Focused Ultrasound for Glioblastoma

Workshop

19–20 May 2021

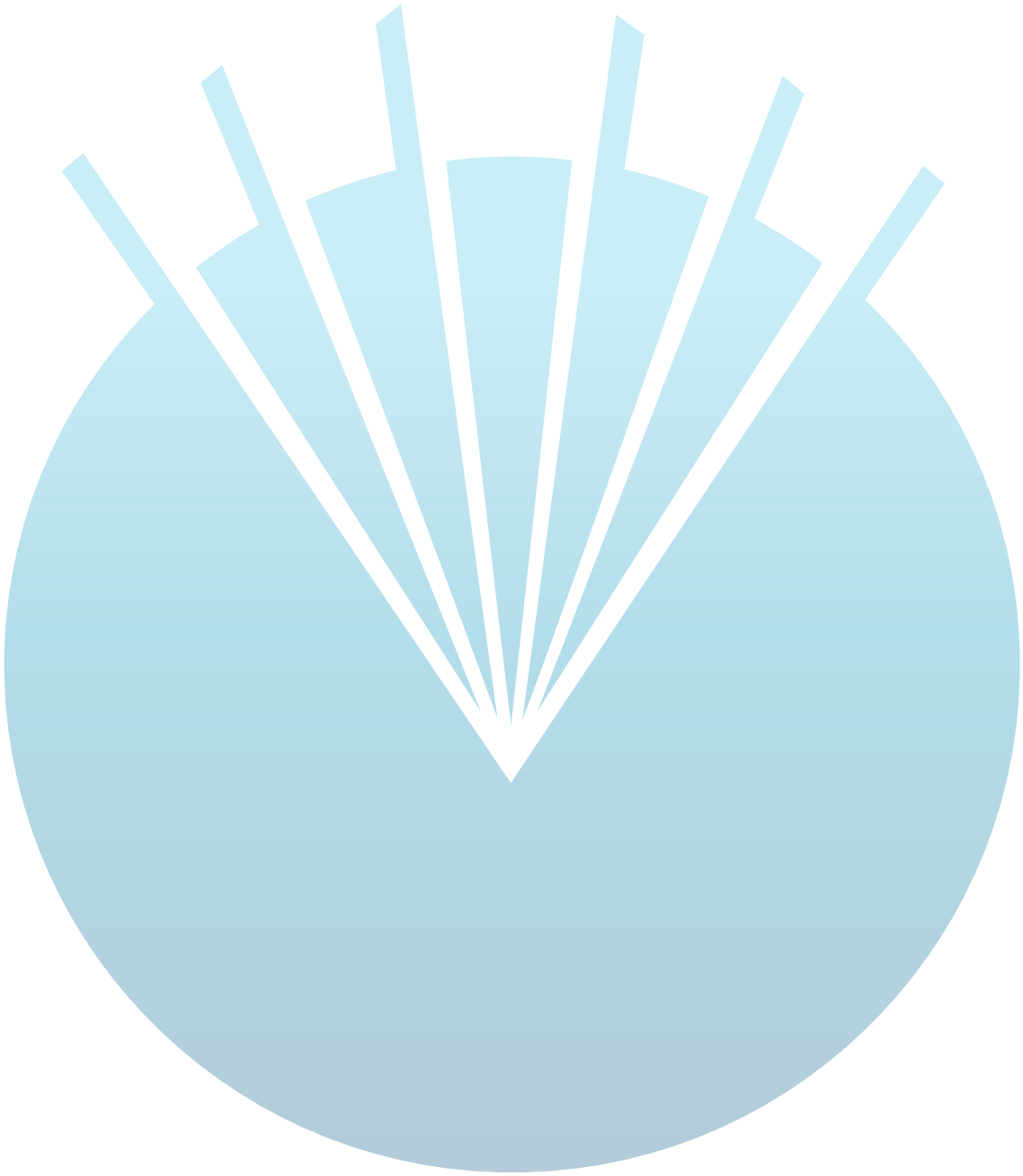
Virtual Meeting

In partnership with

Focused Ultrasound Foundation

Tumor Section 1984 AANS/CNS

SNO The Society for NeuroOncology



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Executive Summary

The Focused Ultrasound Foundation hosted a virtual workshop on focused ultrasound for glioblastoma, GBM, on May 19–20, 2021. The meeting brought together critical stakeholders, including researchers, clinicians, industry, government, and others, to share and combine knowledge to advance the field. Focused ultrasound, FUS, is an early-stage, disruptive, noninvasive therapeutic technology that has the potential to improve the lives of millions of patients with a variety of medical disorders by providing an alternative, or a complement, to existing treatment approaches.

The ultimate goal of the 2-day workshop was to improve outcomes and reduce the cost of care for patients with GBM by reducing the time it takes for FUS to become part of the treatment armamentarium and reach clinical adoption. The workshop identified gaps in knowledge and evidence and created a roadmap for technical developments, laboratory studies, and clinical trials necessary to close these gaps.

There were pre-recorded lectures available one-week prior to the workshop on a variety of topics including the current state of the technology, blood-brain barrier opening, immunomodulation, radiosensitization, ablation, treatment monitoring, and clinical trial design. The live virtual discussions focused on in-depth discussions surrounding the "burning questions" related to each topic. Some common themes that were discussed included:

- Chemotherapy selection for clinical trials;
- Immunomodulation
- Confirmation of blood-brain barrier opening as well as optimal sonication parameters
- Radiosensitization
- Ablation (both thermal and microvascular disruption)
- Microbubbles
- Technology wish list for FUS devices

The group was thoroughly engaged in discussion from the beginning of the workshop until departure. The attendees were asked to continue thinking and collaborating on these issues, and to share any additional thoughts with their colleagues and the FUS.

Virtual Workshop Welcome and Introduction

Jason Sheehan, MD, PhD welcomed attendees and acknowledged that the workshop was developed because of new and emerging treatments in neurosurgery and neuro-oncology. FUS has resulted in a paradigm shift for the treatment of both essential tremor and tremor-predominant Parkinson's disease. The purpose of the meeting was to explore whether FUS can be a paradigm shift for glioblastoma (GBM) and other brain tumors. Lauren Powlovich, MD stated the goal of the meeting was to focus on providing answers to a list of pre-determined "burning questions" on targeted drug delivery across the blood-brain barrier (BBB), blood-brain-barrier opening (BBBO), immunomodulation, immunotherapeutic agent delivery, gene and cell therapy, radiosensitization, ablation (thermal, microvascular disruption, histotripsy, and sonodynamic therapy), technology, patient selection, treatment monitoring (imaging and liquid biopsy), and clinical trial design (regulatory considerations and reimbursement) to move the field of FUS for GBM forward. The meeting program book included several recommended pre-readings.[1-8]

Panel Discussion Take Home Messages

Mechanisms of Action

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Targeted drug delivery across the blood brain barrier

- There are numerous safety and efficacy clinical trials underway that are combining FUS-BBBO with chemotherapeutic agents for the treatment of GBM
- The devices employed in BBBO clinical trials are: Carthera, InSightec and NaviFUS
- Most of the current clinical trials are treating patients with recurrent GBM, but there should be more consideration for upfront treatment since there is less tumor heterogeneity at this time-point
- Additional biomarker and imaging studies as response assessment tools are needed
- Increasing the concentration of TMZ at the site of BBBO will not increase efficacy, as previous studies have shown that greater doses of TMZ do not affect outcome
- A control arm is important to have in FUS-BBBO studies, and one way to minimize the need for numerous control arms is to have a Bayesian designed study to look at multiple therapies at once

Mechanisms of Action

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for panel summary

Blood brain barrier opening

Confirmation of BBBO	• DCE MR imaging or T1 mapping is typically used to confirm BBBO in preclinical models • Fluorescent tracers, mass spectrometry, and acoustic backscatter have also been used to confirm BBBO, but contrast enhanced MRI is the most commonly used surrogate indicator of BBBO • It is more difficult to visualize BBBO in the white matter compared to gray matter given the lack of vascularity in white matter • Further studies should assess the use of radio-labeled drugs/PET scans to confirm drug delivery
Sonication parameters	• At this time, there is no consensus on optimal parameters for BBBO • Optimal parameters likely depend on the size and formulation of the microbubble, as well as the therapeutic agent being delivered
Microbubble administration protocol	• Continuous microbubble infusion is now being used in clinical trials for safety reasons, instead of bolus injections as has been done in a majority of preclinical studies • A microbubble should be designed specifically for use with FUS and BBBO instead of using microbubbles designed for imaging purposes

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Immunomodulation

	• The intersection of FUS with GBM lymphatics needs to be considered going forward • Further research into understanding the interplay between the different FUS modalities and immunomodulation is necessary to move the field forward • Investigation into whether FUS can induce trafficking and activation of immune cells should be pursued • Exploration into whether FUS can activate microglia and if this is beneficial in GBM should be pursued • Determination of the role of FUS immunomodulation should be established

Mechanisms of Action

Immunotherapeutic Agent Delivery

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- There are a variety of different therapeutics that can be studied in GBM patients, including immune checkpoint-directed antibodies, adoptive T cells, natural killer (NK) cells, chimeric antigen receptor (CAR) T cells, and genetically modified antigen presenting cells
- Preclinical models, particularly mouse models, do not sufficiently recapitulate human GBM and it therefore may be too risky to base a large phase III trial off preclinical data
- Neo-adjuvant trials prior to surgery to study whether combination with FUS evokes the desired response might be a better approach
- Performing pathology on a small sample of the tumor could provide misleading results. A key histological question with BBBO is whether there is uniform immune cell dispersal throughout the tumor microenvironment. Without intervention, T cells are limited in the glioblastoma microenvironment to the perivascular space.

Gene and Cell Therapy

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- The heterogeneity of the tumor microenvironment make gene therapy a less attractive treatment strategy for GBM
- CARs directed against a single antigen are unlikely to make a big difference for the treatment of GBM due to antigen heterogeneity and escape
- IDH-mutant GBM may be a subtype of interest for these approaches as wild-type GBMs may be too heterogeneous to effectively treat with

Radiosensitization

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- FUS combined with microbubbles (BBBO) has potential to reverse hypoxia through reoxygenation, thereby inducing radiosensitivity

Mechanisms of Action

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for panel summary

Ablation

Thermal Ablation	• Thermal ablation of GBM's is difficult given the highly vascularized nature of these tumors • Treatment time and temperature need to be slowly adjusted to determine safety limits in these highly vascularized tumors • An incremental clinical trial could be designed with the goal of treating grade 2 lesions, before treating GBM • Thermal ablation is unlikely to be the only focused ultrasound mechanism of action for GBM
Microvascular Disruption	• Microbubbles or emulsions may allow treatment of any brain region by drastically reducing the amount of energy needed to ablate the tissue • There are technical advancements needed prior to microvascular disruption becoming a safe treatment option, including: more accurate monitoring systems and specific transducers for this mechanism
Histotripsy	• Histotripsy is tissue selective and can preserve vasculature • There is some swelling and bleeding following histotripsy in animal models • More data needs to be obtained on the effects of histotripsy at varying ablation parameters (treatment time, energy, frequency of sonications, etc.)
Sonodynamic Therapy	• Sonodynamic therapy in preclinical studies shows promise for treating GBM • IV administration of 5-ALA bypasses the liver and stomach, prevents nausea/vomiting and abnormal liver function, and delivers 5-ALA more efficiently to the tumor • There are several early phase clinical trials in the planning phases to treat patients with sonodynamic therapy

Technology

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for panel summary

Technology gaps and desired features and functionality

- Design patient friendly FUS frames that are comfortable and machines that do not necessitate head shaving
- Consider customizing helmets for each patient's skull characteristics and tumor location
- Ensure mathematical modeling for accuracy should go thru QA process
- Research best methods to quantify amount of drug delivery across the BBB
- Design systems to target larger volumes of tissue including a rind 2 cm around the GBM to treat invasive spread

Patient Selection

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Clinical unmet needs

- Greatest unmet clinical need likely for recurrent GBM but also residual post resection, frontline therapy, and radio necrosis
- Recognize that presurgical trials can inform us of important FUS MOA's to then apply to various stages of disease more successfully
- Future trial designs should include timing of drug administration, duration of BBBO, volume of BBBO and FUS parameters

Treatment Monitoring

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Radiologic Assessment

- Modified RANO predicts tumor growth well
- Differentiating pseudo progression from true progression is challenging, consider using PET, MRI, and machine learning in addition to liquid biopsy

Treatment Monitoring continued

Liquid Biopsy

- Modified RANO predicts tumor growth well
- Design trials recognizing that the detection of blood analytes may be time specific after FUS and/or size dependent based on the amount of BBBO and varying FUS parameters
- LB may assist in longitudinal f/u and to differentiate pseudo vs true progression
- Potential to perform targeted LB in specific areas of the tumor that are resistant or have specific radiologic signatures could direct precision medicine

Clinical Trial Design

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Regulatory Considerations

- An overview of the FDA offices involved in combinatorial trials with focused ultrasound was provided
- Encouraged researchers to schedule a pre-IND meeting to work through any questions and obtain guidance prior to official IND submission
- Submissions must include detailed review of ultrasound technical parameters and safety considerations
- FDA panelists expressed concern with decoupling device and BBBO procedure for approval

Reimbursement

- Obtaining coverage from Centers for Medicare and Medicaid Services (CMS) is mandatory for reimbursement
- Approval by CMS requires preferably 5 years (minimum of 2 years) of durability and US based data
- The American Medical Association (AMA) relies on subspecialty societies to decide on CPT codes

Mechanisms of Action

The workshop was organized into discussion panels to share their thoughts and ideas on topics related to FUS for GBM.

