# Focused Ultrasound for Alzheimer's Workshop

**September 17-18, 2015**
**Bethesda, MD**

**WORKSHOP SUMMARY**

## INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease with pathologic features including neurofibrillary tangles and amyloid plaques. AD is the most common cause of dementia, with over 5 million people affected in the US.[1] The prevalence of AD is predicted to increase to an estimated 13.2 million in 2050. There is a large disease burden cost associated with AD, including not only cost for medical care, but also indirect costs to caregivers. AD is a progressive condition leading to disability and death.[1] Despite decades of progress in defining the pathways responsible for AD, there are few FDA-approved treatments for AD, and the last approval (memantine) occurred in 2003. There are two different pathologies: amyloid-β (Aβ) plaques and tau-containing neurofibrillary tangles (NFT). Because in familial cases of AD pathogenic mutations have been found in genes linked to Aβ formation, the amyloid cascade hypothesis has been proposed in which Aβ has been placed upstream of tau in a patho-cascade. However, there is a role for both tau and Aβ in AD pathogenesis. Amyloid-mediated toxicity is a popular target for disease intervention, and the primary focus of previous research. However, this approach has off-target effects. Weaknesses of the amyloid hypothesis include the fact that a better correlation exists between tau burden and AD onset and severity compared to Aβ burden and AD onset and severity, and that transgenic AD mouse models do not model the entire spectrum of the human AD pathology.

Focused Ultrasound (FUS) is an early stage, revolutionary, noninvasive therapeutic technology. FUS is a possible alternative or adjunct to surgery, radiation therapy, drug delivery, and immunotherapy. It has the potential to improve quality of life and longevity and decrease healthcare-related costs. FUS uses ultrasound energy to treat tissue deep in the body accurately, precisely, and noninvasively. Using imaging techniques (magnetic resonance or ultrasound) the target tissue is identified, guided and controlled treatment occurs in real-time, and the effectiveness of the procedure is confirmed. The main interest of FUS for the treatment of AD is the potential for blood brain barrier (BBB) opening and focal drug delivery. The application of FUS for treatment of AD is currently in the preclinical development stage. FUS is

currently FDA-approved for the following indications: pain from bone metastases, uterine fibroids, and ablation of prostate tissue, although there are many more indications approved outside the US.

On September 17-18, 2015, the Focused Ultrasound Foundation (FUS Foundation), held a workshop to define the role of focused ultrasound in the treatment of AD. Following research presentations and a discussion on the clinical perspective, workshop participants discussed the state of the field, current challenges, and future research directions to move the field forward. The workshop was also intended to foster collaboration by bringing together a multidisciplinary group of thought leaders from fields including focused ultrasound, leading Alzheimer's neurosurgeons, neurologists, neuroscientists, neuroradiologists, and representatives from the FDA, medical research foundations, and industry.

**BACKGROUND**

AD has specific pathologic changes that are targets for disease-modifying therapy: β-amyloid plaques and neurofibrillary tangles. Components of β-amyloid plaques include a core of highly condensed β-amyloid surrounded by a halo of diffuse amyloid, degenerating neurites in the halo, reactive astrocytes, and activated microglia. The major component of NFTs is Tau, a microtubule-associated protein. Tau is hyper-phosphorylated in NFTs. Protein kinases that phosphorylate tau are elevated in AD, and phosphatases that dephosphorylated tau are reduced in their levels and activity. Aberrant tau has a tendency to aggregate, cause synaptic dysfunction, and increase tau pathology in the brain.

