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Executive Summary

Focused ultrasound (FUS) is an early-stage, disruptive, non-invasive therapeutic technology with the potential to improve the lives of millions of patients with a variety of medical disorders by providing an alternative or complement to existing techniques. The precise delivery of ultrasound energy deep in the body can produce more than 18 different biological effects in the tissue, including the ability to temporarily open the blood-brain barrier (BBB).

The use of therapeutic ultrasound for the non-invasive treatment of neurological disorders has a significant history. To date, more than 1,000 patients with various neurological disorders have been treated with non-invasive magnetic resonance-guided FUS via thermal ablation of precise targets deep in the brain. Previous and ongoing clinical studies have used FUS for the precise ablation of dysfunctional brain regions to treat essential tremor, Parkinson’s disease, dystonia, obsessive-compulsive disorder, depression, and brain tumors.

Robust preclinical work is ongoing at several sites throughout the world using FUS in conjunction with microbubbles to open the BBB and deliver large molecules including drugs, DNA-loaded nanoparticles, viral vectors, and antibodies, in models of Alzheimer’s, Parkinson’s, and glioma. Clinical studies are assessing the safety and feasibility of FUS to open the BBB in patients with gliomas and Alzheimer’s disease.

Safe, reliable, temporary, and repetitive opening of the BBB remains an unmet critical medical need for the delivery of therapeutics to the brain and the treatment of a range of neurological disorders. The current methods of getting through this barrier—either via direct brain injections or using mannitol—are far from ideal, and FUS may offer a safer, more controllable and more effective option.

On November 16–17, 2017, the Focused Ultrasound Foundation held a workshop to determine the best path forward to advance ultrasound-mediated BBB opening, either alone or combined with drug delivery approaches (e.g., antibodies, viral vectors, or nanoparticles) for targeted treatment of a variety of neurological disorders. The Foundation convened a multidisciplinary group of thought leaders including ultrasound experts, neuroscientists, neurologists, neuroradiologists, and representatives from US Food and Drug Administration (FDA), National Institutes of Health (NIH), and industry.

The primary objectives of the workshop were to:

- Develop preclinical and clinical roadmaps for treatment of neurological diseases using BBB opening
- Prioritize, streamline, and organize collaborations for future work
- Identify technology and/or knowledge gaps

Participants engaged in lively discussion around the state of the field, the status of critical questions about the safety and efficacy of the technique, standardization of treatment parameters and outcome measures (and reporting these in publications), the most important next steps, and key roles for the Focused Ultrasound Foundation.
Important activities identified that could significantly move the field forward included:

- Develop a streamlined FDA pathway for trials to assess FUS for BBB opening (may need different pathway(s) for different devices/diseases/drugs).
- Organize Foundation-led effort, working with the research community, to propose methods for standardization of ultrasound-mediated BBB opening procedures (FUS treatment parameters, microbubble type/dosing, acoustic/MRI monitoring protocol, etc.) and reporting across device platforms.
- Assess the scientific data available on microbubbles and ultrasound (dosing, differences between microbubble types, safety data, etc.) to enable optimization of microbubble protocol.
- Identify other biomarkers (e.g., exosomes) that could correlate with BBB opening and enable simple blood testing for indication of success.

Participants were encouraged to reach out to the Foundation with any research ideas or project proposals to address the key issues and questions. The Foundation will continue engagement with this community to advance FUS-mediated BBB opening toward clinical adoption.
Workshop Participants

*Please note that the FDA was in attendance only and did not contribute to the views in this publication.*

Raag Airan, MD, PhD  
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Burning Questions

1. Does ultrasound increase the concentration of drug in the healthy brain? The diseased brain? Are there restrictions on what can be delivered? (size, charge, etc.)
   Yes. Objects as large as cells can be delivered across the BBB with FUS. (preclinical and clinical evidence)

2. Can ultrasound safely be used to repeatedly open the BBB in patients?
   Yes. (clinical evidence)

3. Can ultrasound treat a large volume in a reasonable period of time? What is the maximum volume that needs to be treated?
   Improvement needed. A 1-2 cm ring around the enhancing tumor border will likely be needed to prevent recurrence. Volume for effective treatment of Alzheimer’s and epilepsy needs further investigation.

4. Is real time acoustic and visual monitoring of ultrasound-mediated BBB opening possible?
   Yes, monitoring is possible. (cavitation/perfusion based)

5. What is the endpoint for defining a successful treatment? (perfusion, cavitation monitoring, drug delivery)
   Consensus needed.

6. Is it logistically feasible to use ultrasound to repeatedly open the BBB in patients? (equipment and clinician time) What is needed to reduce total treatment time?
   Yes. Systems that do not rely on MR guidance greatly improve the feasibility of repeated treatments.