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Targeted Drug Delivery Across the Blood-Brain Barrier

Overview of current clinical trials

Nir Lipsman, MD, PhD, Moderator
Jin Woo Chang, MD, PhD, **Alexandra Golby, MD**, **Adam Sonabend, MD**, **Roger Stupp, MD**, and **Graeme Woodworth, MD**

The panel discussed safety assessments and monitoring for clinical trials with FUS for GBM. Some important monitoring tools for the Insightec system are real-time MR imaging (T2*), MR thermometry, and clinical neurological examinations performed during the treatment (using minimal sedation). Acoustic emission monitoring is important to understand the mechanical effects. The Carthera implantable FUS device has already gone through extensive safety testing. The device uses less energy as it does not need to penetrate the skull and the procedure is performed in non-sedated patients in under 4 minutes. Many of the concerns relate to potential side effects associated with chemotherapy (paclitaxel). MR imaging is performed following sonication to confirm BBBO and no hemorrhage or other imaging abnormalities have been observed after sonications.

The participants also discussed the optimal timing for FUS in the treatment algorithm. There are a lack of treatment options that prolong survival in patients with GBM. Typically, clinical trials begin in the recurrent setting. Some consideration should be given to clinical trials in patients with recurrent GBM (rGBM), such as window of opportunity trials or proof-of-concept trials to see if FUS can increase drug delivery.

Outcome measures for FUS trials were also discussed by the panel. For example, progression-free survival (PFS) and overall survival (OS) in an upfront treatment model. Patients in the upfront setting have less heterogeneity. The importance of biological endpoints was also mentioned. Additional biomarker and imaging studies are needed in this area.

Participants debated the technological parameters of FUS for BBBO. There are still many unknowns and optimization of parameters has yet to be determined, such as length of opening versus volume of opening.

Discussion
Current protocols and future directions

Priorities were to include clinical trials with sufficient power to detect clinical benefit. Decoupling from MR imaging will allow more patients to access FUS, and perhaps move the treatment to an outpatient treatment setting. Dr. Stupp cautioned that increasing the dose for temozolomide (TMZ) through BBBO will not increase efficacy, as previous studies at greater doses of temozolomide have already shown this. The role for FUS is likely in combination with other therapies for the treatment of GBM.

The panel addressed the need for control arms in each trial, particularly considering that there have been many failed clinical trials in GBM. Participants agreed that using novel trial designs such as Bayesian design to look at multiple therapies at once to minimize control arms should be done. They also suggested using real-world evidence in place of control arms. Too many trials are repetitive single-center trials and thus multi-arm trials should be planned going forward. The panel reiterated the importance of identifying biomarkers to enrich trials for patients likely to respond to a given treatment.

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Blood Brain Barrier Opening

Confirmation of Blood-Brain Barrier Opening

Michael Canney, PhD, Moderator
Nathan McDannold, PhD, **Antonis Pouliopoulos, PhD**, and **Raag Airan, MD, PhD**

Panelists were asked to describe their work confirming BBBO with various methods. Nathan McDannold explained that DCE MR imaging or T1 mapping is typically used to confirm BBBO in preclinical models. Outside of MR imaging, fluorescent tracers have been used (trypan or Evans's blue, fluorescent dextrans). Mass spectrometry has also been used to quantify BBBO. Acoustic backscatter has been explored but is not as reliable as MR imaging. Antonis Pouliopoulos also mentioned that in primate experiments, changes in the diffusion constant of water molecules in the area of the BBBO were observed and the changes also correlate to the contrast-enhanced area. He also mentioned that passive acoustic mapping can be used. Fluorescein dye can also be used in the operating room to visualize BBBO.

Sonication parameters

Nathan McDannold, PhD, Moderator
Kullervo Hynynen, **PhD, Elisa Konofagou, PhD**, and **Francesco Prada, MD**

The panel explained their typical sonication parameters. Elisa Konofagou replied that in mice, 0.45 MPa is the optimal pressure that works with Definity microbubbles for BBBO at safe levels. Kullervo Hynynen stated that they use 10 ms pulses every second, until they

identify subharmonic emissions and then scale back the pressure to 50%. Francesco Prada stated that in clinical trials and *in vitro* they used pulsed sequences at 1 MHz.

At this time, there is no consensus on optimal parameters for BBBO. There is a need to maximize drug delivery and minimize damage. The optimal parameters likely depend on the size and formulation of the microbubble, as well as the therapeutic agent being delivered. Pulse length, frequency, and pressure have to be adjusted to account for larger agents (antibody, gene product, etc.). The carbon length of the microbubble shell also impacts the parameters, and the microbubble size may need to be different when the goal is to achieve larger openings.

Microbubble administration protocol

Continuous microbubble infusion is now being used in clinical trials for safety reasons, instead of bolus injections as has been done in a majority of preclinical studies. There was a suggestion that using the heart rate of the participant could also help increase efficiency of opening with microbubble infusion. Participants agreed that a microbubble should be designed specifically for use with FUS and BBBO instead of using microbubbles designed specifically for imaging purposes.

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Immunomodulation

Discussion

Immune response to FUS and ways to monitor this

Michael Lim, MD, Moderator
Costas Arvanitis, PhD, **Timothy Bullock, PhD**, **Theresa LaVallee, PhD**, and **Tao Sun, PhD**

There is some debate in the literature on whether FUS alone can modulate the immune response. Studies in GBM preclinical models has thus far shown changes in the immune landscape following thermal and mechanical FUS but there were differences in the immune response between regimens. The next piece of the puzzle is to determine whether these responses are durable and meaningful for the treatment of GBM. The panel agreed that the understanding of lymphatic drainage in the central nervous system (CNS) is changing the paradigm for GBM and that the draining lymph nodes play a bigger role than originally thought. The intersection of FUS with GBM lymphatics needs to be considered going forward. In general, FUS-mediated immunomodulation needs to be placed in the context of what we know about the brain and the tumor microenvironment. There may be opportunities at various stages of tumor development, initiation, promotion, and acceleration to intervene with FUS immunotherapy approaches.

The field of immunotherapy is diverse and includes not only checkpoint inhibitors, but also cell and gene therapies. The panel agreed that further research into understanding the interplay between the different FUS modalities and immunomodulation is necessary to move the field forward. Continued preclinical research is necessary to unveil the mechanisms

involved in immunomodulation (e.g., increasing antigen availability and cross-presentation, increasing T cell infiltration into the tumor or modifying the tumor microenvironment) and to determine the best sequencing of combinatorial immunotherapies with FUS.

Instead of focusing on delivering larger amounts of drugs that have failed to show any benefit for brain tumors, investigating if FUS can induce trafficking and activation of immune cells was suggested. The GBM microenvironment has a low number of T cells, particularly CD8+ T cells. Myeloid cells, macrophages, and microglia may also be modulated by FUS. It is also well-known that GBM is rich in myeloid cells. Microglial activation following FUS in patients with Alzheimer's disease indicates that this idea is worthy of investigating in patients with GBM.

In terms of therapeutics, there are approaches that polarize myeloid cells to produce cytokines that support T cell activation (e.g., toll-like receptor agonists, CD40 agonists). Radiation and laser ablation activate microglia, and this may also occur after FUS. However, activation of microglia may not necessarily be beneficial. Further exploration of this topic is needed.

The panel discussed timing of FUS for immunomodulation. Preclinical research shows that within the first 24 hours after BBBO there is a window of opportunity for administering treatments. Preliminary data also suggests that FUS alone does not improve survival in pre-clinical models. Drug administration should occur around the same time as intervention with FUS BBBO. One important step is to characterize the heterogeneity of myeloid cells in brain tumor samples. Theresa LaVallee also proposed using translational research in mice to inform clinical trial design in humans. The field has to decide what the intended role of FUS immunomodulation is in clinical paradigms. There was a suggestion to study damage-associated molecular patterns (DAMPS, i.e., alarmins) in the tumor microenvironment following destructive FUS regimens to see what molecules are upregulated.

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Immunotherapeutic Agent Delivery

Discussion

Immunotherapeutic agents and confirmation of therapeutic delivery

Manmeet Ahluwalia, MD, Moderator
John de Groot, MD, **Amy Heimberger, MD**, and **Patrick Wen, MD**

The panel discussed their thoughts on potential immunotherapeutic agents for GBM. The panel mentioned use of BBBO to activate the immune system. There were a variety of therapeutics mentioned including immune checkpoint-directed antibodies, adoptive T cells, natural killer (NK) cells, chimeric antigen receptor (CAR) T cells, and genetically modified antigen presenting cells. Some of these therapeutics could be delivered directly to the brain with FUS, while others designed to elicit a strong systemic immune response may not necessarily confer clinical benefit if delivered to the brain in greater quantities with BBBO. For example, a chemotherapeutic that already crosses the BBB may not be a candidate

compared with larger agents that do not cross (e.g., antibodies). Preclinical models could provide optimal guidance on immunotherapeutics that we should prioritize on delivering with BBBO, but the panel disagreed on their utility. Panelists felt that preclinical models, particularly mouse models, do not sufficiently recapitulate human GBM and it therefore may be too risky to base a large phase III trial off of preclinical data. Perhaps another approach would be to perform neo-adjuvant trials prior to surgery to study whether combination with FUS evokes the desired response. Pharmacodynamic effects, such as pathway inhibition or suppression of myeloid cells, should also be investigated to inform future clinical trials.

Another panelist commented that FUS has a potential role for combination with immunotherapeutics to either prime the systemic immune system to exert a response against intracranial tumors, or to locally deliver agents for modulation of the local tumor microenvironment.

Amy Heimberger stressed the important consideration of sampling location during neo-adjuvant trials. Performing pathology on a small sample of the tumor could also provide misleading results. A key histological question with BBBO is whether there is uniform immune cell dispersal throughout the tumor microenvironment. Without intervention, T cells are limited in the glioblastoma microenvironment to the perivascular space.

Additional methods being used in clinical trials to determine the effects of immunotherapy in the setting of GBM were discussed. John de Groot mentioned using CEST-MRI to measure pH changes and novel tracers to look at T cell activation via PET. The panel also briefly discussed appropriate endpoints for clinical trials with immunotherapeutics; at this time the optimal endpoint is unknown.

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Gene, Viral, and Cell Therapy

Gene modulation discussion

How can FUS help to overcome clinical barriers of gene and cell therapy?

Amy Heimberger, MD, Moderator
Stephen Bagley, MD, **Isabelle Germano, MD**, and **Natasha Sheybani, PhD**

The literature is sparse on the combination of FUS with gene, viral, and cell therapy in GBM. There is some early preclinical work with CAR T cells that BBBO may enhance delivery to the tumor. The panel agreed that long term, CARs directed against a single antigen are unlikely to make a big difference for the treatment of GBM due to antigen heterogeneity and escape. IDH-mutant GBM may be a subtype of interest for these approaches as wild-type GBMs may be too heterogeneous to effectively treat with CAR or gene therapy.