The first human clinical trials to explore the safety and efficacy of anti-amyloid therapy was an anti-amyloid vaccine (AN1792), an active immunization consisting of an amyloid peptide with adjuvant. During a phase II clinical trial, several patients developed evidence of brain inflammation (autoimmune encephalitis), a likely side effect with immunization against a brain peptide. At autopsy, these patients had reduced amyloid burden, but NFT pathology remained. Treated AD patients showed some evidence of cognitive improvement and stabilization of patients with mild disease also occurred. Later development work on anti-amyloid vaccines attempted to maximize benefit and minimize risk. One strategy included passive immunization (humanized monoclonal anti-amyloid antibody), which has shown little benefit in large studies (bapineuzumab and solaneuzumab) although there may be a benefit in sub-groups. Another strategy is active vaccination to restricted regions of amyloid peptide. Transgenic mouse models (Tg-SwDI) show a greater clearance of amyloid when antibodies were intracerebrally injected directly into the hippocampus compared with systemic injection.[2]

There are several FDA-approved treatments for AD-related symptoms. However there are no successful disease-modifying therapies for AD, despite a variety of successful approaches in preclinical models. One hypothesis for failed translation is that potentially effective therapies were administered too late in the disease course. There is also a lack of useful biomarkers for AD that would allow pre-symptomatic diagnosis (allowing earlier treatment) and measurement of clinical trial endpoints. A variety of imaging biomarkers are available including amyloid binding ligands, NFT binding ligands, and volumetric imaging of the hippocampus. However, it is unknown how these imaging biomarkers correlate with clinical endpoints.

There are many potential disease-modifying therapies for AD currently under study that do not cross the BBB. These include:

- Both anti-Aβ and anti-Tau antibodies
- Stem cells (mesenchymal, neuronal progenitors, and umbilical cord blood cells)
- Gene therapy
- APP/amyloid cleaving enzymes (neprilysin and insulin degrading enzyme)
- Growth factors

Minor abnormalities of the BBB in AD are well described, but of unclear significance. [Since this workshop Ryan Watts and colleagues published a paper in Neuron that suggests a lack of widespread BBB disruption in AD[3]]. Amyloid angiopathy is commonly associated with AD and is a major cause of intracerebral hemorrhage. MRI can identify micro-hemorrhages, which were observed as a result of anti-amyloid vaccination along with edema.

***Focused Ultrasound for the Treatment of Alzheimer's Disease***

Several brief presentations by workshop participants provided an overview of the current state of FUS AD research, and the potential to use FUS alone or in combination with other disease-modifying treatments for AD.

Kullervo Hynynen at Sunnybrook Health Sciences Centre described FUS-induced BBB opening, safety, and efficacy in a mouse model of AD (TgCRND8). FUS-induced BBB opening has been demonstrated in a variety of preclinical models.[4] In short, microbubbles are injected into the bloodstream, focused ultrasound is applied, and the pressure difference causes the bubbles to oscillate, stretching the endothelial cells apart and opening the BBB. A procedure using the lowest possible power levels that results in BBB opening without damage to the surrounding neurons has been developed. A hydrophone (receiver) is used to detect acoustic emissions generated during microbubble-mediated FUS disruption of the BBB, which can be used to control acoustic pressures in real-time and serve as a feedback control algorithm to safely modulate pressure during treatment.[5]

The Hynynen and Aubert groups have shown that FUS can deliver therapeutic agents and endogenous therapeutic molecules to the brain leading to plaque reduction and improved cognition.[6-8] For example, FUS alone or combined with an anti-Aβ antibody, BAM-10, reduced the number of plaques within 4 days after treatment.[6,7] Multiple treatments of FUS (once per week for three weeks) could improve behavior (memory task, Y-maze) and increase neuronal plasticity in older AD mice.[8] This study demonstrated an increase in the number of immature neurons in the hippocampus, and FUS also increased dendrite length and arborization of these developing neurons. In a rat study, GFP-labeled stem cells were capable of maintaining a neuronal lineage when delivered to FUS-targeted brain regions (hippocampus and striatum).[9] Human patients with AD have compromised vasculature, but mouse models of AD did not show any differences in the probability of BBB opening compared to healthy controls.

