Of note, PET is not reimbursed through healthcare in most Canadian provinces but this may be changing soon with the prospect of disease modifying therapies. Single-photon emission computed tomography is often used in its place and shows typical perfusion reductions in AD. TDP-43 is often observed in the brain prior to cognitive impairment and may present with very severe memory loss and/or as apathy, which have been previously considered as the temporal variant and frontal variants of AD. Posterior cortical atrophy occurs mostly in patients that are younger, such as in their 50s.

What Should be the Inclusion Criteria for Upcoming Studies?

Sandra Black, MD, provided background on AD and inclusion criteria for future work. Understanding the status of vascular disease in the brain is critical to considering inclusion criteria for AD studies. The neurovascular unit is constituted by endothelial cells, myocytes, neurons, and their processes, astrocytes, and perivascular cells (microglia, macrophages, mast cells, etc.). The functional integrity of this unit is crucial for normal brain function. Aβ 42 and hyperphosphorylated tau work together for pruning in development and protecting the brain from injury in ways that are still not fully understood. There are many types of cerebrovasculopathies. Small vessel disease can lead to lacunes, white matter hyperintensity, perivascular spaces, microbleeds, and microinfarcts. Arteriolar sclerosis can result in remodeling of arteries in the brain. Thalamic infarcts are also a significant and under-recognized contributor to impairment in the elderly when they involve the left dorsal medial nucleus especially on the left. Lobar enlarged perivascular spaces in white matter versus basal ganglia implicate the penetrating arterioles vs the larger basal ganglia arteries. Likewise basal ganglia microbleeds relate to vascular risks like hypertension and diabetes, whereas lobar microbleeds and siderosis suggest Aβ angiopathy and can also be associated with subarachnoid hemorrhage.

Another consideration is that blood also flows out of the brain. There is a gradient of blood flow from the cortex towards the ventricles (watershed). The periventricular area shows white matter hyperintensities associated with increased risk of stroke, cognitive decline, dementia, and death. These are also associated with a faster decline in global cognitive performance, executive function, and processing speed. White matter hyperintensities seen with aging and vascular risk factors are not a result of an adaptive t-cell mediated inflammatory process as in multiple sclerosis, but rather reflect autoimmune inflammation-related processes relating to pericyte and microglial activation but much more study of these mechanisms is needed. Some peripheral pro-and anti-inflammatory cytokines (e.g. IL-8) are elevated in patients with a clinical AD diagnosis and who also have extensive white matter hyperintensities.[48] The deep medullary veins drain blood from white matter to the deep venous system and may be part of the glymphatic system that helps clear toxic amyloid beta 40 and 42 out of the brain during deep sleep. Periventricular white matter hyperintensities co-localize with the deep intramedullary venules which develop collagenosis of their walls based on recent neuropathological studies.[49,50]

In 2022, a severity rating scale for ARIA was revised for use in clinical trials of aducanumab.[51] Monitoring for ARIA during treatment with anti-Aβ antibody treatment is important to practicing clinicians. ARIA with edema (ARIA-E) can be caused by parenchymal hyperintensity, sulcal hyperintensity, and swelling. ARIA with hemorrhage (ARIA-H) can be caused by microbleeds, superficial siderosis, and lobar hemorrhage. Dr. Black reviewed the inclusion and exclusion criteria used in the CLARITY AD study and the corresponding proposals for appropriate use recommendations for patients treated with lecanemab.[52] The recommendation is to treat patients with early-stage disease. There is controversy surrounding the use of anticoagulants, but these may increase the risk for macro hemorrhage. Management of ARIA-E or ARIA-H was also discussed.

Depending on severity, dosing is suspended or discontinued. The resources needed by a clinician and the medical center for safe treatment are also provided in the appropriate use recommendations.[52]

The key points to consider for future combination trials of FUS BBBO treatment include:

- Despite the fact that low-intensity FUS in AD may only target key signature areas, it is advisable to follow the appropriate use rules for stage of disease, concomitant, white matter hyperintensity load, and exclusionary criteria.
- Follow the appropriate use recommendations for when to suspend or discontinue treatment if ARIA-E or ARIA-H develops.
  - The simpler guidelines for both ARIA-E and ARIA-H are more easily implemented and are therefore preferred.
- These rules should also be followed when antibody infusions are added to the treatment regimen.