FUS therapy may play a role in gene delivery but viral vectors have thus far not been particularly efficacious in GBM. This is in part mediated by lack of uniform distribution throughout the microenvironment. As such, BBBO may help to optimize viral delivery. Single-agent gene therapy trials are expensive and time consuming, and efficacy with

single-agents needs to be shown prior to combination therapies. There have not been many combination trials to date, but recombinant oncolytic poliovirus (PVS-RIPO) with pembrolizumab may be promising.

Phase 0 trials may provide more meaningful data relative to preclinical studies, since the animal models of GBM are not predictive of human GBM clinical trials.

For nanoparticle delivery, e.g., brain penetrating nanoparticles could be considered for delivery with FUS but this needs further preclinical testing. Natasha Sheybani indicated that there are also several new approaches emerging in the literature at the junction of FUS and synthetic biology. These diverge from how we traditional think about leveraging FUS, as they seek to remotely modulate genes or gene products. One of these approaches involves using FUS as a 'remote control' to direct CAR T cells.

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Radiosensitization

Gene modulation discussion

Role of FUS in radiation treatment

Gregory Czarnota, PhD, Moderator
Hao-Li Lui, PhD, and **Frédéric Padilla, PhD**

Radiotherapy is a mainstay of GBM treatment, and tumor hypoxia is a common feature that causes resistance to radiotherapy. FUS combined with microbubbles (BBBO) has potential to reverse this hypoxia through reoxygenation thereby inducing radiosensitivity.

Another mechanism of radiosensitization is through FUS-MBs endothelial cell damage followed by vascular shutdown. In preclinical xenograft models—not of GBM—suppressive effect on the tumor growth after FUS combined with RT was observed, using FUS-induced hyperthermia, FUS-MBs BBBO-type of treatments, or FUS MBs destruction.

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Ablation

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Thermal ablation

Is there still a role?

Suzanne LeBlang, MD, Moderator
John Ragheb, MD

For benign brain lesions, a few patients have been successfully treated (hamartoma and low-grade lesions) with thermal ablation using FUS without significant complications. However, these were small lesions (<1.5 cm^3). The goal is to work back towards malignant lesions with greater vascularization, but it will be a slow process. In terms of treating GBM, vascularity is the biggest concern. The greater the vascularity, the more difficult thermal ablation would be. It is unknown if temperature and time can be increased simultaneously for ablation in highly vascularized tumors. An incremental clinical trial could be designed with the goal of treating grade 2 lesions, for example an anaplastic ependymoma or anaplastic astrocytoma without evidence of hemorrhage, before treating GBM.

There is a planned trial for WHO I and II lesions that will measure response not only with gradation of temperature and time but other technical parameters as well. Thermal ablation also has the potential to be combined with immunotherapy, radiotherapy, and therapeutic delivery. Thermal ablation is unlikely to be the only FUS strategy to treat GBM.

Microvascular Disruption/Non-thermal ablation

Current protocols and future directions

Tyrone Porter, PhD, Moderator
Nathan McDannold, PhD, and **Frédéric Padilla, PhD**

The panel discussed 'big picture ideas' for mechanical ablation as a GBM therapy. Thermal ablation is currently limited to central brain regions but adding microbubbles or emulsions to expand the therapeutic envelope seems promising. Microbubbles or emulsions may allow treatment of any brain region by drastically reducing the amount of energy needed to ablate the tissue. The application of thermal ablation could be useful prior to surgery or prior to treatment with various therapeutic agents. The biggest obstacle now is controlling the energy delivered to the brain.

The panelists also explained the technological advances that are needed to move thermal ablation forward. In terms of ablation, effective means to monitor the treatment are needed. Determining if the energy is directed at the correct location in the brain at the correct dose is vital for safety. Thermal ablation is a linear measurement, but when treating with microbubbles or emulsions, the energy needed to treat is variable. Passive acoustic mapping can be used in place of thermometry. On the device side, once the treatment energy is more certain, specific transducers for non-thermal ablation could be designed. The biggest roadblock now is the need for image guided FUS.

Dr. Padilla mentioned that quantifying perfusion before and after FUS is ongoing. Droplet-based ablation looks promising as a surgical tool, based on preliminary data in preclinical models of GBM. The focus of current research is on use of microbubbles with monitoring methods for combination with radio- or chemotherapy. The panelists mentioned a need for deeper understanding of the mechanisms involved in GBM immunotherapy as a prelude to determining how FUS microvascular ablation can be used in this context.

Histotripsy

Zhen Xu, PhD, Moderator
Tatiana Khoklova, PhD, **Joan Vidal-Jove, MD, PhD**, and **Eli Vlaisavljevich, PhD**

The panel discussed their thoughts on the differences between cavitation histotripsy and boiling histotripsy. Intrinsic histotripsy is thought of as using short, high pressure, single cycle pulses to rise above the intrinsic threshold of nucleating cavitation, which is approximately 25 to 35 MPa for all soft tissues that are water based; this excludes fatty tissues. Shock-scattering histotripsy uses between 5 to 20 cycle pulses at lower pressures based on tissue stiffness (10- 20 MPa) that generates large bubble clouds. The efficiency of the ablation is tied to the properties of the bubbles within the cloud. Boiling histotripsy uses lower pressures with longer pulses (~1 ms) to rapidly boil the tissue up to 100°F. The bubble dynamics are based on thermally induced cavitation, but the biological effects remain very similar.

Histotripsy is tissue selective and can preserve veins within the ablated area, which is different from what is possible with thermal ablation. Histotripsy is also confined to the ablated area without the swelling and edema that happens in the peripheral tissues with thermal ablation. Antigens and immune cells are also found within the ablated area. In animal models treated with histotripsy, there is inflammation and swelling following treatment that may be related to the size of the treatment area versus the size of the organism. Animal models also show localized but extensive bleeding from disrupting small blood vessels confined to the lesion itself. Larger ablated volumes show swelling that dissipates quickly. It remains to be seen whether treatment will consist of a single large ablation, or a series of smaller ablations.

The immune response after histotripsy was also briefly mentioned. There is little evidence that histotripsy releases antigens that may slow tumor growth. Mouse studies in GBM with histotripsy suggest an immune response due to tumor antigen release/preservation and recruitment/activation of other immune cells.

Sonodynamic therapy

Current protocols and agents

Jason Sheehan, MD, PhD, Moderator
Kullervo Hynynen, PhD, **Hao-Li Liu, PhD**, **Stuart Marcus, MD, PhD**, and **Francesco Prada, MD**

Sonodynamic therapy in preclinical studies shows promise for treating GBM. The mechanism appears to be related to microbubble effects and not thermal effects. The intensities are small for initiating inertial cavitation. Kullervo Hynynen theorized that there may be very small bubbles in the tissue that are collapsing, but this has not been confirmed. Another possibility is that sound generates light in tissue and allows activation of the sonosensitizer in addition to mechanical stimulation of the immune system, essentially mimicking photodynamic therapy. In a preclinical study, immune activation was observed without a sonosensitizer, suggesting multiple mechanisms.

Potential sonosensitizing agents were discussed. Fluorescein has been used in some pilot trials, and it is an already approved agent in the clinical setting. The panel also responded to concerns surrounding the precision and accuracy required to treat GBM with sonodynamic therapy. Neuronavigation-guided FUS can provide accuracy similar to MR imaging-guided FUS (MRgFUS). Neuronavigation is easy to use and suitable for the outpatient setting. Similar to the strategy that neurosurgeons use for GBM resection, sonodynamic therapy aims to deliver sonodynamic agents to the peripheral regions around the tumor.

The panel responded that sonodynamic therapy could be combined with any other therapy. Sonodynamic therapy in a preclinical pig model with sensitizers (5-ALA and fluorescein) did not result in damage to brain tissue because the sonication parameters were very low. There was no accumulation of the sensitizer nor was there mechanical damage in this setting. To study mechanisms and pathways involved in sonodynamic therapy, additional preclinical work at a variety of time points should be carried out.

Apoptosis occurs with sonodynamic therapy and has great potential as a future treatment for GBM. Apoptosis occurs as soon as photons are emitted in the cell. The sonication parameters are versatile, some researchers use continuous wave and some use pulse wave parameters. Both high and low pressures can produce apoptosis.

Oral 5-ALA results in nausea and vomiting and increases in liver function tests. Patients with liver function abnormalities may not be able to use this agent. IV administration of 5-ALA bypasses the liver and stomach, prevents nausea/vomiting and abnormal liver function, and delivers 5-ALA more efficiently to the tumor. 5-ALA may also potentially treat other cancers such as melanoma as there is preclinical evidence that other tumors also uptake 5-ALA.

There are several early phase clinical trials in the planning phases to treat patients with sonodynamic therapy. There is a planned clinical trial with 5-ALA and sonodynamic therapy to treat metastatic melanoma in the brain. There is another planned trial for safety and feasibility of sonodynamic therapy prior to tumor resection. Several other trials are also in the planning phases for the treatment of GBM with sonodynamic therapy.

Technology

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Technology gaps and desired features and functionalities

Elisa Konofagou, PhD, Moderator
Kullervo Hynynen, PhD, **Ying Meng, MD, PhD**, **Graeme Woodworth, MD**, and **Fred Wu, MD, PhD**

The panel discussed FUS parameters that need to be determined to move forward. For thermal ablation, the deep portions of the brain cannot be ablated, and further translational work is needed.

For BBBO, further work to optimize microbubbles needs to be carried out. Sonodynamic therapy is still in the early stages and optimal sonication parameters are yet unknown.

Tumor type is extremely important in designing treatment. GBM has micro-metastases that need to be treated by targeting a 2 cm margin around the primary tumor, but brain metastases do not have these and need to be treated focally. Clinical trials generally select healthy patients, but many patients with GBM will have cognitive deficits and require sedation, etc. and more consideration should be given to these kinds of patient needs. Consideration should also be given to creating a patient-friendly set-up for FUS; currently most systems use stereotactic headframes. Fred Wu also advised that the mathematical modeling for the treatment plan should go through QA to ensure accuracy.

Acoustic monitoring has helped to increase the treatment envelope and may also be useful to quantify the amount of BBBO. However, the ability to quantify the amount of drug delivered is not yet optimized. The current standard to quantify the amount of drug delivery is through the use of radioisotopes. Preclinical work should be used to study additional methods to increase drug delivery, such as the use of pre-sonications, pulsed sequences, longer exposures, multiple frequencies, etc.

Demonstrating benefit may prove challenging, and the panelists suggested that early phase trials could use measures of clinical benefit such as quality of life to overcome some of these challenges. Another option is to design trials for specific patient populations that would be more likely to respond, including patients with IDH1 mutations or patients with MGMT hypermethylation.

FUS technology development is outpacing what is known about GBM treatment. Early phase studies applying FUS prior to surgical resection could allow the study of potential mechanisms and underlying biology of FUS in GBMs. The panel cautioned against designing clinical trials in the rGBM setting as well as selecting therapeutic agents that have been tested and failed because they did not cross the BBB. One of the more promising FUS applications to pursue in GBM is BBBO, as this has been found to be safe after extensive translational development. That being said, the long-term effects of BBBO have not yet been explored.

The panel discussed the maximum treatment volume for GBM. There are a lot of factors, but between 60 to 80 cm^3 is likely the maximum volume that can be treated at one time.

Microbubble dosing depends on the drug used. For example, DEFINITY® microbubbles were developed for cardiac imaging and the maximum dose was set by the manufacturer. Resected tumor tissues are under activation investigation to look for immunological markers. Preclinical research could also be useful for informing GBM trials in humans to look at different FUS modalities (mechanical and thermal) and at different time points to understand the biology in a deeper way.

Technologies currently in development for FUS include custom-printed helmets for each patient that are optimized for the target and use the phased-array technology capable of detecting microbubbles with high spatiotemporal resolution.

For patients, the biggest drawbacks to FUS treatment are head shaving, the use of a headframe, and the extensive time in the MRI. The implantable SonoCloud system developed by Carthera does not have these drawbacks and sonications can be performed in 4 minutes outside of the MRI without head shaving.