Jürgen Götz and Gerhard Leinenga at the University of Queensland have also shown that FUS treatment reduces plaque burden in a mouse model of AD (APP23).[10] This study showed extensive internalization of Aβ into the lysosomes of activated microglia in FUS-treated animals, with no concomitant increase observed in the number of microglia suggesting that endogenous microglia may have been activated to phagocytose the plaques. Behavioral tasks (Y-maze, active place avoidance, and novel object recognition) were also improved with FUS treatment. Future planned studies include larger animals (sheep), mechanistic studies in mice (role of microglia), and therapeutic studies in mice. Götz's group has also developed a tau antibody currently being investigated as a therapeutic treatment in combination with FUS. Early data suggest that FUS facilitates the uptake of the single-chain antibody into the interstitial space and into neurons.

Elisa Konofagou's group at Columbia University has also studied FUS for the opening of the BBB for drug delivery in both AD and Parkinson's disease. In a mouse model FUS-induced BBB opening was produced with a frequency of 1.525 MHz, 20 ms pulse, pulse repetition frequency of 10 Hz, with BBB opening threshold occurring around 0.3 MPA.[11] They have also found that the FUS-induced BBB opening was dependent on both microbubble size distribution within the injected volume and the specific targeted brain region.[12] Larger microbubbles (4-5 µM, 0.3 mPA) had greater permeability. Direct targeting of neurons with brain derived neurotrophic factor (BDNF) and FUS was investigated in an AD mouse model (tau), resulting in new neurons and dendrites in the hippocampus. In a Parkinson's disease model (1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP)), viral vectors (glia derived neurotrophic factor (GDNF)) in combination with FUS prior to MPTP treatment resulted in neuroprotection in the substantia nigra. When FUS plus viral vectors (neuturin) was applied after MPTP treatment, the combination resulted in neurorestoration. The group also looked at FUS in combination with either intranasal delivery or IV delivery of a model drug (40-kDa fluorescently labeled dextran)

and observed no differences in delivery efficiency. Konofagou has also shown that FUS can be combined with MRI real-time monitoring for BBB opening in larger animals (nonhuman primates).[13] Gray matter is an easier target for BBB opening in nonhuman primates compared with white matter. In nonhuman primates, pressure up to 450 kPa has been shown to be safe. Preliminary studies of repeated BBB opening in nonhuman primates has also been shown to be safe.[14]

### *FUS Strategies for Treating Brain Diseases: Lessons Learned*

Workshop participants also briefly presented on the use of FUS-induced BBB opening to enhance drug delivery in brain tumors and the potential for delivery of nanoparticles to the brain.

Nathan McDannold at Brigham & Women's Hospital, Harvard Medical School, provided an introduction to the use of FUS-induced BBB opening to enhance drug delivery in brain tumors. FUS enables therapeutic levels of chemotherapy, including large particles (antibodies and liposomes), to cross the BBB in rodent brain tumor models. FUS enhanced delivery of liposomal doxorubicin in a rodent glioma tumor model.[15] Adverse events were consistent with known effects of doxorubicin such as skin toxicity and a risk of hemorrhagic tumor. FUS plus Herceptin enhanced delivery of the drug in a mouse model of breast cancer brain metastases (HER2/neu-positive human breast cancer cells (BT474)).[16] Therapeutic effects of FUS plus chemotherapy are variable, depending on tumor stage, and other factors. A safety study of functional cognitive testing in nonhuman primates (rhesus macaques) demonstrated no behavioral deficits, visual deficits, or loss in visual acuity.[17] However, 6 weekly sessions (0.73 mPa) of FUS in rodents resulted in tissue damage and scar tissue in rodents, indicating a narrow window for safe exposure. Overall, safety studies show little or no histological or functional changes. The studies described here indicate the safety of FUS for glioblastoma patients; similar studies in preclinical models are needed to answer safety questions in AD.