7. What are the key parameters we can use to measure or describe BBB opening that will permit translation between systems/devices? (peak negative pressure, microbubble dose, etc.)
   Consensus needed.

8. What evidence is needed to achieve regulatory approval? What evidence is needed to achieve third-party insurance reimbursement? What is the maximum cost per treatment that the market will support?
   Questions remain. Approval for microbubble use in conjunction with the device is a key issue.
9. Do demographics (age, sex, race/ethnicity, co-morbidities, etc.) affect inter-patient variabilities (skull geometry & thickness, vasculature, etc.)?

Yes. Additional clinical data are needed.

10. What is the optimal disease target(s) for treatment with ultrasound for BBB opening? In which disorders is it most likely to provide unique value? Which diseases should we treat now?

Glioblastoma, Alzheimer’s, Parkinson’s, Epilepsy. Good disease candidates for ultrasound for BBB opening are those with few/poor treatment options, well-identified targets within the brain, and potential therapeutics that do not cross the intact BBB.

11. Disease specific questions: What is the optimal drug(s)? What is the optimal dosing schedule? What is the optimal number and frequency of US treatments? What is the clinical endpoint?

More preclinical/clinical data are needed.
Workshop Presentations

Several presentations provided an overview of FUS for BBB opening, the state of the field for its use in preclinical and clinical studies, and key topics on the safety of the technique.

Overview of FUS for BBB Opening

Kullervo Hynynen, Sunnybrook Research Institute, gave an overview on the use of FUS for BBB opening. The first observations of FUS-induced increase to BBB permeability were observed around FUS-induced lesions.1

Over time, multiple experiments were carried out to develop a technique using microbubbles that could open the BBB without damage to the surrounding tissue.2 Microbubbles in the capillaries expand and contract within the ultrasound pressure field. The expansion and contraction forces open the tight junctions in the walls of the capillaries, enabling temporarily increased flow between the bloodstream and the brain.

Permeability-glycoprotein (Pgp) expression is transiently downregulated by FUS exposure, which might slow down drug efflux after delivery to the brain.3 Repeated BBB opening in non-human primates demonstrated the feasibility of BBB opening for clinical applications.4

Exposure monitoring and control can be achieved through monitoring of acoustic emissions.5,6 Transducer arrays can be used to develop accurate targeting of discrete brain regions.7

BBB opening for therapeutic treatment has been extensively studied in preclinical models. For example, trastuzumab can be locally delivered to the mouse brain using an MRI-guided FUS (MRgFUS) technique.8 MRgFUS can also be used for targeted delivery of nanoparticles (containing therapeutic agents) to the brain.9

After demonstrating safety and efficacy in preclinical models, FUS-induced BBB opening is now under investigation in clinical trials. One trial is investigating the use of MRgFUS for BBB opening to deliver chemotherapy to brain tumors. Another clinical trial is investigating low intensity pulsed ultrasound, via an implantable ultrasound device, to deliver chemotherapy to brain tumors.10

In addition to chemotherapy delivery, ultrasound enhanced the delivery of antibodies in mouse models of Alzheimer’s disease (AD) and ultrasound effects by themselves were found to reduce pathology, promote neural plasticity and improve cognition.11-14 Based on these preclinical findings, FUS entered clinical trials for the treatment of AD patients (NCT02986932, NCT03119961) and is being investigated for the delivery of a range of therapeutic agents.
### BBB Opening

**Approaches and Mechanisms**

Several presenters discussed the current status of preclinical research on FUS-mediated BBB opening, including several disease models and delivery of a range of therapeutics.

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**Elisa Konofagou, Columbia University**, discussed neurorestoration of the nigrostriatal pathway via FUS-facilitated drug delivery to the brain in a mouse model of Parkinson’s. Konofagou’s research has focused on controlling and predicting the size of BBB opening based on both the microbubble diameter and the applied pressure.

Neurturin (NTN) administration after FUS-induced BBB opening restored degenerated neurons in the substantia nigra and caudate putamen. FUS was also used for gene delivery (adeno-associated virus (AAV) vector) to a targeted brain region in a mouse model of Parkinson’s.

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**Nathan McDannold, Brigham & Women’s Hospital**, discussed closed-loop control of FUS drug delivery.

The ‘magnitude’ of BBB opening is related to the amount of drug delivery, the size of the drug delivered, the penetration depth, the duration of opening, and the duration of drug retention. These factors depend on acoustic parameters such as pressure, frequency, burst length, duration, and microbubble dose.