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Patient Selection

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Discussion

Clinical unmet needs

Jason Sheehan, MD, PhD, Moderator
Manmeet Ahluwalia, MD, **Mitchel Berger, MD**, **Henry Brem, MD**,
Susan Chang, MD, **Michael Lim, MD**, and **Roger Stupp, MD**

FUS-related publications continue to grow exponentially, and there has also been growth in publications on brain tumors and GBM. Jason Sheehan discussed results from a poll of meeting participants. Participants felt that the greatest unmet clinical need for patients with GBM was recurrent tumors and 'all of the above' (frontline therapy, residual tumor post-resection, recurrent tumor, and radionecrosis). Participants did not agree on what stage is best suited for initiation of the next FUS clinical trials in GBM. Responses were mixed between recurrent tumor, residual tumor post-resection, and frontline therapy.

The panel discussed the survey results and the path forward. FUS has the potential for treatment at each stage: initial, residual, and recurrent. A major opportunity is for BBBO to enable treatment of the tumor in a manner that slows disease progression. Another major opportunity is being able to treat areas of the brain that cannot be removed surgically with the aid of real-time monitoring. Further down the line, when the technique has been sufficiently refined, direct ablation of the tumor or micro-metastases represent yet another good application for FUS.

Other avenues for treatment include using FUS in different ways to elicit an immune response. First, understanding the immune response of the tumor will be essential for determining

candidate immunotherapies to combine with FUS. These efforts could also be directed at finding ways to reduce pro-tumorigenic inflammation.

The recurrent setting has proven difficult for drug development and has thus far yielded no effective treatments. However, this also means that it is an area of high unmet need wherein even minor incremental benefits could help patients. GBM is different from other tumors with high mutational burden because, so far, single-agent immunotherapy has yielded no significant clinical benefit. Single-arm trials without biomarkers are unlikely to yield results that will move the treatment forward. It was also suggested that stakeholders in the field collaborate on the design of future clinical trials so that each trial can inform other clinical trials (synergy), even if the trials are not being run at the same institutions; this can also help prevent redundancy.

There was discussion around optimal future trial designs. Questions that should be answered in future clinical trials pertain to timing of drug administration, duration of BBBO, the volume of opening, and various FUS parameters. Current needs are focusing on the technology and figuring out what needs to be done to move it to the clinical setting.

The panel stressed effective clinical trial design, for which considerations include

1. selecting patients for the specific treatment being tested and
2. measuring appropriate outcomes.

Proof-of-principal is important for FUS trials. The pre-surgical trials that are underway should inform FUS mechanisms for GBM, as well as enable examination of heterogeneity in the GBM microenvironment. They present an opportunity to survey the primary tumor tissue, imaging, and liquid biopsy specimens in order to better understand the biology of GBM and potential biomarkers.

Treatment Monitoring

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Radiologic assessment

Current protocols and future directions

Patrick Wen, MD, Moderator
Benjamin Ellingson, PhD, **S. Ali Nabavizadeh, MD**, and **Max Wintermark, MD**

Monitoring treatment response in GBM has been a significant challenge. Given the complexity of chemotherapy, immunotherapy, and FUS in the treatment of GBM, there are a lot of unknowns. For example, even selecting the tumor area to treat with FUS is complex. Embracing experimental imaging techniques will help to understand the tumor microenvironment.

The panel reiterated that GBMs are very heterogeneous tumors—even within the same tumor, there can be regions with different microenvironments.

PET with specific tracers could be helpful in understanding how FUS works for the treatment of GBM, particularly in the pre-surgical setting. Downstream targets could also be assessed, such as tumor metabolism. Eventually, imaging biomarkers could be helpful as a proxy to determine efficacy of a given treatment.

Long-term responses could be assessed with modified Response Assessment in Neuro-Oncology (RANO) criteria. The modified RANO criteria assesses if the tumor is growing beyond transient changes; this measure has performed well in predicting tumor growth in patients with GBM. To differentiate progression from pseudo-progression, PET tracers can be used as well as machine learning techniques. Pseudo-progression remains a major issue in treating GBM.

Liquid Biopsy

Current protocols and future directions

Chetan Bettegowda, MD, PhD, Moderator
Hong Chen, PhD, **Ying Meng, MD**, and **Houtan Noushmehr, PhD**

The panel discussed the types of analytes that are close to clinical translation that could be used with FUS-enhanced liquid biopsy for GBM. The biggest challenge for identifying analytes is the limitation of protocols on what can be collected and analyzed; future trials should design protocols with the ability to collect blood for analyte analysis. Some analytes are time specific (i.e., can only be detected immediately after BBBO), so the ability to detect them will depend on when the blood is sampled. Another consideration is the size of the analyte, and this may depend on the nature of FUS parameters. FUS parameters could be optimized to elicit the analyte of interest. Epigenetics can be leveraged following extraction of cell-free/circulating

tumor DNA from plasma or serum. Some preliminary studies are collecting and storing blood for further analysis of analytes. In the future, it will be helpful to generate a repository of samples from clinical trials to aid in robust identification of key analytes.

Liquid biopsy for discernment of pseudo-progression from true progression could be useful. A unique advantage of FUS lies in its ability to target a discrete region of the tumor, following which blood samples can be collected. One long-term goal is to have a progression monitoring tool. Details of the protocol for blood collection are important and should be standardized (e.g., sample tube types, etc.).

Clinical Trial Design

Regulatory considerations

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Combinatorial Trials Using Focused Ultrasound

An FDA Perspective

Gautam Mehta, MD and **Gregory Clement, PhD**

The Oncology organization at the FDA is made up of several cross-disciplinary offices including the Center for Biologics Evaluation and Research (CBER), the Center for Drug Evaluation Research (CDER), the Center for Devices and Radiological Health (CDRH), and the Oncology Center of Excellence (OCE). Regulatory review in oncology uses a multi-disciplinary approach for patient-centered decision making. The Office of Science and Engineering Laboratories (OSEL) does regulatory research as well as scientific reviews. OSEL has an individual program for research on therapeutic ultrasound. The laboratory evaluates devices on power, pressure, intensity, temperature, etc. For combination products, early phase concerns are related to safety. Once a product is assigned to a center (IND or IDE), this continues for the duration of the regulatory process. When it is unclear whether a combination product is a drug or device, the Office of Combination Products can help make the determination. The primary mode of action determines the center.

For INDs reviewed by CDER, the review process includes non-clinical toxicology and pharmacology, clinical experts, statisticians, product quality, clinical pharmacology, and engineering. For IND applications, the regulatory review process spans drug development with the goal of protecting clinical trial participants as well as the evaluation of the quality of the scientific study in later phases. The IND review process includes a 30-day safety review that determines if an IND is "safe to proceed" or placed "on hold." The review criteria require that a device does not pose an unreasonable or significant risk of illness or injury and is adequately designed to meet its stated objectives. The FDA also considers the patient population and the availability of therapies, seriousness of disease, known toxicities and/or toxicity in animals, and special populations (e.g., age, pregnancy). Dose is also reviewed

as part of the IND review. Dose-limiting toxicity (DLT) should be clearly defined. Safety monitoring is also important to the IND review. Applicants need to provide a calendar of events (testing schedule); first-in-human studies may need frequent monitoring and labs due to possible toxicities. Informed consent is also important. It is vital not to oversell benefits and minimize risks.

For complex studies, a pre-IND meeting should be requested prior to IND submission, the meeting will include the team that will review the application. Applicants should prepare specific questions and prepare detailed procedures, or a complete protocol, for topics to be addressed.

Ultrasound safety considerations were also discussed. There are existing standards for high intensity focused ultrasound (HIFU) devices. In terms of ultrasound safety, mechanical and thermal (total energy) safety is the primary consideration. Device considerations that are almost always considered under an IND for any ultrasound system:

- Full device description, including transducer and standoff materials
- Instantaneous and time-averaged acoustic output powers
- Output frequency (or frequencies)
- Calibrated plots of acoustic pressure along with focal dimensions
- The peak pressure of the underrated field (in water) while operating at clinical-level powers
- Duty cycle, and any other relevant pulse parameters
- Total sonication time(s)
- Measurements/simulations estimating clinical acoustic and thermal fields
- Data supporting safety under "the worst-case operating conditions"

A list of ultrasound-related standards and guidelines was also described. Guidance based on physiotherapy and diagnostics may be relevant to some devices. The Medical Device Development Tools program was devised to streamline development. Qualifying tools, applied within their context of use, are accepted by FDA.

Panel Discussion

Jessica Foley, PhD, Moderator
Amy Barone, MD, **Bennet Blumenkopf, MD**, **Greg Clement**, **Subha Maruvada, PhD**, **Gautam Mehta, MD**, and **Matthew Myers, PhD**

Panelists responded to a question on what is needed for applications of combination products with respect to FUS and BBBO. In terms of non-clinical data, it is important to evaluate the exposure of the drug to the brain before and after FUS is delivered. Particularly if the drug has not been given to humans, high doses and attempts to assess any CNS toxicity are important. Applicants were encouraged to take advantage of the pre-submission process; this

is a good opportunity to work through questions prior to a formal submission. For example, the pre-submission process on the device side is a good opportunity to discuss the volume of BBBO and doses of microbubbles prior to formal submission.
A participant asked the panel if there was a path to decouple the device and BBBO procedure from the drug. The panel responded that there would be concerns with device interaction and compatibility with drug. Some devices are approved as tools, but not when they are considered as a therapy, such as for essential tremor. Each drug would be looked at individually in terms of FUS and BBBO. Another issue was the FUS and microbubble interaction; at this time there is not enough known about safety to separate the different components. There were also concerns that drug concentrations in the brain would be higher than previous research, if any had been done, so that would also have to be considered.

The panel was asked about the use of microbubbles and things that would be required for applications. At this time, the patterns between BBBO and microbubble parameters (size and constituents) are unclear. Important questions to answer include which bubble parameters produce a BBBO of a given duration, etc. in a predictable way.

There was a question on how different devices that produce reliable BBBO would be considered and whether they would be interchangeable. The panel responded that as long as the performance of one device is the same as another in terms of important parameters it would be considered. Preclinical work would be required to demonstrate safety at incremental volumes equivalent to the human.

The panel was asked about potential for use of liquid biopsy in the GBM and FUS space. Liquid biopsy would be considered an experimental endpoint as it has not yet demonstrated clinical benefit.

There was a question on how to study systemic immune effects with FUS. This likely depends on whether safety or efficacy is the focus. Safety is a key consideration due to the risk for heightened immune responses and that needs to be addressed in an IND, including rules for stopping treatment if there are safety concerns.

Adaptive clinical trial design for device/drug combination trials were considered by the panel. These kinds of trial designs are better for later-stage development. FUS device/drug trials are earlier in development at this time. At later stages, this would likely be feasible. Pre-submission meetings could help to make sure that the trial has a design that will satisfy safety and efficacy requirements. Another challenge for FUS and GBM is that drugs are not labelled for GBM. Panelists responded that the concerns with this were the unknown interaction between the device and the drug as well as the efficacy of the drug for GBM. Safety of the drugs was also a concern particularly considering that dose levels might be higher in the CNS with FUS-mediated delivery. In conclusion, participants were encouraged to reach out to the FDA early in the clinical trial application process.

Reimbursement

What evidence is required for reimbursement?

Jessica Foley, PhD, Moderator
Stephanie Kennan, MBA, and **Dee Kolanek, AAS**

It is important to remember that in order to have reimbursement, coverage also has to be in place for the Centers for Medicare and Medicaid Service (CMS). Whether to obtain a national coverage decision or local decisions (Medicare contractors individually) is another consideration. A national coverage decision will require 5 to 10 years of data, and a negative decision will make it difficult to get reimbursement from local contractors. Reimbursement levels are frequently updated by Medicare and need to be monitored. Early engagement on regulatory issues related to coverage was recommended. There is no preferred formula on the data required, but safety, efficacy, and durability (5 years preferred, 2 is the minimum) will be key in the decision-making process. For CMS, clinical trials need to be based in the US and published in US-based journals. The challenges for FUS were often the number of patients in the study and the length of the study. For reimbursement, coding, coverage, and payment all have to be in place for market access.