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Panel Discussion

Panelists
Sandra Black, **Paul Fishman**, and **Marc Haut**

Q. Should the presence of ARIA be an exclusion criterion for FUS trials in AD?

- Dr. Black indicated that per the guidelines, some ARIA is allowed, and she recommended that investigators follow these recommendations.s.

Q. What are some exclusion criteria that should be considered for trials of FUS in AD?

- Patients with preexisting vasogenic edema seen as periventricular confluent white matter hyperintensities reflecting vasogenic edema and likely perivascular stasis should be excluded as this can impede amyloid clearance and increase this side effect of amyloid removing antibodies.

Q. For patients treated with BBBO, has ARIA been observed?

- The investigators replied that they have not seen ARIA outside of sites targeted by FUS, but aducanumab has only been administered at lower doses (6 mg/kg).
  - All patients are screened for APOE4 as this is a known risk factor for AEs. For FUS alone patients were allowed to have one copy of the gene, but with the combination of FUS plus antibody, patients are not allowed to have any APOE4.

Q. Do differences in ARIA occurrences happen between the anti-Aβ antibodies?

- ARIA is not well reported in clinical trials, so it is unknown.

Q. For patients that experience ARIA that suspend treatment and then restart treatment, do the ARIAs reappear in the same places?

- ARIAs can happen in new locations, not just places where they occurred before.

Q. What is the reversibility of ARIA?

- Typically resolves within a few months, and if ARIA recurs, it can happen in a new location.

Q. What are some of the additional exclusion criteria for the FUS plus aducanumab study?

- One consideration is if there is enough tissue for targeting, patients with too much atrophy would be excluded.

Q. In terms of targeting, is there any evidence to suggest optimal brain areas for FUS targeting versus those that should be avoided?

- The parietal lobes seem to acquire T2* signals earlier and at lower cavitation levels, so frontal lobes are preferred. Frontal lobe treatment with FUS has also produced the greatest decreases in Aβ.

Q. Has the amount of Aβ in plasma been looked at in combination for FUS plus aducanumab?

- The blood has been collected, but not analyzed.
- Dr. Konofagou mentioned that after FUS alone there was an increase in Aβ and tau in serum in humans, and this is also seen in preclinical trials. Dr Götz mentioned that regarding Aβ and tau, his team found mechanisms in the brain (such as microglial uptake of Aβ or autophagic clearance of tau) rather than clearance into the periphery.

Q. How do you quantify or grade perivascular spaces, white matter disease, and atrophy?

- There are grading systems for medial temporal lobe atrophy, particularly the hippocampus, and the entorhinal cortex. The team has also constructed their own clinical database of patients with mild-cognitive impairment (MCI) and AD with their 3T scanner. There are also semiautomatic ways to quantify perivascular spaces.[53,54]

Q. Are the vessels more fragile after FUS?

- This is hard to differentiate on MRI and the vessels could be weak due to cerebral Aβ angiopathy.

Q. There was a question on whether tau PET was in use for the combination FUS and anti-Aβ antibody trial.

- Tau PET is not being used in that study because of logistical challenges, and Tau tracers are not as well-developed as Aβ tracers. There is only one FDA-approved

ligand for tau, which is manufactured in California, and there is no method to get it to the study site quickly enough for use. In place of this, they use tau measured in CSF.

Q. What other markers for use with PET would be useful?

- The panelists listed several including microglial activation, α-synuclein, TDP-43, and pericyte markers.
- Panelists also discussed the importance of biomarkers for recruitment so that the patient population could be standardized between sites.