After establishing the factors that affect drug delivery, they were able to reliably deliver a pre-determined amount of liposomal doxorubicin into the brain, which caused tumor regression and improved survival in a mouse glioma model.

Passively-recorded acoustic emissions can be used to control FUS-induced BBB opening. McDannold’s group is developing a 128-element passive acoustic monitoring array for Insightec’s ExAblate system.

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**Richard Price, University of Virginia**, discussed BBB opening with FUS for nanoparticle delivery.

Using nanoparticles loaded with therapeutics in combination with FUS-induced BBB opening has several advantages for delivery including versatility, tunable controlled-release properties, high payload capacity, reduced need for multiple treatments, and enhanced tissue penetration. Brain-penetrating nanoparticles (BPN) (60 nm) cross the BBB when combined with FUS.

Recent experiments have demonstrated that this MRgFUS nanoparticle delivery approach enhances tumor delivery of cisplatin and inhibited tumor growth and invasion in a rat model of F98 glioma.
Price has also investigated delivery of neurotrophic growth factor (GDNF) in a rat model of Parkinson’s disease (PD) that restored the dopaminergic neurons in the striatum and improved motor function.\(^{21}\)

Additional work on the potential for FUS-mediated immunoregulation for cancer treatment demonstrated that BBB opening around the tumor in a mouse model of glioma may cause enhanced therapeutic immunologic responses.\(^{22}\)

Gerhard Leinenga, University of Queensland discussed FUS as a treatment modality for AD and other proteinopathies.

Repeated scanning ultrasound reduced amyloid β (Aβ) protein and improved memory in a mouse model of AD (APP23).\(^{23}\) Preliminary data suggest that ultrasound in very old APP23 mice (2 years) demonstrates safety without increasing the spontaneous microhemorrhage risk. Repeated FUS treatments did not show any detrimental effects (neuronal morphology) in wild-type mice.\(^{24}\)

FUS-mediated delivery of antibody fragments (scFvs) to target tau protein reduced phosphorylated tau levels in the amygdala.\(^{25}\) Antibody treatment also reduced anxiety-like behavior in pR5 tau transgenic mice.\(^{26}\)

A large animal model (sheep) is under development to establish BBB opening with low frequency ultrasound (286 kHz) in combination with Definity® microbubbles.

Isabelle Aubert, Sunnybrook Research Institute, discussed the critical parameters for using FUS as a therapeutic treatment for AD (microbubbles, imaging/monitoring, and endpoints).

In a transgenic mouse, (TgCRND8), FUS increased the delivery of antibodies, injected intravenously at clinically relevant doses, by approximately four-fold in several brain regions. Small molecules, which do not cross the BBB when injected intravenously, were also successfully delivered to specific brain regions using FUS. These therapeutics were effective at reducing amyloid pathology and stimulating plasticity.

Further work to characterize the effect of FUS alone is ongoing.

Aubert cautioned that research will need to consider potential effects in peripheral organs and in the peripheral nervous system because all therapeutic agents to be used in combination with FUS are currently being administered via the bloodstream.

Discussion from Presentations

- There was some discussion on whether the combined FUS and viral vector administration affected peripheral nerves. Due to the synapsin promotor on the viral vector, there was no evidence of gene expression to peripheral organs.
- Future studies need to assess whether neurodegeneration can be dampened following effective gene delivery.
Clinical Experience

Speakers discussed the current state of clinical trials for ultrasound-mediated BBB opening.

Nir Lipsman, Sunnybrook Research Institute, discussed ongoing clinical trials with FUS for BBB opening at Sunnybrook Health Sciences Centre in Toronto, Canada. BBB opening is performed with the ExAblate Neuro system from Insightec.

An early phase study to assess the safety and feasibility of FUS-mediated BBB opening to deliver chemotherapy in patients with high-grade glioma is ongoing. Recruitment challenges resulted in protocol changes; patients were allowed prior chemotherapy to increase eligible patient numbers.

Eligible patients were surgical candidates, and FUS is used in combination with temozolomide prior to tumor resection. Five patients have been treated to date. The infiltrative edge of the tumor was sonicated, and post sonication MR imaging demonstrated successful BBB opening.

Lipsman also discussed an ongoing phase I safety and feasibility trial in patients with AD. Patients must have Aβ deposition in the non-dominant frontal lobe. Eligible patients had a mini-mental state examination (MMSE) score between 18 and 28, and were in the early stages of the disease.

Patients received two FUS treatments, one month apart, in the non-dominant frontal lobe. MRI was performed one day after FUS sonication, and positron emission tomography (PET) scan was performed 7 days following sonication.

Six patients have been treated. The average age was 67, average length of disease was 3 years, and the average MMSE was 22.