The panel responded to the challenges of reimbursement unique to drug-device combinations. Early engagement on the concept will be vital. Detailed information on the science behind the treatment will be helpful. To get a Current Procedural Terminology (CPT) code, medical specialists need to be involved. The CPT process is decided by the American Medical Association (AMA) and relies on the specialties to advocate for new CPT codes. The specialty societies are the drivers for new CPT codes. One successful strategy that has worked in the past, is to get the specialty societies to publish a position statement. Private payers often will not meet with companies. There are different categories of codes for CMS and the Healthcare Common Procedure Coding System (HCPCS) can be used for CMS reimbursement prior to receiving a CPT code. The panelists also cautioned that when using the 510K process for approval from the FDA, consider the reimbursement implications for the predicate product.

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Roadmap & Discussion

Led by **Suzanne LeBlang, MD** and **Lauren Powlovich, MD** via Zoom

The FUS Foundation created a list of 'Burning Questions' to guide the discussion throughout the workshop. All participants were given the opportunity during an open discussion forum to comment on these questions and other important considerations to move the field forward.

Targeted drug delivery across the Blood Brain Barrier

What new drugs/therapeutics should be explored?

- Drugs with off-target neurotoxicity
- Two spaces: cancer neurobiology and immunotherapy; there is a need to learn more about the interaction between tumor and other cells in the microenvironment. Further research could look at T cell coordination and other immunomodulation techniques
- There should be the ultimate "home run" goal of curing GBM, but also the practicality of what is possible in a clinical trial. Cancer biology drugs ("base hit"), start with drugs that are already approved for other tumors rather than experimental ones for FUS. Studies should focus on these high hit targets, for example computational efforts that focus on finding targets for known drugs

What is the timing between dosing of the therapeutic and FUS delivery?

- Dose is dependent on the size and formulation of the drug
- FUS BBBO with microbubbles and drug delivery should be started at a consistent time in preclinical trials. Currently, there are discrepancies in timing of therapeutic delivery relative to FUS in both the preclinical and clinical literature
- Timing should also be considered from the standpoint of what is practical/feasible in clinical workflows
- For orally administered medications (pediatrics): you have to know the pharmacokinetic (PK)/PD and when the drug will reach its max concentration in the blood. This will provide further information on timing of administration. Clinical trials of FUS and BBBO using drugs with known safety data are easier to design than studies combining a procedure and a drug with little prior safety data

How is confirmation of drug delivery performed?

- Labeling of the carrier (droplet or bubble) for subsequent MR imaging
- Metabolic labeling allows visualization of the target
- Radiolabeling of therapeutics for quantitative spatiotemporal mapping by PET imaging
- Optical imaging techniques

Blood Brain Barrier Opening

How do you confirm focused ultrasound blood-brain barrier opening?

- T1 weighted images, DCE, and SPECT
- However, do not focus only on a T1 weighted image
- Ferumoxytol, will also tell you about microglial activation and lymph node activation
- Magnetic transfer T1 weighted sequence allows visualization of contrast-enhanced white matter, consider going to a 3D sequence
- Contrast free techniques such as diffusion tensor imaging (DTI), which correlates with the volume of BBBO
- Post-contrast FLAIR

What are the sonication parameters needed to open the BBB?

- Different systems will have different parameters, but it is important to find ways to monitor the emissions generated by the microbubble given certain settings that the system might have
- AI or machine learning was suggested. At this time, there has not been much effort in this domain yet due to limited data. Predictions are more reliable when you have a linear system (microbubbles are non-linear). Gather the data first before using AI for confirmation
  - At this stage, standardizing outputs that could eventually be integrated into machine learning algorithms could be useful
  - At this time, some researchers are using AI for confirmation of BBBO in order to give dramatically reduced doses of gadolinium and move towards contrast free methodology

Immunomodulation

Can focused ultrasound stimulate an immune response to GBM?

- Participants agreed that FUS alone may not be able to stimulate an immune response in the GBM setting, but the results of the GBM consortium project will speak to this more definitively

What modalities are most effective at stimulating an immune response?

- Mechanical modalities show the most promise
- Additional preclinical data is needed across FUS regimens and experimental parameters
- Part of the challenge is that the preclinical models currently used are responsive to checkpoint blockade monotherapy independent of BBB—unlike human GBMs
- Consider the sterile inflammation response

How do you monitor the immune response

- There are guidelines developed for clinical and preclinical studies that can be found on the FUS website. Standardization is very useful, and researchers should start with these guidelines
- Monitoring is different for patients vs animals
- Geo-spatial profiling is a new technique that is gaining traction for preservation of spatial context in the process of high-dimensional immune profiling (e.g., Zellcanner One)

Immunotherapeutic Agent Delivery

How do you confirm delivery of the agent?

- Tagging CD8+ T cells with radioisotopes or using metabolic approaches in vivo
- CAR-T's can be labelled ex vivo
- One challenge is the ability to detect antibody in the bloodstream to measure immune response for GBM. Another challenge is finding a biomarker in the bloodstream that correlates to increased immune response against GBM. It will also likely be context context dependent pending the immunotherapy paradigm under consideration

Radiosensitization

What is the role of focused ultrasound in radiation treatment in GBM?

- Reduce radionecrosis
- Increase effectiveness of radiation therapy in combination with chemotherapy or radiosensitizers
- Further study on whether FUS can increase oxygenation penetration of GBMs is needed as this is a major reason radiation treatment of GBM fails and overcoming this barrier is an area of unmet clinical need

What is the dosing?

- Depends on the modality, i.e., hyperthermia (HT) vs FUS/microbubble
- BBBO will likely be the first modality to enter clinical trials since it is furthest along clinically in other areas
- HT may provide an additional boost, but still has technological limitations related to off-target heating of tissue in the brain setting

- In need of safety studies focused on cavitation to demonstrate feasibility
- Giving a clinical dose of radiation (or alternative dosing for frail/elderly) in combination with FUS and BBBO in a preclinical model for safety has to be done before moving to clinical trials

What is the timing?

- Recent research suggests FUS plus microbubbles (cavitation) before radiation may be the best approach, but this has not yet been shown in a model of GBM

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Ablation

Thermal Ablation

Does thermal ablation have a role in GBM treatment?

- Studying thermal ablation in less vascular tumors than GBM
- Partial ablation to stimulate an immune response
- Use of microbubbles to enhance thermal ablation is worth exploring to expand the treatment envelope

Microvascular Disruption

What are the focused ultrasound settings used to achieve microvascular ablation?

- Nanodroplets and microbubbles are both being explored
- Determine if it can be used alone or in combination with radiotherapy, etc.
- Study whether microvascular ablation should be used to replace surgical debulking, or otherwise combined with surgery

What is the optimal microbubble infusion protocol?

- The peak contrast between the tumor tissue and brain tissue when using microbubbles is during the peak enhancement of the microbubbles
- A bolus injection might work better for ablation protocols, infusion may work better for BBBO
- Microbubbles require lower pressures compared with perfluorocarbon nanoemulsions are different

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Technology

What are the desired features and functionality of a device for the treatment of GBM?

- Techniques to improve the patient experience are needed as previously mentioned; avoidance of head shaving and long amounts of time in the MR imaging machine

What are the technology gaps?

- Closed loop acoustic feedback monitoring
- Must have microbubble control (in space and time) with real-time capabilities

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Roadmap

Attendees brainstormed ideas to move the field forward. They discussed drug selection, microbubbles, and preclinical modeling of GBM.

- To strengthen the hypothesis that FUS + microbubble-mediated delivery of therapeutic agents to GBM has safety and efficacy, there will need to be dose escalation studies and corresponding tumor responses. This will be key to regulatory approval. Preclinical studies also need to demonstrate the same principle
- Another high-value target could be the delivery of drugs that are not absorbed into the bloodstream but only accumulate in tumor tissue
- Drug selection is important but there was some debate about the best strategy. There are drugs with potential to treat GBM if they are delivered across the BBB. There was a suggestion to select drugs with known safety data, such as those drugs that have at least gone through phase 1 and 2 trials
- Better preclinical models of GBM need to be developed and employed in FUS research. The model needs to have a microscopic infiltrative component to match the clinical scenario
- The bolus technique for microbubble dosing will not work with transcranial FUS systems as BBBO requires multiple sonications and the half-life of microbubbles is too short to accommodate this. Bolus is currently used by the Carthera system which requires only a single microbubble infusion
- Participants agreed that the field needs to determine the best kind of microbubble to use with BBBO. Should diagnostic microbubbles continue to be used, or is a FUS-specific microbubble needed? Factors to consider include PK, level of oscillation, and mechanical properties of the bubbles
- For the early phase trials, it is vital to collect samples for RNA-seq to understand what is happening at a multidimensional level based on varied FUS modalities and parameters, etc. It is also important to collect data on BBBO alone
- Creating a database of MR images from FUS treatments would be helpful

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Outcomes and Next Steps

Participants were encouraged to reach out to the Foundation with any research ideas or project proposals in this area. The FUS Foundation will continue engagement with this community to move the research forward.

Pre-workshop Education Content

Current State of the Technology

A Portable and Flexible Transcranial Focused Ultrasound Device for Blood Brain Barrier Opening

Elisa Konofagou, PhD opened the presentation with an overview of FUS-induced BBBO with microbubbles. The ultrasound beam engages microbubbles, leading to oscillation, allowing the vessel walls to become more porous and permits drugs to reach GBM cells. In this method, IV microbubbles are administered first. Then, FUS is applied to induce BBBO through oscillation of microbubbles within the acoustic field that then disrupts the BBB. Lastly, the drug is delivered either IV or intraperitoneally, and extravasates from the blood vessels to reach neurons, cancer cells, or other targets.

The UltraNav® device provides neuronavigation-guided FUS BBBO. The procedure involves 3 steps:

1. pre-planning (simulation),
2. targeting (neuronavigation), and
3. monitoring of microbubble activity in the acoustic field via real-time acoustic cavitation mapping.

An MRI and CT scan help to predict the amount of attenuation expected and adjustments to the output pressures can be made. The tumor must be located on the MR image right before the procedure to ensure that it is targeted effectively. The ultrasound regime is then activated, and cavitation, bubble activity relative to the target, and safety are monitored throughout the procedure. The procedure is noninvasive, low-cost, does not require an incision, can be performed in the neurological clinic or patient point of care, and takes approximately 30 minutes. UltraNAV® is already approved by the FDA to perform clinical trials in Alzheimer's disease.

In a preclinical mouse study of glioblastoma (GBM), researchers showed that FUS-enhanced etoposide (5 mg/kg) resulted in a 5-fold higher concentration at the tumor site relative to etoposide alone; this also reduced tumor volume by two-fold, which increased mean survival to 30% relative to etoposide or BBBO alone.[2] Englander and colleagues showed a similar effect in a mouse model of diffuse midline glioma (DMG). Etoposide concentration increased 8 to 10 times at the tumor site as a result of FUS BBBO and increased tumor uptake.[9] When given in conjunction with panobinostat, FUS improved local tumor control in GBM. A clinical trial of FUS and oral panobinostat has been approved in pediatric patients with DMG and will be launching very soon (NCT04804709).

Minimally Invasive Focused Ultrasound for Neurosurgical Treatments *From the Lab to a Startup and Beyond*

Amir Manbachi, PhD and **Nao Gamo, PhD** , presented the device being developed by NeuroSonics Medical. The goal for NeuroSonics Medical is to develop a miniaturized FUS device to allow neurosurgeons to treat brain tumors in a minimally invasive manner with enhanced efficiency.

Brain tumors affect 700,000 patients in the US, of which 80,000 are newly diagnosed. Approximately 167,000 of these patients are candidates for surgery. The standard of care in patients with brain tumors is craniotomy, an invasive surgery with associated risks of infections, damage to healthy brain tissue, and prolonged post-operative recovery. Available devices on the market include ExAblate Neuro® by Insightec, Visualase® by Medtronic, and NeuroBlate® by Monteris Medical. These devices may be effective in essential tremor, Parkinson's, and epilepsy but are not optimal in brain tumors. The brain is a sensitive target with substantial eloquent tissue; and these devices remain expensive, invasive, and require a MR imaging suite. Challenges remain in depositing ultrasound at the target region and energy loss (>90%) occurs as it passes through the skull. Tumors located in the center of the brain are very difficult to treat with these devices.