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Roadmap Discussion

Moderators
Suzanne LeBlang and **Lauren Powlovich**

Q. What Focused Ultrasound parameters should be employed for clinical trials?

Sonication Parameters
- Frequency = 200-700 kHz, small volume 1-2 MHz
- Burst length = 5-10 ms, single cycle – continuous wave
- PRF: 0.2-10 Hz
- Pressure amplitude: >0.45MI<subharmonic threshold<inertial cavitation
- MRI: >T1C enhancement<T2*
- Duration: 50-102 s
- Parameters need to be tailored to the specific brain region
- Definitely Report: cavitation dose

Microbubbles
- Recommend commercial bubbles for clinical trials.
- For custom bubbles, make sure to report properties of the custom bubbles (size, size distribution, how fast they clear, carbon shell length, type of gas, administration details such as bolus or continuous infusion and site of injection, etc).
- Nanodroplets: have a liquid center instead of gas and can turn into microbubbles with FUS, safety is better.
- There are intellectual property issues with bubble manufacturers—a request was made by a participant that the FUS Foundation could potentially work with bubble companies to supply them to researchers to avoid the IP issues.
- Consensus on microbubble properties that are ideal for FUS BBBO is needed.
- Infusion is better than bolus.

Volume
- Current: 40 cc (target) but consider increasing volume as this may be necessary to become clinically relevant.
- Limited by patient tolerance and knowledge of safety data.
    - Consider sedation if attempting larger treatment volumes.
    - Consider treating various locations within the same session.
- Important to standardize reporting of volume: define the target and report target coverage.
    - This can be difficult to quantify.
- More is not necessarily needed, especially around the motor cortex.

Devices

- There needs to be standardization of reported parameters in the literature, including reporting of the cavitation dose
    - See Gail ter Haar's recommendations[48]

Q. What therapeutics should be considered in conjunction with FUS and BBBO for AD?

Therapeutics

- Drugs that have already been proven to work (e.g., aducanumab, lecanemab and donanemab).
- Antisense oligonucleotides.
- Smaller companies with new agents that FUS BBBO could enhance.
- IVig.
- Targeting tau should be a future goal.
    - Accumulation of Aβ can occur without symptoms, until tau also starts to accumulate.
    - Could be difficult to engage with pharma on this topic.

Dose, Timing, and Frequency

- FA standardized way to report timing is needed.
- FAdministration of drug should be performed as quickly as possible after BBBO.
- FCurrent clinical timing is not ideal.
- FLook at the FDA product jurisdiction algorithm.
- FLook at how typical medication (donepezil) safety data is reported.

Confirmation of Delivery

- PET labeling.
- Gadolinium based contrast enhancement.
- T2 FLAIR.
- T2* changes?
- DTI, to look at fractional anisotropy, can be correlated to BBBO.
- Cavitation mapping for confirmation of opening.
- Blood and CSF biomarkers that prove efficacy (neurotrophic factors?)

Q. Should FUS BBBO alone be further considered as a treatment option for AD?

- Identify key areas to target – default mode network.
- Develop technology to perform FUS on the entire brain.
- Confirm BBBO and minimize T2* changes.
- Use and optimize standard microbubbles.
- Further research MOA.
- How often to perform BBBO.
- FUS neuromodulation with or without BBBO.

Q. What should the inclusion and exclusion criteria be for upcoming studies?

Stage of disease

- Mild-to-moderate disease (cut-off levels for Aβ and tau).
    - Might prefer higher levels of Aβ
- No APOE4 hetero/homozygous carriers for Aduhelm study.
- Consider baseline FDG PET – biomarker in addition to Aβ and phosphorylated tau in blood.

Inclusion of other dementias
- Limit to only AD or MCI at this time

Comorbid white matter disease
- Prefer 3T: consider perivascular spaces, white matter disease excludes Fazekas 3, sometimes 2, and excludes only significant global atrophy and sometimes parietal if cannot safely target Aβ.

Microbleeds/edema
- Follow guidelines for managing various forms of complications even if not symptomatic (i.e., various rules for stopping for ARIA), < 5 microbleeds and only one area of siderosis
- No vasogenic edema (that may be too extreme as Fazekas score for WMH could be included but not score 3 These areas represent vasogenic edema around collagenized veins as discussed above.)
- Use of the appropriate use guidelines with therapeutics for stop and start.