Richard Price, University of Virginia, discussed the potential to deliver nanoparticles to the brain via MRI-guided FUS. Due to the electrical charge and tight junctions in the brain, it is difficult to get large or electrically charged nanoparticles across the BBB. Brain penetrating nanoparticles (BPN) densely coated with low molecular weight polyethylene glycol (PEG) used in combination with FUS were able to cross the BBB and enter targeted brain regions and has led to further investigations of non-viral delivery of genes via a BPN PEG-polyethylenimine (PEI) nanovector.[18,19] Toxicity testing (glial fibrillary acidic protein (GFAP) staining for reactive gliosis) did not reveal any signs of toxicity 1 week after treatment. Approximately 45% of cells (both neurons and glia) were transfected with the PEI DNA-BPN. These PEI DNA-BPNs plus FUS have been used to deliver neurotrophic factors in a rodent model of Parkinson's disease, resulting in

increased delivery of GDNF in the 6-hydroxydopamine (6-OHDA) rat model (treated 2 weeks after 6-OHDA injection into the striatum). GDNF delivery inhibited neurodegeneration (striatum and pars compacta portion of the substantia nigra) and improved behavioral assessments (apomorphine rotation test).

**Overall Discussion and Evidence Gaps**

*Preclinical Models of Focused Ultrasound and Alzheimer's Disease*

- There was a discussion of potential mechanisms that underlie the effects of FUS on AD in preclinical models, particularly the transgenic mouse models.
- Defining the mechanisms of Aβ plaque removal in FUS also needs to be detailed.
- Concern over hypertension was raised; this has not been investigated in nonhuman primate models. There was a comment that, at least for clinical trials, blood pressure would be controlled during the procedure and that hypertensive patients would likely be excluded.
- There was some discussion over whether any research has looked at the safety of microbubbles alone without FUS. Preclinical research indicates that there is no effect on plaque load alone with microbubbles.
- There was a discussion regarding the use of commercial microbubbles. There are two commonly used commercial options for microbubbles that are FDA-approved for cardiac diagnostic imaging, Optison and Definity. These microbubbles contain a gas core stabilized by a shell comprised of proteins, lipids, or polymers. Each type of microbubble has unique advantages tailored for specialized functions. Larger microbubbles (4-5 microns) are easier to work with because they allow BBB opening at lower pressure. The FDA-approved microbubbles are commonly used in preclinical research by Toronto and Boston groups but not by several others due to cost concerns.

***Pilot Clinical Trial: Overview of Protocol and Discussion***

Nir Lipsman, Sunnybrook Health Sciences Centre, gave an overview of the proposed protocol for a phase I study of safety and feasibility of MR-guided FUS for the treatment of AD. The protocol was designed by the FUS Foundation Alzheimer's Steering Committee. Inclusion and exclusion criteria are similar to other FUS clinical trials. The proposed study will be a prospective single-arm non-randomized feasibility phase I trial, with two phases. First, demonstrate (N=6-10) safety of small volume BBB opening in a pre-selected brain target and then demonstrate reproducibility of acute BBB opening, safety of larger volume BBB opening, and efficacy signal in the same group of patients. The objectives will be to evaluate the safety of BBB opening using transcranial MR-guided FUS in conjunction with an IV ultrasound contrast

agent in patients with mild AD. The entire study will last 90 days. The committee aims to finalize the protocol in the next 1 to 2 months, and enroll patients in early 2016. Current topics that are being debated are the inclusion/exclusion criteria, staging, and outcomes.
There was a discussion on the proposed design of the clinical trial.

*Targeting*

- There was extensive discussion over which brain region should be targeted by a clinical trial. The consensus of the group was to target cortex (gray matter) in the right frontal lobe. Reasoning for this was due to the presence of Aβ in the frontal cortex, preclinical safety data, the relative safety of superficial targeting, and the ability to titrate to higher volumes while maintaining safety. The same brain target will be used in both phases of the study.
- There were suggestions for future studies (post phase 1) to target memory related structures, such as the hippocampus. Although there was also some concern that AD patients may have a high level of progressive atrophy in the hippocampus, which would make FUS treatment difficult in this brain target.

*Volume*

- Positron emission tomography (PET) imaging for Aβ will be used to choose brain regions of interest for future clinical trials.
- The first stage in this study will involve sonication of a roughly 1 $cm^3$ region in the cortex of the right frontal lobe.
- There was a suggestion that the second stage should sonicate a larger volume (approximately 3 $cm^3$) similar in size to the hippocampus, in order to establish the safety of a larger volume BBB opening.