NeuroSonics proposes to place the FUS device through burr holes and a trocar device. The device is a minimally invasive FUS probe with improved steering capability and can create a focal point 4 to 5 cm away from the device. In non-operable tumors, FUS transducers can be placed anywhere the burr holes are created and can be used to treat tumors. The NeuroSonics therapeutic device will be inserted within BrainPath™ trocars and focus sound where it needs to go. The BrainPathTM trocars are already FDA approved for patients undergoing minimally invasive brain surgery. This solution uses a minimally invasive toolkit resulting in less invasive surgery, reduced risk of infection, shorter operating time, shorter hospital stays, faster return to normal activities, and improved aesthetics. The first prototype has shown that minimally invasive FUS is viable.[10]

NeuroSonics is partnering with device companies to build the optimal device and move faster in the translational world. To this end, extensive field research was performed to understand the needs of the market. The US market for minimally invasive neurosurgical devices is expanding and estimated to reach $57 M in the next few years. Initial customers are assumed to be 1,200 hospitals that treat most Medicare patients, and this service addressable market is estimated at $216 M. At a later phase, the target addressable markets can reach $998 M and include > 5,700 neurosurgical centers in the US. So far, 3 versions of early prototypes have been designed, built, and tested and the company has raised over $500,000 in grant funding. The group is preparing for a pre-submission meeting with FDA to confirm the regulatory pathway and clinical trial design. Once preclinical safety and efficacy studies commence, findings will be used to move forward with design and manufacturing.

MR-guided Drug Delivery

Rafi de Picciotto, PhD provided an overview of the key elements required for an ultimate BBBO solution. A medical device designed to deliver drugs to the CNS must be noninvasive, facilitate safe and reversible BBBO, and permit repeated BBBO according to scheduled treatment regimens. Focal therapy may represent a significant advantage in certain indications. The optimal device must deliver the desired drug amount to a target location and confirm its delivery.

The MR-guided drug delivery program started at Insightec 5 to 6 years ago with Sunnybrook in Canada and has since expanded to a growing network of research sites, with numerous clinical protocols. Despite chemotherapy and radiotherapy for GBM, remnant tumor will infiltrate neighboring tissue resulting in rGBM and further spread into vast areas of CNS.

The first challenge is the need to treat large tissue volumes. Additionally, there is a limit on the microbubble amount that can be used in FUS BBBO. The procedure also needs to be completed within a particular time window.

In neurogenerative disease, treatment must be targeted at precise brain areas, possibly eloquent tissue, and the FUS device must be equipped to target drug delivery within very small spots. GBM tumors may be located right next to the skull, and skull aberration correction will be important to maintain focal therapy and facilitate precise drug delivery. In brain metastasis, multiple foci need to be targeted. The FUS device must be able to switch quickly, treat some targets simultaneously or twice. Advanced transducer design can overcome the problem of "hot spots" due to limited steering or grating lobes, and cause sound waves to concentrate in areas that are not targeted or planned. These newer designs allow large e-steering of focal spots, maintain tightness, and permit quick steering between and parallel treatment of different foci.

In addition, the uniformity of drug delivery in heterogenous tissue must be maintained. Within a large target area, microbubble activity might be non-homogenous due to local changes in tissue or vasculature, showing a heterogenous local response. MRgFUS possesses sophisticated mechanisms that can control parameters locally and assure uniform bubble activity map. In a pig model, the controller was shown to control the entire target on average vs local control activity. These features are applicable for drug delivery to a large volume of tissue in GBM.

Neuronavigation-guided Focused Ultrasound *A Platform for Accelerating the Treatment of Central Nervous System Disease*

Tim Liu presented the NaviFUS device (also referred to as NAviFUS-001). NaviFUS uses a novel neuronavigation technique to help simulate the optimal opening of the BBB near a tumor. The neuronavigator uses optical tracking to visualize the position of a surgical instrument by recognizing fiducial markers (registration), which are custom NaviFUS markers.

The standard treatment workflow includes pre-treatment MR imaging to confirm the tumor and select target location, CT scan, personalized treatment plan, and skull penetration estimation that is integrated into the neuronavigator. The procedure takes 30 minutes and consists of 4 steps:

1. setting up the neuronavigation guidance,
2. IV microbubble injection,
3. FUS sonication, and
4. post-treatment MR imaging.

Three NaviFUS hardware technologies were highlighted: focused point steering, passive cavitation detection (PCD) feedback control, and passive imaging of FUS energy. NaviFUS is a phased array with a 256-element transducer, up to 32 elements can be used as a receiver for different functions.

NaviFUS transducer design confers several advantages to the procedure: each element can control output energy and relative phase difference among elements independently, precise control of the focusing point resulting in improved and more efficient dynamic scanning effect, and multiple focal points can be targeted for distribution of ultrasound energy allowing increased flexibility of therapy. The system has a frequency of 500 KHz, burst length of 300 μsec, and an output acoustic pressure of 0.1 to 3 MPa. A single focal beam can have a focus distance of 140 mmm, a focal beam dimension of 3 x 3 x 20 mm, a focal scanning matrix of 43 beams, separated by 3 mm between each other, so that an overall 20 mm a focal beam steering video can be built.

PCD control has been used to validate safe levels of exposure in animals and was shown to reliably open the BBB and minimize the risk of side effects. PCD control is currently being utilized in a first-in-human clinical trial using FUS with bevacizumab. The system also uses transcranial passive imaging to visualize FUS energy. It utilizes 32-channel receiving to construct passive imaging of US energy, a technical breakthrough compared with previous technologies with 4 receiving channels, unable to reconstruct the passive image. This feature allows transcranial mapping of the FUS focal beam target position, resulting in significantly higher signal-to-noise ratio and improved visualization of acoustic emissions.

The software enables users to customize treatment in two- and three- dimension views and estimate transcranial penetration rates. The NaviFUS PCD function can provide personalized acoustic output. The effectiveness of the device and safety parameters were based on extensive translational studies from animal studies. NaviFUS is being tested in clinical trials in brain tumors (BBBO), epilepsy (neuromodulation), and Alzheimer's disease. NaviFUS first-in-human clinical trial in rGBM demonstrated safety in human use, and BBBO was noted immediately and returned to baseline with 24 hours. Another trial in epilepsy completed in 2020 (n=6) showed decreased EEG power in 2 patients after sonication, and decreased seizure frequency in another 2 patients at 1 to 3 days from baseline.

SonoCloud System

Michael Canney, PhD discussed the SonoCloud-9, an implantable ultrasound device designed to induce a temporary disruption of BBB, developed by Carthera. There is a pipeline of clinical programs evaluating the device in CNS indications, including GBM, brain metastasis, and Alzheimer's disease. Results of the first phase 1/2 clinical trial in rGBM have been published.[11,12]

SonoCloud-1 is a small implant designed to fit in a burr hole in the skull. The earlier SonoCloud-1 design was used in pilot trials in GBM and Alzheimer's disease. The SonoCloud-9 (SC9) is a larger device, designed to sonicate a much larger volume of tissue. It is now being evaluated in 3 different clinical trials in GBM. The device consists of 9 US emitters spaced over a 6 x 6 cm grid, each emitter operating at 1 MHz and sequentially activated for a 4-minute duration to perform BBB disruption. The device is compatible with MR imaging. A transdermal needle is plugged for each activation, passes through the skin, connects into the port, and provides power to drive the device. An external generator with touch screen guides the user through the treatment, when microbubbles are injected, and activates the device. Three early phase clinical trials are evaluating SC9 in combination with various therapies such as carboplatin, nab-paclitaxel (Abraxane®), and TMZ in newly diagnosed and rGBM patients. An ongoing phase 1/2 trial is exploring carboplatin in combination with SC9 in rGBM, in an international multicenter setting (US, France), with plans to recruit 33 patients. Another phase 1/2 trial is planned and will evaluate albumin-bound paclitaxel in combination with SC9 in rGBM. This trial plans to recruit 34 patients (NCT04528680). The third program, SC9-SONOFIRST, is a multicenter trial recently launched in Europe in patients with de novo GBM exploring SC9 with TMZ (NCT04614493). The SonoCloud treatment has been well tolerated with approximately 50 patients being treated, including the elderly population. The sonication procedure is easy, takes less than 10 minutes, and has been repeated in some patients for up to 12 sonications. There is no need for imaging or guidance or head shaving and BBB disruption can be done in a chemotherapy infusion suite by a skilled nurse or physician.

Progress in Developing NextGen Brain FUS Systems

Kullervo Hynynen, PhD opened the presentation by providing a rationale for developing the NextGen helmet. While the Insightec device and other devices are effective in brain tumors, they can only perform ablation in the center of the brain leaving other brain areas out of reach. MR imaging is expensive, not widely available, and cannot be applied on the long term. In contrast, the NextGen device can ablate throughout the brain, deliver multiple treatments, control bubble activity, and functions outside of a MR imaging scanner. It is also a portable and low-cost system. All aspects of design including beam steering were optimized via computer simulation. In vivo experiments of sonication and acoustic beam in the brain showed enhancement of BBBO.

The device provides for acoustic monitoring during bubble-induced ablation. Researchers are constructing a spectrum that allows them to either increase percentage under received harmonic signal, keep the same power, or increase by 50%, 100%, 150% of that level. Signals

generated from microbubbles show the sonication location from which researchers create algorithms to predict the location of tissue damage. The acoustic monitoring feature helps assess the volume of damage as a function of the percentage of exposure level (no tissue damage at lower exposure levels, starts at 100-150%). Passive acoustic mapping can generate time information for each location and create 4 dimensional maps of bubble activity that can be used to calculate tissue damage. This principle of concept for the NextGen helmet showed its feasibility and the possibility for using this technique to target tissue and control exposure. This technique can be used to create a custom helmet, though additional studies will be needed to translate it to the clinic.

Transcranial Histotripsy Devices for Brain Applications

Zhen Xu, PhD stated that in contrast to transcranial HIFU thermal approach, histotripsy uses internal cavitation to liquefy target tissue via microsecond-length pulses at low-duty cycle and high pressure. The use of very low-duty cycles avoids overheating the skull surface. Hence, transcranial histotripsy can treat a wide range of locations and volumes, including those close to the skull surface without overheating. Transcranial histotripsy device requirements include the ability to generate 1-cycle pulses, a peak negative (peak-) pressure > 26 MPa, and a duty cycle < 0.1% through the skull. This creates an intrinsic threshold to generate cavitation confined in small zones and increased the accuracy compared with shock cycles. A duty cycle of < 0.1% through the skull allows for a long cooling time between pulses reducing heating, an important factor in treatment large areas of the brain. Transcranial histotripsy devices are required to be MR imaging compatible to help guide treatment.

The latest transcranial histotripsy system is a 360-element array, with a frequency of 700 KHZ and a focal distance of 15 cm. The system can achieve a peak- pressure > 26 MPa, an electronic steering range of 55 mm x 57 mm x 64 mm allowing treatment of a volume up to 105 ml using electronic focal steering and intrinsic threshold cavitation through skull.

Preclinical research in pigs have been carried out. A truncated array was used to 0.74 (128 elements) due to the size of the pig brain. An excised human skull was used, and craniotomy performed. Through the skull aberration correction, this system can generate 73 MPa, with a focus on electronic steering range, 37 x 35 x 50), peak- pressure > 26 MPa. Compatibility with MR imaging use has been demonstrated. In this in vivo pig brain treatment, histotripsy ablation was successfully observed in 8 pigs through the excised human skull. Treatment zones were visualized post-treatment and 2 hours post-treatment on T2 weighted and T2*w images. The histology location and size of treatment zone matched MR images. No excessive hemorrhage or edema were observed.

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Blood Brain Barrier Opening
General

Microbubble Distribution and Administration Protocols During FUS

Francesco Prada, MD stated that there are several devices currently employed in the clinic for BBBO in brain tumors including MR imaging-guided systems, implantable devices, and external navigation devices. All of them have been used in conjunction with bolus injections of microbubbles. Prada reviewed protocols of administration of microbubbles, including infusion of microbubbles. BBBO can be achieved with microbubbles delivered as an infusion without damage.[13] Understanding microbubble distribution and behavior within brain tumors (gliomas) is valuable to define microbubble-mediated treatments.