Early assessments suggest that the FUS treatments were safe, and all patients went home after an overnight hospitalization. Repeated treatments demonstrated feasibility and reproducibility of FUS-mediated BBB opening.

The patient recruitment process is challenging, and future trials will depend on collaboration with local regulatory authorities.

Michael Canney, CarThera, described the early clinical trial for the CarThera SonoCloud® device.

The SonoCloud system consists of an implant designed to fit in a skull burr-hole that emits low intensity pulsed ultrasound (LIPU). The implant is connected to a generator and used in combination with microbubbles to induce BBB opening to deliver chemotherapy (carboplatin) in glioblastoma (GBM) patients.
The device can be placed during a tumor biopsy or surgical debulking procedure. The sonication procedure lasts about 10 minutes, and it can be done by a nurse because it does not require image guidance.

A safety and feasibility study was conducted to look at escalating pressure doses with T2*, FLAIR, and diffusion MRI for 6 months with treatment every 4 weeks. The study included patients with GBM who had previously failed therapy with surgery and/or radiotherapy and temozolomide, with a contrast-enhanced tumor of <35 mm diameter and eligible for carboplatin therapy.

Nineteen patients have been treated repeatedly with the SonoCloud system. Preliminary data from the trial suggests safety and tolerability of the SonoCloud LIPU procedure.10 There were 6 patients (31%) that had progression-free survival ≥ 19 weeks.

A limitation of the current device is the single transducer design that prevents coverage of the entire tumor volume. A bridge study is looking at the safety and efficacy of implanting three devices. There is also a larger device in development that could treat an entire tumor.

A large multicenter trial is planned for the larger device.

A clinical study with the SonoCloud device is in development for AD without drug (BBB opening only).

Canney concluded that for US FDA approval of BBB-opening devices, a clear regulatory pathway would be helpful to bring these devices to the US market.

Discussion from Presentations

- There were a few questions on details of the Sunnybrook trials.
  - There were no adverse events or severe adverse events reported in either tumor or AD trial.
  - No blood or cerebrospinal fluid (CSF) was collected as part of this phase I trial.
  - Consider dialysis catheter placement to determine FUS BBB opening effects on the drug concentration in the parenchyma.
  - Consider fluorodeoxyglucose (FDG) PET results in tumor trials to determine if improved drug delivery resulted in a treatment effect when compared to non-sonicated tumor.
  - FUS was applied one hour after temozolomide injection.
  - Amyloid PET ligand was assessed before and after FUS treatment and results are currently being analyzed.
Safety of FUS for BBB Opening

Several speakers discussed topics related to the safety of FUS-mediated BBB opening.

Joe Frank, National Institutes of Health, discussed safety concerns associated with FUS for BBB opening in combination with microbubbles.

Recent preclinical studies have found that FUS plus microbubbles results in a state of sterile inflammation in the brain. Sterile inflammation consists of the release of damage-associated molecular patterns (DAMPs) and the resulting pro- and anti-inflammatory cascade.

After FUS-mediated BBB opening in rats, there was an immediate release of the DAMP response including elevations in pro-inflammatory, anti-inflammatory, and trophic and neurogenic factors. These elevations persist for 24 hours, but they disappear by 48 hours after treatment.

Frank’s group has also looked at the pharmacokinetics of microbubbles. Microbubble dosing information (in rodents) is inconsistently reported in the published literature. Depending on the infusion rate and the timing of sonication, there were differences in the area under the curve for the number of microbubbles that were sonicated.

Future work should consider a standardized microbubble dose, infusion rate, timing of sonication after infusion, oxygenation state, MRI metrics and sequences, and gadolinium dose for imaging.

Frank has also looked at longer term effects of BBB opening after both single and repeated sonications. Ventricular volume changes occur after repeated sonications. There was also an increase in phosphorylated tau after repeated sonications.

Nathan McDannold discussed BBB opening and delivery of irinotecan in a rat model using a clinical transcranial MRgFUS system (Insightec’s 230 kHz Exablate system).

Cavitation monitoring was used for real-time feedback control. Acoustic power and time trajectories depended on the brain target. Less than 0.1% of all bursts had subharmonic or wideband emissions.

T1 mapping was used to assess the amount of BBB opening, and was localized with slight variability in the target (error was less than one millimeter in 25/30 sessions). The striatum had the largest amount of BBB opening (highest energy density) and the cortex had the smallest amount of BBB opening (lowest energy density).

There was minor vessel damage (12/40), yet most of these were less than 100 microns. In one severe example, there was vascular damage that resulted in a small scar in the striatum. The closed-loop feedback control based on 2nd and 3rd harmonics ensured that BBB opening occurred with minimal vascular damage. Additionally, irinotecan delivery to the brain was not neurotoxic.
Elisa Konofagou discussed safety of FUS-mediated BBB opening in mice and non-human primates.