*Inclusion/Exclusion Criteria*

- Patient recruitment is anticipated to happen quickly, and there has been some early interest from patients. However, there was some concern that the stringency of exclusion criteria will affect patient recruitment. Lipsman explained that the exclusion criteria were created from surgical criteria to insure a population in adequate health to undergo the procedure.
- Major discussion points included raising mini mental state examination (MMSE) upper limit to 28 (in the context of a positive amyloid PET study) and removing the requirement for 3/3 genotype.

*Peripheral Amyloid-β Markers*

- Concern over CSF collection (would require lumbar puncture) was expressed. CSF is an indicator of global Aβ, and FUS may not influence total Aβ. The group suggested that

CSF not be collected as part of the phase I study, but included in future studies. It was also suggested that plasma/serum Aβ could be measured instead.

*Monitoring*

- The optimal monitoring approach was discussed. It is not feasible to do MRI monitoring on every patient outside of the clinical trial situation.
- BBB opening could be monitored through gadolinium enhancement immediately following sonication with microbubble contrast injection and acoustic monitoring through the detection of subharmonic frequencies, detected by sensors within the FUS system.

*Study Objectives*

- There was discussion around the fact that the primary objective of this study is safety. Safety will be assessed in two ways:
    - Clinically through the presence of device-related adverse events, both prior to and following individual sonications as well as short and long-term follow up
    - Radiographically through the presence of bleeding, swelling, etc. both following individual sonications and in short and long-term follow up

*Additional Comments*

- There was discussion over whether Tau imaging was possible. The consensus was that it is possible, but not practical at this early stage.
- There were comments made by representatives from the US FDA indicating that in the US, this treatment will likely be considered a combination product - the FUS device in combination with microbubbles. Stopping criteria for intensity should be well defined to avoid inertial cavitation (to enhance safety). Targeting accuracy will also need to be well defined.
- Future trials will include serial treatment (i.e. weekly, monthly) parameters alone or in combination with therapeutic agents. Although participants cautioned that the risk of hemorrhage is additive with each new technique.

*Preclinical Research Needs to Support Clinical Trials*

There was discussion on whether there was sufficient preclinical research to support future clinical trials. There was consensus that more preclinical work is needed to determine long-term effects of BBB with FUS. There is a sense of urgency surrounding AD treatments, as it is a severe disease with global impact. Current preclinical data suggests that FUS is safe. The following is a list of remaining preclinical questions

- Comparison data for the addition of therapeutic agents versus FUS plus microbubbles alone is needed.
- The question of whether serial treatments (weekly, monthly) are more effective, and the optimal parameters for efficacy would be informative.
- The question of optimal timing of FUS treatment during the disease course would also be informative for designing human clinical trials.
- Preclinical behavioral studies are needed to look at whether FUS targeting of the hippocampus is better than widespread treatment.
- Additional work on FUS should also be done in aged AD mice.
- Comparison data to identify potential therapeutics likely to have efficacy when combined with FUS is needed.
    - Isabelle Aubert, Sunnybrook Research Institute, mentioned that that the addition of a BAM-10 antibody or intravenous immunoglobulin (IVIG) improved Aβ plaque load reduction, but the two agents were not compared in the same study. Aubert also welcomed suggestions for additional therapeutic agents to test in mouse models.

**Role for FUS and Future Directions**

The consensus of the discussion suggests three distinct tracks for future research:

- Future clinical trials in humans will likely include FUS plus microbubbles with a brain target in the hippocampus
- Preclinical research that can inform the optimal design of future clinical trials
- Preclinical research is needed to understand the mechanisms responsible for transient BBB opening and Aβ clearance after FUS treatment

Historically, the breakdown of the BBB has been a sign of disease with negative consequences. FUS-mediated BBB opening and subsequent closing is a highly controlled and reversible process, it will be important to communicate this to non-experts going forward. One suggestion was to discuss failed studies of BBB opening with mannitol, and contrast this with the safety and reversibility of FUS. The size of the BBB opening can be controlled through FUS parameters: pressure, number of pulses, and duration.