When injected as a bolus, the levels of microbubbles peak initially, then circulate throughout the body, wash out from organs, then reach a slowly decreasing steady state.[14] In contrast, when administered as an infusion, microbubbles reach a steady state.[14] For the same amount of microbubbles, the microbubble duration in the blood stream is twice as long after an infusion than a pulse injection, though the peak is much stronger in a bolus administration compared with an infusion.[15]

Applications of microbubble infusion in the human brain were reviewed. Potential applications include the feasibility of quantifying cerebral microbubble distribution, BBB disruption that result from microbubble infusion, and noninvasive biomarkers of microbubble distribution. Quantitative analysis of intra-operative data provides information about microbubble distribution in time and space. Studies in large cohorts of patients have shown that different areas of the brain show different concentration of microbubbles. For example, microbubbles are less concentrated in the corpus callosum than the periventricular white matter, and the basal ganglia concentrates similar amounts of microbubbles to brain tumors (anaplastic glioma shown here). In addition, different amounts of MBs can be found within the same tumor; more MBs are observed in the center than in the periphery or necrotic area. More preclinical data is needed to determine whether bolus or infusion is more effective. Finally, noninvasive biomarkers and 3D maps of MBs distribution might give insight into MBs disruption.

Parameters for Blood Brain Barrier Disruption

Nathan McDannold, PhD discussed the parameters that affect BBBO:

- Pressure amplitude (typically Pa< 1 MPa)
- Frequency (typically 200-700 kHz)
- Burst length (typically 1-10 ms); lower range of bursts have been used (0.5, 1, and 5 seconds) leading to smaller end BBBO
- Pulse repetition frequency (typically 1-10 Hz)
- Duration of sonication (typically 60-120 seconds); a longer duration results in larger opening but increases the incidence of damage. A maximum duration of sonication should be in place

- Sonication duration as short as 6 seconds have been applied
- Microbubble agents: different brands (Definity, Sonovue, Optison, etc.) induce similar BBBO
- The microbubble dose (larger doses lead to better opening than smaller agents)
- The typical dose ranges from a fraction of the clinical dose up to 100 times the clinical dose

There are complicated factors that impact the BBBO, such as anesthesia and oxygen, steroids, and pre-sonication of the brain before applying the microbubble and sonication. The BBB closed faster in steroid-treated subjects than those without steroids, and dexamethasone was shown to cause disruption. Researchers noted that pre-sonication improved disruption and boosted BBBO.

BBB disruption can be achieved across a wide range of acoustic parameters however there is no consensus on the optimal parameters. The magnitude of BBB disruption depends on a number of factors: the amount of drug or tracer delivered, the size or other properties of drug or tracer delivered, the penetration, the depth or extent of infiltration of the drug into brain tumor cells, the duration of opening, and the parameters used.

In small molecules of MR contrast agent, the $t_{1/2}$ is 4 hours. The barrier closes to larger agents more quickly. Depending on the time of evaluation, BBBO will be different for different molecules. In brain tumors, the BBB and the blood tumor barrier might affect disruption post-sonication and delivery of MR contrast agent. In a study of liposomal doxorubicin, a large increase in the delivery of the agent was observed. There is a detection threshold for BBBO, and under this threshold, BBBO might be not detected, hence the need to improve the sensitivity and detection. Determining "optimal" parameters to detect BBB disruption and microhemorrhage to ensure the safety window is important.

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Enhanced Delivery of Chemotherapy Using BBBO *Clinical Trials*

Focused Ultrasound in Neuro-oncology *Early Experience in Glioblastoma FUS Trials*

Nir Lipsman, MD, PhD discussed the early trials of FUS for GBM. The field of neuro-oncology has rapidly expanded in the last 30 years with new technologies being developed to overcome the challenge of the BBB and the delivery of therapies to the brain. Significant advances in FUS technology have been made, from an original increase in the BBBO from a size of 1 to 2 cm^3 to 65 cm^3.

The ExAblate helmet developed by Insightec, which includes several features for real-time BBBO and represents a less invasive, spatially targeted approach. The procedure is done under MR imaging guidance and combines a stereotactic procedure, anatomic, and spatial approaches that provides access to brain pathology. Earlier studies have shown that this

approach shortens the procedure time and is technically feasible and safe. Drawing on previous experience with this technique, a multicenter trial was conducted using FUS to enhance TMZ delivery in patients receiving treatment for 6 months. The procedure allowed researchers to target the resection cavity of these tumors. This approach shortened the duration of treatment and an increase in BBBO order of magnitude was recorded. The procedure was well tolerated and showed feasibility of BBBO.

rGBM is a challenging-to-treat, highly malignant condition for which very few effective treatments exist. A phase I multicenter feasibility study was conducted to investigate FUS-induced BBBO and concurrent carboplatin patients with rGBM. The study showed that large volumes of drugs can be delivered near the resection cavity of recurrent brain tumors. FUS was also evaluated in metastatic breast cancer to enhance the delivery of the HER2-targeted immunotherapy trastuzumab. In this phase 1, two-patient cohort trial, FUS was shown to enhance drug delivery to the posterior fossa in progressive intracranial disease and was well tolerated. Results supported safely and reversible FUS BBBO in highly eloquent areas of the brain. Future directions will include combination of FUS with additional chemotherapy drugs and the potential to label the chemotherapy drugs and visualize drugs' passage through the BBB.

The experience in BBBO applications in human oncology trials has been positive overall. Over 150 patients have been treated for different indications including neurodegenerative conditions. The majority of the procedures were outpatient and well tolerated with no serious adverse events (AEs). An important safety mitigation strategy is the acquisition of T2* to measure blood products, the presence or absence of T2 changes that also allow technical fine tuning. T2* and gradient recalled echo changes disappear in most patients. These results support FUS safe targeting of large areas of the brain including eloquent areas of the cortex (motor, sensory, brainstem). Notably, the positioning of the helmet relative to the tumors is important; very large or displaced tumors may require repositioning of the helmet.

The key remaining questions include the timing of chemotherapy administration relative to FUS and subsequent BBBO. In the research described here, chemotherapy was administered either concurrently or shortly before sonication to maximize its onboard concentration. Interest in brain tumor research is growing, particularly pediatric brain tumors such as diffuse intrinsic pontine glioma. FUS technology has the potential to be implemented throughout the continuum of treatment in the upfront, maintenance, and recurrence settings. Beyond therapeutic applications, FUS may also have diagnostic applications and facilitate finding peripheral biomarkers for metastatic or primary disease.

Microbubble-enhanced Focused Ultrasound for Brain Tumors

Graeme Woodworth, MD discussed new technology for FUS treatment of brain tumors. Treatments of GBM are limited by the BBB, which limits entry of most (> 90%) therapeutics in the GBM-invaded brain regions. The mainstay of treatment in brain tumors is surgical resection of contrast enhancing lesions, typically followed by a brief tumor-free period. Unfortunately, recurrence occurs within 17 months, with new enhancements indicating tumor spread observed in the 2 cm around the resected cavity

and across the midline. By improving the delivery of chemotherapy in and around the resection cavity where most recurrences occur, researchers hope to stall brain disease. For these technologies to advance to the FDA approval process, it will be critical to establish the accuracy, safety, and efficacy of BBBO, perhaps as separate parameters.

The safety and feasibility study of ExAblate-induced BBB disruption for planned brain tumor surgery was presented. In this trial, patients undergo BBB disruption prior to surgery to aid individualization of the planned surgical resection volume. The technique can turn non-enhancing T2 hyper-intense lesions into enhancing tissue, enabling the future potential use of intravascular fluorescent dyes to visualize these regions. This method allows for controlled BBB disruption in a region with planned resection and enables researchers to evaluate tissues for safety and drug delivery applications. Patients enter the standard treatment workflow starting with preoperative MR imaging scans (within 6-8 hours of surgery for best results), neuronavigation MR imaging scans, then delivery of MRgFUS.

MRgFUS BBBO has been tested in the first brain tumor patient in the US (NCT03322813). T2 hyper-intense non-enhancing tissue underwent BBB disruption and new T2* changes were observed. MRgFUS neuronavigation scans allow researchers to navigate to T2 hyper-intense areas, and accurately sample treated tumor tissue for comparison with other tissues (controls) within the surgical resection volume. Importantly, as researchers navigate to these regions during surgery, new fluorescence and contrast enhancement was observed. In the future, researchers envision the potential ability to deliver numerous therapeutic agents, including nanoparticles, small molecules, antibodies, and stem cells agents.

In another trial, researchers are investigating the repeated use of FUS to enhance monthly chemotherapy regimens of TMZ, following standard radiation (NCT03551249). The possibility to safely use FUS-enhanced treatment modalities in a repeated fashion opens the door to new therapeutic opportunities for brain tumors. This system provides controlled BBB disruption in patients with GBM undergoing standard chemotherapy. It allows closed feedback loop control during treatment to guide and dose energy delivered within the targeted region.

Phase 1-2 Clinical Trial of Blood Brain Barrier Disruption with Implantable Ultrasound to Enhance Paclitaxel Delivery in Recurrent Glioblastoma

Adam Sonabend, MD discussed disruption of the BBB using an implantable ultrasound device. Prior clinical trials confirmed BBB disruption with the CarThera SonoCloud implantable device.[11] The 2nd generation SonoCloud 9 (SC9) is an implantable ultrasound device that was previously used in clinical trials in combination with carboplatin.

Paclitaxel is one of the most potent drugs used in gliomas and is 1,400 times more potent than TMZ and carboplatin. However, in previous experience using paclitaxel in rGBM, systemic delivery of paclitaxel has shown poor BBB penetration. Both cremophor paclitaxel and albumin-bound paclitaxel (nab-paclitaxel) were tested in preclinical models.[12] In xenograft models, BBBO and co-administration of paclitaxel was shown to increase drug

concentration in the brain parenchyma ~4 to 6 times compared with non-sonicated brain, achieving therapeutic levels in line with treatment in glioma cell lines. Nab-paclitaxel is better tolerated over the course of treatment and causes less peripheral neuropathy than cremophor paclitaxel. In preclinical models, a modest survival benefit with nab-paclitaxel plus sonication was observed.

These observations led to a phase 1/2 clinical trial of BBB disruption with the SC9 device to enhance paclitaxel delivery in rGBM (NCT04528680). The trial aimed to determine the maximum tolerated dose (MTD) and DLT during dose escalation regimens to reach the approved paclitaxel dose of 260 mg/m^2. The primary endpoint was clinically significant CNS toxicity of nab-paclitaxel after sonication with the SC9. The phase 2 expansion cohort (n =15) aimed to enroll additional patients at the MTD plus 9 evaluable patients from phase 1 with the hypothesis that 65% OS would be reached at 1 year. The Bayesian optimal interval design of this phase 1 study allowed researchers to redesign the trial after patients finished the DLT period. To date, 5 patients have been enrolled for the study and the treatment was well tolerated.

Preliminary Results of the Combination of NaviFUS System with Bevacizumab in Patients with Recurrent Glioblastoma

Ko-Ting Chen, MD presented data from a clinical trial of the NaviFUS system in combination with bevacizumab. Clinical trials have shown that FUS BBBO can enhance drug delivery in rGBM using different ultrasound systems. Data about FUS BBBO in conjunction with bevacizumab in rGBM are lacking. In a mouse model, FUS significantly enhanced penetration of bevacizumab into the CNS compared with the non-exposed brain.[16] FUS significantly increased median survival time by 135%. In a pilot trial, researchers were able to precisely open the BBB in rGBM patients and suggested a potential immunostimulatory effect by FUS alone.[17]

NaviFUS BBBO combined with bevacizumab will be evaluated in a first-in-human study in patients with rGBM (NCT04446416). The study plans to recruit 10 patients with first recurrence GBM and will be performed in the outpatient setting with a primary endpoint of PFS. Given that >90% of recurrences occur at the margin of resection, the strategy will be for FUS exposure to cover as much of the tumor peripheral region (ROI) as possible. A newer, 2nd generation of array beams were designed to create a larger ROI. To maximize safety, real-time acoustic emission was used with image feedback exposure level control. The NaviFUS system was to be used within 1hr of IV bevacizumab administration. Two patients have been recruited for the study so far. Study results confirmed a larger BBBO up to 2 cm at the target region on contrast-enhanced MR imaging. In patient 1, residual tumor was seen in the frontal lobe. The patient is stable and PFS is 9 months. In patient 4, the recurrent tumor was more extensive in the left temporal parietal region and the beam path was designed to cover these temporal regions. The patient had a PFS of 4 months.