Because of the injection of microbubbles, cavitation mapping can be used during sonication. Safety correlation with acoustic pressure can be assessed with MRI and haematoxylin and eosin (H&E) histology in a mouse model. In this study, they found that acoustic pressures between 300-460 kPa were the safest for BBB opening. At high pressure, 750 kPa, there was leakage from tight junctions. Repeated weekly sonications for 6 months did not cause any changes in a motor and anxiety assessment.

In a non-human primate study, real-time cavitation monitoring of FUS-mediated BBB opening was possible. White matter tracts have low permeability during BBB opening. Vital signs (heart rate, respiration, and oxygen level) showed no change during sonication. BBB opening was reproducible and safe after 20 months of monitoring.

Kullervo Hynynen discussed large animal evaluation of clinical-scale methods for FUS treatment of AD.

A clinical-scale prototype system has been developed for BBB opening in combination with simultaneous cavitation mapping. An acute study in rabbits detected red blood cell extravasation in the perivascular space in a very small number of focal spots one week after sonication.

A chronic study in pigs (2-4 weekly sessions in the hippocampus) found no abnormal findings on neurological exams and no abnormalities on T2* MRI, but there were low levels of microhemorrhage.

Future work will combine large animal testing with ex vivo human skullcaps and will also investigate other cavitation-mediated brain therapies.

Discussion from Presentations

- There was a discussion on appropriate safety biomarkers moving forward.
  - Real-time monitoring and control (e.g. acoustic cavitation mapping) may be necessary for ensuring safety of transcranial FUS for BBB opening, at least at this early stage.
  - Repeated BBB openings (65) with the SonoCloud demonstrated the safety of these procedures.
  - Petechiae are usually the first damage that can be visualized during sonication.
  - Behavioral testing could be important depending on the disorder under treatment.
  - Clinically meaningful adverse events particular to the field of interest should be defined.
  - Monitoring the immune system may provide predictive biomarkers.

- Imaging parameters should be standardized across both preclinical and clinical studies.
There was a comment that relevant neuropsychological batteries could be very informative in clinical trials.

There was agreement that detailed mechanistic studies are necessary.
  - Molecular analysis in animal models may be informative, and some of these studies are underway.

From the clinical perspective, there are critical unmet needs that FUS-mediated BBB opening could address. A risk assessment for the current standard of care in each potential FUS indication can be informative for the level of safety that would be acceptable. The acceptable risks will depend on the disease in question and the current standard of care.

**Technology**

**Mor Dayan, Insightec**, discussed the company’s perspective for safety of MRgFUS for BBB opening.

There are four key safety features for BBB opening using the Insightec system: rely on real-time acoustic monitoring and control, use of MR imaging for treatment safety and efficacy, local and precise targeting, and the skull remains fully intact.

Real-time acoustic control allows treatment parameters to be adjusted as necessary and prevent unintended damage. The use of a hemispheric transducer shape allows for a focal effect and electronic beam steering.

The next R&D steps are to integrate real-time acoustic monitoring, replace thermometry with real-time T2* MRI during sonication, treatment of large volumes with well-defined margins in a single session, and design tools and workflow specifically for BBB opening.

Long-term future directions are to make the procedure frameless and remove the necessity for hair removal from the scalp.

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**Group Discussion**

**What is the key indicator for safety monitoring?**

- Real-time acoustic monitoring and post-sonication MRI are essential for safety monitoring using transcranial ultrasound devices, although the usefulness of such systems is limited unless measured in 3D and correlated with vessel and tissue type.
- There was a lengthy discussion on whether the FUS-mediated BBB opening procedure must take place in the MRI. The consensus was that initial studies will benefit by taking place in the MRI. After safety and efficacy are demonstrated, it should be possible to design the procedure to occur outside of the MRI.

**What evidence of safety is necessary for FDA approval?**

- Overall, standardization of the procedures is important. For preclinical data, targeting accuracy is important.
- Safety evidence should be clinically relevant.
• For safety consideration, it would be helpful to know the maximal safe threshold (maximal ultrasound pressure for safe BBB opening).

• Consider the changes occurring in the brain. For example, cerebrospinal fluid (CSF) sampling or PET imaging.

• There are many aspects to each sonication procedure and no single measure to report safety. The creation of a safety index (combining multiple measures) might be useful for comparison purposes.

• The metrics for successful BBB opening are necessary going forward. For example, (e.g., subharmonics and others).