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Clinical Outcome Measurements

Scales
- ADAS COG – learning trials, recognition trials, and memory sensitive but hard to show changes without a large sample size; imaging surrogate but need to document clinical improvement (trial versus treatment). Clinical Dementia Rating scale (CDR) sum of boxes is widely used in the pharma trials and could be considered
- FDG is a reasonable clinical surrogate.
- Patients and caregivers reported outcomes.

Liquid biopsy
- In addition to measuring Aβ and tau, hypothesize creating assays with proteomics and metabolomics in targeted areas.
- Optimize collection time points for samples in blood and/or CSF.

Imaging biomarkers
- Standardize MRI protocol.
- Use Aβ and tau PET scans for inclusion/exclusion and monitoring (centiloid scales), FDG PET.
- Percent decrease and baseline level comparisons.
- Use of AI and machine learning to investigate large datasets, such as the UK biobank.

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Open Discussion

Microbubbles
- Participants highlighted the lack of commercial bubbles for use with FUS BBBO. The available bubbles were created for and only approved for use by the FDA for cardiac imaging. It is difficult to obtain commercially available bubbles for research purposes.
    - The use of custom, "home-made" bubbles in preclinical or clinical research makes replication across laboratories difficult and the FDA typically may not rely on data from custom bubbles for safety and efficacy decisions.
    - Properties of bubbles that need to be reported include size and carbon length on the shell.

- An optimized bubble could increase the efficacy of FUS BBBO in the clinic.
- The FDA does not recommend commercial versus custom made and encourages researchers to submit with the kind of bubble they think is best.
- The FDA may evaluate microbubbles either as drugs or devices, it depends on the specific situation.
- There was a request for the FUS Foundation to assist in the distribution of commercial microbubbles to bypass the IP issues.

FUS Parameters

- Sonication parameters need to differ between grey and white matter; white matter is less vascular so the opening will be smaller, and permeability is lower.
- The area of the skull that is being targeted is an important consideration. It is important to know both the trajectory and area of targeting.
- There was consensus on increasing the volume of opening for patients with AD. Volume of opening is not limited by technical ability, it is limited by the study protocols. Patient procedural time in the device and the amount of bubbles would also need to increase.
- To move the field forward, efforts should be coordinated. Pooled data, standardized reporting, and division of efforts could push the field forward in a faster way. The volume of opening and microbubble parameters could be investigated in parallel.
- Participants noted that FUS BBBO is also being done in other brain diseases such as amyotrophic lateral sclerosis and brain tumors. It would be interesting to compare these sonication parameters with the parameters for AD.

Additional thoughts

- The field needs to determine the optimal way to confirm delivery of therapeutic agents via FUS and BBBO. In the long term, PET labeling will not work for pre- and post-treatment.
- Participants discussed the fact that neuromodulation occurs through BBBO alone.
- Neuromodulation alone in healthy primates can improve cognitive function.
- There was mention of the fact that patients with AD have better outcomes in clinical trials because they receive comprehensive care as well as social stimulation.
- By the time Aβ has accumulated, the brain has already been damaged. It is key to know the pathogenic processes that lead to this accumulation. Preclinical research is still needed.
- here was some discussion on the cost-effectiveness of AD agents combined with FUS, it may be easier for patients in earlier stages of disease.

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Action Plan

As a result of this workshop, the Focused Ultrasound Foundation is committed to the following action items:

1. Publish a consensus paper on FUS BBBO parameters.
2. Investigate the microbubble landscape to ease availability, standardize reporting for microbubble usage, and work to optimize a FUS specific microbubble.
3. Pursue additional clinical trials which use larger treatment volumes or additional therapeutics such as Lecanemab.

Through these actions, FUSF hopes to catalyze the clinical adoption of FUS for Alzheimer's Disease.