There was a discussion on the mechanism responsible for Aβ clearance after FUS, whether it was due to microglial activation or infiltrating monocytes. Overall immune system changes after FUS plus microbubbles should be determined, and after each of those treatments alone. The immune response after FUS should be characterized both by measuring cytokine responses in blood and brain. Some of these experiments could likely be investigated using existing tissue. Cynthia Lemere, Brigham and Women's Hospital, proposed the use of a mouse model

developed by Richard Ransohoff in which monocytes are labeled red and microglia are green. Lemere suggested that a longitudinal study of microglial activation at multiple time points after FUS treatment should be performed. If Aβ is cleared through transport across the BBB, it might be detectable in the blood. Götz's group looked at blood samples at a single time point after FUS treatment in mouse models, and found that Aβ levels were very low (lower than the levels of detection for the ELISA being used). Future work should look at this at multiple time points after FUS. Aubert's group has also started looking into the immune response with a transgenic AD mouse model. There was a suggestion that long-term studies of Aβ clearance would also be useful. Lemere proposed an experiment with 16-month old mice, to look at the effects of FUS on immune system activation and memory.

There was a general discussion on the role of Aβ in the pathogenesis of AD. Twenty years of research on Aβ has led to treatments that remove some Aβ from the brain, but with regards to cognition, the studies are so far not conclusive. There may be additional factors that could be measured in AD patients that directly correlate to improvements in the pathology of the disease. Some of the previous clinical trials were done in patients with moderate to severe AD, and that removing Aβ in patients with progressive disease is unlikely to affect cognition. More recent trials have shown cognitive improvements in patients with mild AD. Selecting the right population of patients will be key to the success of FUS trials. FUS treatment is unlikely to improve AD in patients with moderate to severe disease.

Another issue of interest is whether there is a dose-response relationship with the size of BBB opening. Specifically, whether one large opening could work as effectively as multiple small openings.

The difference in AD plaque pathology between mice and humans was raised, and how these differences affect translation from the lab to the clinic. Mouse models used in preclinical research should model human disease as closely as possible. Another issue is that AD develops over a very long period of time in humans, which is accelerated in mouse models with a shorter lifespan. There may be no clinical significance between different pathologies, but this work has not yet been performed.

## OUTCOMES AND NEXT STEPS

One participant mentioned that Congress is discussing a substantial increase in funds for AD research in in NIH's appropriation, and this could be a potential resource for FUS research for the treatment of AD over the next few years.

There is a lot of work to be done in the next year, but there is a great potential to use FUS in combination with therapeutic agents for the treatment of AD and other indications through direct delivery across the BBB. It is also vital to present this work to researchers in the AD field,

as the awareness in the field is very small at this time. Specific recommendations to increase awareness were at the Alzheimer's Association International Conference (AAIC) and through ISTAART. The FUS Foundation will continue engagement with this community to move the research forward.

**ABBREVIATIONS**

| | |
|---|---|
| AD | Alzheimer's disease |
| APP | Amyloid precursor protein |
| BBB | Blood brain barrier |
| BPN | Brain penetrating nanoparticles |
| BDNF | Brain derived neurotrophic factor |
| CSF | Cerebral spinal fluid |
| ELISA | Enzyme-linked immunosorbent assay |
| FDA | U.S. Food and Drug Administration |
| FUS | Focused ultrasound |
| GDNF | Glial cell derived neurotropic factor |
| GFAP | Glial fibrillary acidic protein |
| IVIG | Intravenous immunoglobulin |
| MAP | Microtubule-associated protein |
| MMSE | Mini mental state examination |
| MPTP | 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine |
| NFT | Neurofibrillary tangles |
| NIH | National Institutes of Health |
| PEG | Polyethylene glycol |
| PEI | Polyethylenimine |
| PET | Positron emission tomography |

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