• The device plus a drug is considered a combination product by the FDA.
  - Participants requested assistance from the Focused Ultrasound Foundation to coordinate with the FDA for combination submissions and streamline the process.
Overall Discussion and Evidence Gaps

Participants were guided to discuss key questions regarding FUS-mediated BBB opening.

Does FUS increase the concentration of drug in the healthy and diseased brain?

- There was agreement that FUS-mediated drug delivery is achievable and reproducible in the healthy brain. In the diseased brain (e.g., GBM, AD) there are still many questions unanswered, particularly related to disease-specific parameters required for safe and efficient delivery leading to treatment efficacy.

When FUS plus BBB opening is used in combination with drug treatment, what is the increase in the amount of drug that enters the brain?

- Several preclinical studies have demonstrated an increase of various magnitudes. For example, a rodent study that administered fluorescent doxorubicin in a glioma model showed a two-fold increase in doxorubicin. However, the FUS-induced increase has not been measured in humans. The participants agreed that whenever possible this should be measured in future clinical trials.

- In preclinical studies, the amount of drug entering the brain after BBB opening ranged from 1 to 5 micrograms/g tissue, although this value will depend on the total amount of drug delivered and the characteristics of the drug (size, charge, etc.). Some drugs do not get into the brain at all without BBB opening.

- The amount of drug delivery to the brain to achieve a clinically significant therapeutic effect is a high priority question for future research.

- We need quantitative metrics for the amount of BBB opening (e.g., T1 mapping, DCE mapping) matched with quantitative amount of drug delivered.

- In preclinical models of cancer, the amount of drug entering the brain depended on the tumor stage. Therapeutic dose will depend on the specifics of the agent and the brain environment for the desired application.

- FDA is concerned with local drug exposure, and this should be taken into consideration in future trials.

Can FUS and other ultrasound approaches be safely used to repeatedly open the BBB in human patients?

- Participants agreed unanimously. However, more data are needed to confirm safety.

What indications should be pursued for FUS plus BBB opening?

- Glioma, pontine glioma, brain metastases, benign tumors, AD, PD, Huntington’s disease, epilepsy, amyotrophic lateral sclerosis (ALS), stroke (stem cell recruitment), and cerebral palsy (underlying neuroinflammatory component) were all mentioned.

- There was a suggestion to aim for the major disorders such as GBM, AD, and PD. After that, consider other disorders that have no treatment such as Huntington’s disease, multi-system atrophy, or other orphaned diseases.
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• Direct injection of cells into the brain can be problematic, and FUS may be useful for directing these cells to their intended targets. Peripheral entrapments and effects of the cells remain to be considered when injected intravenously.

• Terminal diseases with very little to no treatment options may be better targets for new technology. Some of the possibilities mentioned were adrenoleukodystrophy, lysosomal storage disorders, and ALS.

For glioma, what are the key findings and what should be studied next?

• Overall survival and progression-free survival are the most important outcome measures. Other measurements include tumor volume measurements and metabolic imaging.

• The Sunnybrook experience suggests that for FUS-mediated BBB opening there are limitations of the microbubble dose and the BBB volume that can be opened. This limitation is not reported by CarThera with the SonoCloud.

• The goal is to use FUS plus BBB opening to keep the tumor from progressing. This will require occasional treatments to maintain this state. Ideally, a 2-cm rim around the tumor would be sufficient depending on tumor location.

What other agents for GBM should be considered for use with FUS and BBB opening?

• Irinotecan, doxorubicin, carboplatin, and BCNU may have some efficacy. There are very few drugs approved to treat GBM. It is likely better to prove the efficacy of FUS plus BBB opening with already approved agents at this stage. Combining an experimental technology with an experimental drug could be very difficult to gain regulatory approval.

• Carboplatin was used in the CarThera trial because of its safety profile.

• Consideration should be given to gene therapy delivery for GBM.

For GBM, is there an optimal number and frequency for FUS BBB opening?

• CarThera’s protocol performs repeated LIPU-mediated BBB opening until disease progression. Patients are treated monthly. Up to 10 consecutive treatments have been performed in 2 patients.

• GBM patients may need ongoing maintenance treatments. It’s unknown if this is feasible, but there was some concern regarding the potential for cumulative toxicity of chemotherapy.

Is AD a priority for FUS plus BBB opening?

• Participants agreed that preclinical models show safety and efficacy, and this should be investigated as a potential treatment option for clinical trials.

  ■ Preclinical work in PD is also promising.

• FUS treatment is attractive for AD since the brain itself is not exposed (i.e. non-invasive procedure). There are concerns for inflammation and other side effects associated with exposing the AD brain to the environment.

Are there additional AD preclinical studies that are needed to move the field forward?