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Abbreviations

AAV	Adeno-associated virus
AD	Alzheimer's Disease
AE	Adverse events
AI	Artificial intelligence
ARIA	Amyloid-related imaging abnormalities
BBB	Blood-brain barrier
BBBO	Blood-brain barrier opening
DBS	Deep-brain stimulation
FDA	U.S. Food and Drug Administration
FUS	focused ultrasound
FLAIR	Fluid-attenuated inversion recovery
FUS	Focused Ultrasound
MCI	Mild-cognitive impairment
MRS	Magnetic resonance spectroscopy
NGF	Nerve growth factor
PET	Positron emission tomography
SPECT	Single-photon emission computed tomography
VCID	Vascular contributions to cognitive impairment and dementia

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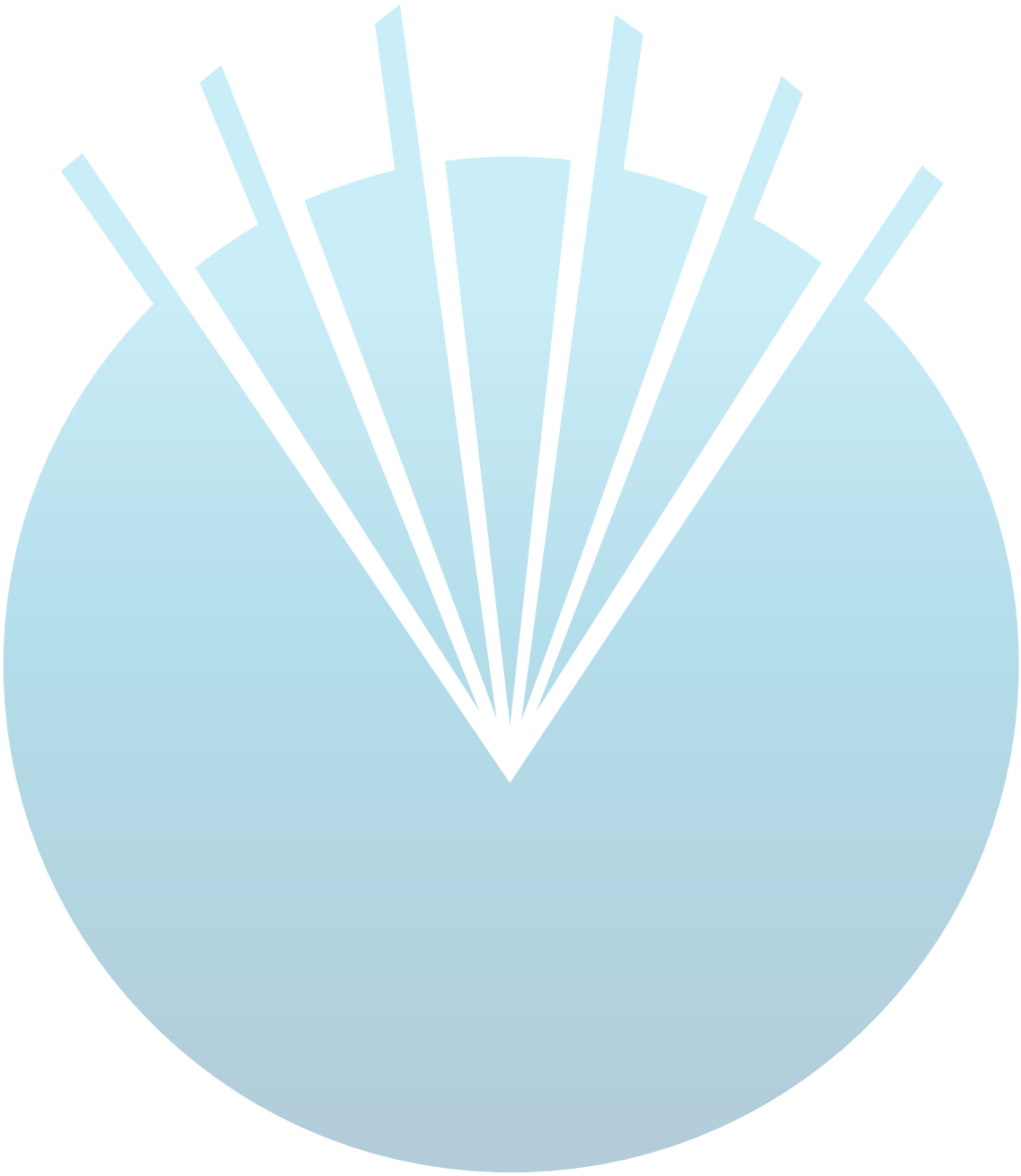