• There are likely immune system changes that accompany AD, and preclinical models should explore this further. Measuring peripheral blood mononuclear cells (PBMCs) in the blood before and after sonication may reveal predictive biomarkers. In a failed
AD trial, mRNA sequencing in PBMCs found predictive changes in TNF-α signaling in those patients that developed meningoencephalitis.

- Preclinical research could look at timing and type of immune cells that enter the brain after repeated sonications. This should also be assessed in regional lymph nodes.
- Repeated PET scanning for microglial activation translocator protein-18 kDa (TSPO) in mice could also inform the timing of immune cell activation and infiltration in the brain.
- More work to assess the vasculature and BBB opening at different stages of AD pathology should be done.
- There was a suggestion to collect blood in the clinical trials, particularly to look for changes in plasma and red blood cells. Specifically, to look at the effects of age, BBB opening, and the immune system.
- Patients with APOE4 genotype have a higher risk for vasogenic edema, and this might be an appropriate screening tool for the initial clinical trials.
- Exosomes in the blood could be a potential biomarker to show BBB opening. There was a suggestion to collect blood samples at multiple time points following sonication in the clinical trials that are ongoing. Exosomes can contain Aβ and phosphor-tau.
  - Exosomes can also be used therapeutically as anti-angiogenic treatments in combination with FUS.
- The Focused Ultrasound Foundation should follow up with interested researchers to share information regarding exosome testing in blood samples (the availability of commercial testing platforms, associated costs, and other details).

**Should FUS and BBB opening be considered for the treatment of epilepsy?**

- Approximately one-third of new patients with epilepsy will be unresponsive to available pharmacological treatments, a condition known as medically-intractable epilepsy. For some of these patients, surgery has been shown to be a very effective intervention, reducing or eliminating seizures.
- A possible application for FUS-induced BBB opening is to allow for the focal delivery of a neurotoxic agent that is tolerated systemically, but capable of killing neurons in a seizure-generating area of the brain.33
- The advantages of assessing FUS plus microbubbles for this application are:
  - the target is an identifiable, restricted area of the brain,
  - only a single, focal sonication in a given area would be necessary,
  - the mechanism of action of the toxin is known,
  - the functional and structural effects can be monitored,
  - the impact of the treatment should be demonstrable within a practicable period of time (days to weeks).
  - The approach avoids potential complications associated with alternative, invasive neurosurgical procedures, and procedures.
  - The strategy could be more widely embraced by patients, who do not wish to undergo invasive surgery or receive ionizing radiation.
Can FUS be used to treat a large volume in a reasonable period of time?

- To treat larger volumes, faster beam steering is necessary. Insightec indicated that the hardware to achieve this can be designed after once a clinical need has been identified.
- CarThera indicated that with the SonoCloud-9, a larger volume would be treated in 4 minutes.

What is the maximum BBB opening volume necessary for treatment of GBM?

- Assuming that the tumor would be surgically resected, a 2 cm margin around the tumor periphery should be sufficient.
  - FUS treatment around the tumor would need to be uniform.
- A similar strategy can be used to treat brain metastases, but the margin around the tumor may be smaller (around 1 cm).

What is the maximum BBB opening volume necessary for AD treatment

- The human hippocampus is around 2.0-6.0 cm³.
  - There was some discussion on the fact that the hippocampus is surrounded by many other brain structures and these may influence BBB opening.
  - Vascular amyloid may affect parameters.
- The volume needed for treatment will depend on many factors, including the treatment agent and state of pathology.

How should we standardize microbubble protocol used in BBB opening (dosing and reporting methods)?

- In studies with Definity® or Optison® microbubbles, safety has been shown for the imaging dose. When adverse events have occurred, the dose is usually higher than the imaging dose. However, these microbubbles are most typically used to image peripheral organs such as heart, liver, and kidney, and less often in the brain.
- The scientific literature has a large degree of variation, and preclinical studies in mice tend to use higher doses of microbubbles for ease of injection of small volumes.
- There was consensus that researchers should report microbubble dose and infusion rate as well as the FUS acoustic parameters. The size and composition of the microbubbles themselves should also be reported.
- Ultrasound contrast agent dose and infusion rate for BBB studies should be defined. Ultrasound microbubble dosing is well defined for imaging but not yet for FUS-mediated BBB opening. This requires further exploration.
- A participant mentioned that the microbubbles may be considered a new drug in the context of a new device by the FDA. An FDA attendee stated that discussions are ongoing at the FDA to consider microbubbles not as a drug but as a device.
- Infusion of microbubbles might work better than bolus doses, because this helps to achieve a stable concentration. Patients with higher body mass index tend to have a lower concentration of microbubbles reach the brain.
- Microbubble dosing will likely be disease specific. Patients with AD might have different vasculature compared to patients with GBM.
Different areas of the brain show different microbubble concentration. For example, the basal ganglia tend to have a greater concentration of microbubbles. GBM tumors also have a higher concentration of microbubbles compared to the surrounding brain tissue.