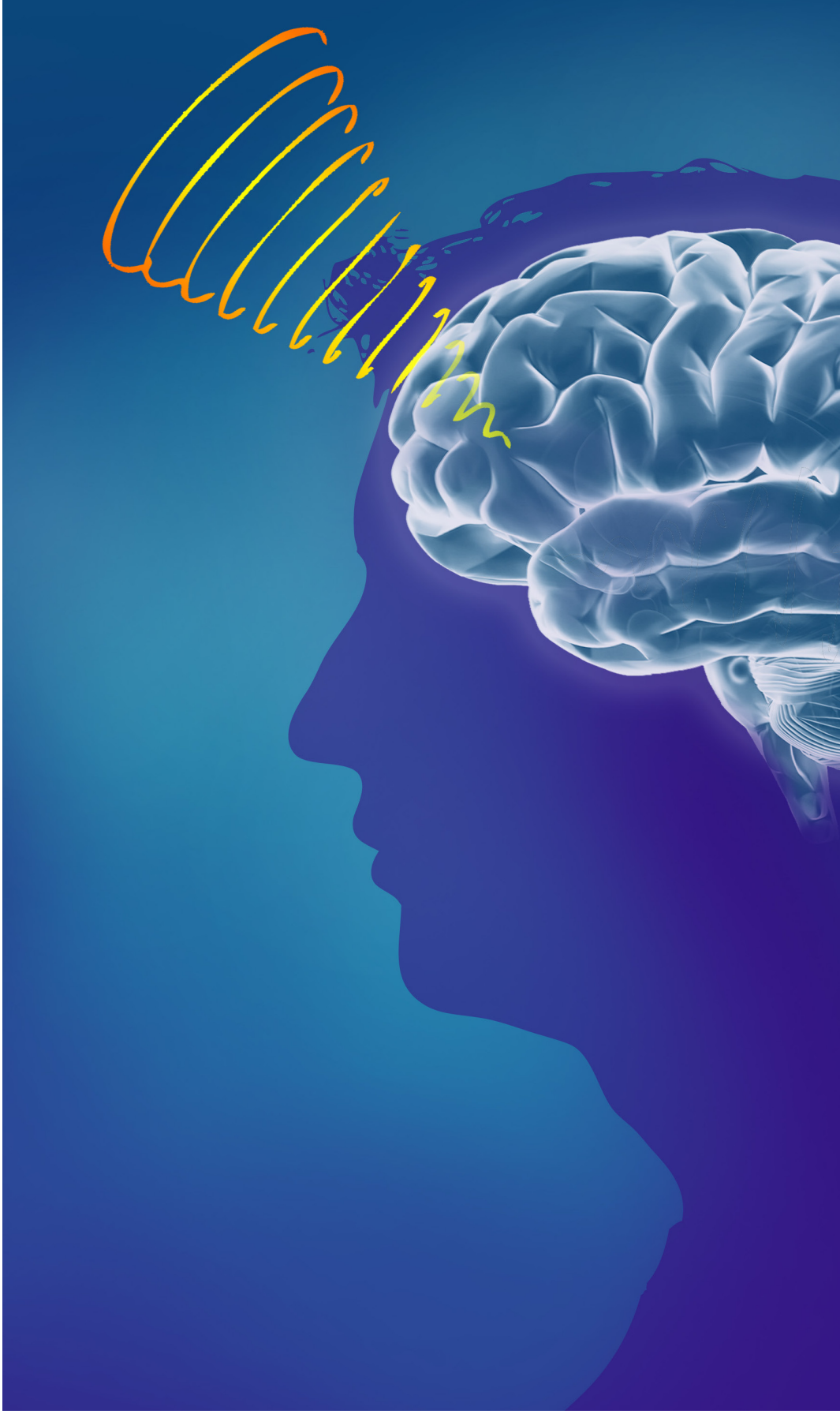

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