There was discussion on the half-life of a microbubble in a mouse versus a human. This is being measured in the Sunnybrook clinical trials.

As the field moves forward, microbubbles should be designed specifically for use with FUS and BBB opening. The microbubbles currently in use were designed for different applications, but are FDA approved. These bubbles were selected for clinical trials to gain faster approval from regulatory authorities.

Ultimately, there are two questions that we should answer:

- What is the known maximum tolerated dose of microbubbles that has demonstrated safety? A literature review might help to answer this question.
- What is the optimal microbubble dose for BBB opening?

What are some methods for the standardization of MRI imaging?

- Gadolinium concentration T1 mapping can confirm BBB opening and drug delivery. There are standard MRI sequences that researchers should use.
- T1-weighted with contrast imaging (gadolinium enhancement), T2*, and FLAIR MRI sequences are being used during sonication at Sunnybrook.
- A clear standard MR protocol to confirm BBB opening is necessary for comparative purposes across clinical trials.

FUS parameters for BBB opening

- The Focused Ultrasound Foundation created a list of parameters for BBB opening treatment: focal depth and volume; acoustic power; mechanical index; acoustic pressure; frequency; number of sonications; length of sonications; duty cycle; pulse repetition frequency; burst duration; microbubble size, dose, and dispersion; passive cavitation detection, contrast enhancement, H&E histology, TUNEL staining, Ktrans, and animal model.

There was some discussion on the creation of a single index to measure safety. At this stage, there are too many unknowns and it is better to report raw numbers for comparisons. However, for regulatory purposes it would be easier to have a few indexes that allow comparison across devices and studies.

Other factors that could help move the field of FUS and BBB opening forward?

- A streamlined process from the FDA for developing FUS for BBB opening.
- Work with investigators to propose methods for standardization of procedures and reporting across platforms.
Outcomes and Next Steps

Important activities identified that could significantly move the field forward included:

■ Develop a streamlined FDA pathway for trials to assess FUS for BBB opening (may need different pathway(s) for different devices/diseases/drugs).

■ Organize Foundation-led effort, working with the research community, to propose methods for standardization of FUS-mediated BBB opening procedures (FUS treatment parameters, microbubble type/dosing, acoustic/MRI monitoring protocol, etc.) and reporting across device platforms.

■ Assess the scientific data available on microbubbles and FUS (dosing, differences between microbubble types, safety data, etc.) to enable optimization of microbubble protocol.

■ Identify other biomarkers (e.g. exosomes) that could correlate with BBB opening and enable simple blood testing for indication of success.

Participants were encouraged to reach out to the Foundation with any research ideas or project proposals to address the key issues and questions. The Foundation will continue engagement with this community to advance FUS-mediated BBB opening towards clinical adoption.
References


### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AAV</td>
<td>Adeno-associated virus</td>
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<tr>
<td>AD</td>
<td>Alzheimer's disease</td>
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<td>ALS</td>
<td>Amyotrophic lateral sclerosis</td>
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<td>BBB</td>
<td>Blood-brain barrier</td>
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<td>BPN</td>
<td>Brain-penetrating nanoparticles</td>
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<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<td>DAMP</td>
<td>Damage-associated molecular pattern</td>
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<td>DCE</td>
<td>Dynamic contrast enhanced</td>
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<td>FDA</td>
<td>US Food and Drug Administration</td>
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<td>FLAIR</td>
<td>Fluid-attenuated inversion recovery</td>
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<td>FUS</td>
<td>Focused ultrasound</td>
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<td>GMB</td>
<td>Glioblastoma</td>
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<td>IVIG</td>
<td>Intravenous immunoglobulin</td>
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<td>LIPU</td>
<td>Low intensity pulsed ultrasound</td>
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<td>MMSE</td>
<td>Mini-mental state examination</td>
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<td>MRgFUS</td>
<td>MRI-guided focused ultrasound</td>
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<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>NIH</td>
<td>National Institutes of Health</td>
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<td>PBMC</td>
<td>Peripheral blood mononuclear cells</td>
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<td>Parkinson's disease</td>
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<td>PET</td>
<td>Positron emission tomography</td>
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<tr>
<td>SAE</td>
<td>Serious adverse events</td>
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<tr>
<td>TUNEL</td>
<td>Terminal deoxynucleotidyl transferase dUTP nick end labeling</td>
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