In conclusion, BBBO with the NaviFUS system is feasible to combine with bevacizumab in rGBM as an outpatient procedure, with the goal of repeatedly opening the BBB safely and accurately. A trend of normalizing T2 signal hyperintensity was observed in beam

concentrated regions, which might suggest that FUS BBBO has the potential to decrease edema or tumor invasion through enhanced bevacizumab delivery to the treated site (n=2). More data are needed to analyze whether combination of FUS plus bevacizumab offers survival benefit (PFS).

Immunomodulation

FUS for Immunotherapy in GBM

Kelsie Timbie, PhD presented a literature review of FUS for immunotherapy in GBM and discussed how different FUS modes impact the immune response, including thermal and mechanical regimes. Depending on how each mode affects biological tissue, FUS may interact with the cancer immunity cycle through different mechanisms:

- Direct killing of cancer cells via thermal or mechanical ablation
- Cell death that triggers antigen release
    a. Once released, antigens are sampled and cross-presented by dendritic cells (DCs) for priming and activation of T cells
    b. Activated T cells traffic to the tumor and attempt to infiltrate the tissue.

Low-power mechanical FUS protocols like BBBO can activate the tumor vasculature or disrupt tumor stroma to facilitate the process.

The recognition of cancer cells by T cells can be augmented by immunotherapeutics that FUS can deliver to the tumor. FUSF funded multiple projects to elucidate how FUS interacts with immune response in glioma through thermal and non-thermal mechanisms. Thermal ablation conferred acute GBM growth control but did not significantly affect the immune response in the tumor. HT increased infiltration of activated NK and effector CD8+ T cells, but also myeloid derived suppressor cells (MDSCs). Histotripsy decreased MDSCs and increased interferon (IFN) gamma production, and finally, low-intensity mechanical protocols (e.g., BBBO) increased CD4+ and CD8+ T cells and enhanced dendritic cell activation. These studies showed that different FUS mechanisms have vastly different effects on the immune system.

Sheybani et al, used HT to trigger the release of extracellular vesicles from glioma cells. Extracellular vesicles play a significant role in the cancer immunity cycle, carrying signals from the tumor to other cell types throughout the body—including immune cells. HT increased the number of extracellular vesicles released from glioma cells.[18] When cultured with dendritic cells, HT-exposed extracellular vesicles stimulated their release of IL-12—indicating that extracellular vesicles may play a role in the anti-tumor immune response following FUS.

Several studies have been published on the use of FUS to deliver immunotherapeutics to the brain. FUS can be used to deliver across the BBB anything from small molecules, chemotherapeutics, and nanoparticles to viruses, antibodies, and other products. In one study, FUS was used to deliver IL-12 to the C6 rat glioma models.[19] Results showed

significant increases in intra-tumoral CD8+ T cells and regulatory T cells (T_{regs}). The ratio of cytotoxic T cells to T_{regs} increased. FUS and IL-12 treatment induced tumor growth control and significantly extended survival. Another study combined radiolabeled antibodies and immuno-PET to optimize mCD47 to the brain, unveiling important insights regarding timing of antibody delivery relative to BBB), as well as resulting in tumor growth control and extended survival in glioma-bearing mice.[20] FUS was used to significantly enhance the delivery and distribution of intranasally administered anti-PDL 1 antibodies in a mouse glioma model.[21] The benefit of this approach is reduced systemic toxicity, often a concern for immunotherapy. Another study tested the ability of FUS to enhance NK cell trafficking and infiltration to a metastatic brain tumor model.[22] FUS was shown to improve NK cell trafficking and infiltration, but aggressive treatment must begin in early-stage tumors to provide survival benefits. Front-loaded regimens showed delayed tumor growth with extended survival compared with a biweekly regimen, which did not confer benefit.

Gene and Cell Therapy

Natasha Sheybani, PhD gave a brief overview of FUS and gene therapy and its role in cancer treatment. Cancer diseases comprises 65% of indications among gene therapy clinical trials (as of 2019). The different categories of gene therapy include gene replacement (replacing dysfunctional genes with healthy genes); gene silencing, (knocking inappropriate genes); gene addition (introducing or overexpressing a gene); and gene editing (permanent manipulation of gene at specific locations in the patient genome using tools such as CRIPR-Cas9).

Several strategies have been used in vivo to deploy gene therapies such as suicide gene and prodrug therapy, oncolytic viral therapies, immunomodulatory therapy, and deposition of genetic material through synthetic non-viral vectors and nanopeptides.[23] CAR-T cells are one example of gene therapy done ex vivo. Peripheral T cells are sampled from patient's blood, genetically modified to produce CARs, then infused back into the patient to attack tumor cells expressing the antigen they are programmed against. Various challenges exist in the delivery of gene therapies: the lack of localized, minimally invasive targeting; genotoxicity; the need to improve transfection and transduction efficiency; invasiveness of in vivo delivery; and immunogenicity.

FUS can be leveraged to improve the deployment of gene therapy. Several FUS mechanisms of action relevant to gene therapy have been supported by the recent literature:

1. improvement of gene vector delivery and transfection and transduction,
2. potentiation of gene editing, and
3. remote spatial control and activation of genetic circuits.

Proof-of-concept for non-viral vector delivery using FUS BBBO in preclinical GBM trials has been published. In a GBM study, delivery of brain penetrating nanoparticles (BNPs) bearing plasmid DNA encoding for a reporter gene was shown to improve transgene expression after one round of BBBO. Compared with systematically delivered brain-penetrating nanoparticles (BPNs) alone, FUS-delivered BPNs improved the penetration far beyond the tumor-confined microvessels and into the tissue.[24] Yang, et al. showed that the use of lipid

polymers, hybrid nanoparticles to refine delivery of CRISPR-Cas9 substantially improved delivery and transfection efficiency over control counterparts.[25]

Another category of emerging FUS applications seek to remotely control gene products or activate genetic circuits in a spatially controlled manner. These tools capitalize on the remote capabilities of FUS and the deep tissue penetration of sound waves. Recent work established the ability of FUS-induced BBBO to enhance cell delivery in a tumor model. NK cell accumulation was quantifiably improved by delivery with FUS BBBO, which translated into restricted tumor growth and survival benefits in the animals. A growing body of literature supports the immunomodulatory effects of FUS on cytokine expression via mechanisms of activation of the vascular endothelium, liberation of antigens, and phenotype or functional changes in immune cells that are peripheral or local to the tumor microenvironment. Studies support the hypothesis that FUS may eventually be able to improve delivery as well as persistence of genetically engineered cell products in GBM.

Additional preclinical work in FUS has investigated delivery of therapeutics with the assistance of microbubbles bearing a gene product encoding for immunomodulatory genes, to address bottlenecks in the cancer cycle as well as enhance the performance of immunotherapy in solid tumors. One such study combined low frequency FUS with tumor targeted microbubbles to partially destroy the tumor then transfect remaining viable tumor and stromal cells with plasma DNA encoding IFN-β. This FUS-mediated cytokine transfection was shown to be feasible and effective in potentiating checkpoint blockade. It triggered an immune response that resulted in recruitment of macrophages and CD8+ T cells as well as tumor control when combined with PD1 blockade.[26]

Radiosensitization

Research Overview
Role of FUS for Radiosensitization of GBM

Frédéric Padilla reviewed that the standard of care in GBM is surgery with radiotherapy and TMZ, but the prognosis remains dismal. Advantages of radiosensitization are improved efficacy, possibility to reduce the dose in special population such as in pediatrics, reduced risk of radionecrosis, and improved response to treatment. Radiosensitization can be delivered via thermal (using HT) and non-thermal mechanisms mediated by interactions of FUS and MBs. HT is a radiosensitizer that sensitizes cells to radiation.

The mechanism of action of HT consists of impairment of DNA damage repair mechanisms, increased perfusion, increased oxygenation of tumors, cell death and activation of the immune response (immunogenic cell death [ICD]). The safety and feasibility of HT in combination with radiotherapy for GBM has been evaluated in phase 1 trials. Efficacy was assessed in one phase III randomized controlled trial which evaluated surgery + external beam radiation therapy (EBRT) + interstitial brachytherapy (iBT) with or without HT in high-grade GBM. The combination with HT was shown to improve efficacy compared

with radiotherapy alone. However, HT remains an invasive method limited by side effects together with the high rates of radiation necroses with iBT are limitations of this treatment scheme. Thus, there is a need for a safe and efficient technique to produce low level of HT. FUS is an efficient, noninvasive strategy to induce HT and has clinical applications for drug delivery. FUS-mediated HT is an effective adjuvant to radiotherapy (RT) for several cancers (such as head and neck cancers, brain cancer, prostate cancer), it improves tumor control and increases response rates, and is well tolerated with minimal toxicity. In 1991, a phase 1 trial showed feasibility of FUS-HT radiosensitization for GBM; patients received weekly FUS- HT + daily EBRT. Limitations of this technique included the requirement for craniotomy for FUS application and thermal sensors, the difficulty to heat large tumors, and side effects arising from treatment of temporal lobe tumors (such as temporalis or pinna heating). Alternative ways to control HT include MR-guided FUS with thermometry. Microvascular ablation and BBBO are also alternatives for radiosensitization.

Several challenges remain in HT, mainly the feasibility to deliver FUS to the brain in the clinic, as well as technological challenges of delivering the treatment volume and a controlled HT for 30 minutes. Treatment parameters/logistics need to be validated (sequence of treatment, HT duration, timing between HT and RT, frequency of HT and RT). The most appropriate mechanisms of delivery need to be defined (thermal vs mechanical), as well as pathways, drug delivery, immune activation. FUS may play a role in drug delivery through increased BBBO in GBM and represent a possible avenue for the delivery of radiosensitizers (such as PARP inhibitors) and modulate pharmacokinetics for improved efficacy.

Ablation by Microvascular Disruption

Focal Non-thermal Ablation of Brain Tumors

Tyrone Porter, PhD discussed delivering successful thermal ablation in the brain using transcranial FUS as a driver for investigation of non-thermal modalities. The use of continuous wave HIFU is not ideal for brain tumors and is limited by absorption of the US beam by the skull and thermal damage in the cortex. This is due to the very high powers needed (e.g., 1,000 Watts) and long treatment time (hours) depending on tumor size. Researchers are evaluating the use of cavitation to reduce acoustic power and enable ablation inside the brain, with increased localization and precision.

Porter described the use of phase-shift nanoemulsions (PSNE) as cavitation nuclei in his lab. PSNEs consist of polyfluorocarbon (PFCs) microbubbles condensed to submicron size. PFCs are contained within a lipid shell particle, which results in prolonged circulation after systemic administration and improves the stability of the particle. The focused ultrasound vaporizes liquid PFCs droplets that reconstitute into PFC gas bubbles, to later achieve cavitation and facilitate thermal ablation. Compared with microbubble facilitated ablation, PSNE allows spatial control of the vaporization of PFC, such that the cavitation field is highly localized to the focal spot of the transducer and can avoid the intervening tissue between transducer and the focal spot. Advantages of PSNE include unparalleled spatial control of

cavitation at pressures < 10 Mpa, significantly longer circulation time than microbubbles, and the possibility to inject them at higher concentration than microbubbles (10^9 vs 10^6/ml).

It is hypothesized that PFC PSNE could improve spatial control of cavitation-mediated non-thermal alation of brain tumors, avoiding pre-focal damage. Early experiments were conducted in mice with no established brain tumors, to compare ablation size in focal and cortical regions after treatment with microbubbles vs PFCs PSNE. Successful ablation and edema were observed in focal and cortical regions of microbubble-treated brains. In contrast, there was no pre-focal lesions generated with PSNEs and no ablation or edema in these regions. Edema volume was larger in the microbubble-treated regions compared with PSNE. Microbubble and PSNEs solutions were each diluted at 30% or 5% concentrations. For similarly concentrated solutions of microbubbles and PSNEs, significantly smaller lesion volumes or edema were seen in PSNEs compared with the microbubbles group. Study results showed average lesion size was smaller and better controlled with PFC PSNE and supported the used of spatial control with PSNE compared with microbubbles.

The next study evaluated microbubbles as ultrasound contrast agents or PSNE in F98 rat cell lines systemically serving as cavitation nuclei for local non-thermal ablation. MR images show edema accumulation reflective of successful ablation. In microbubbles, damage was limited to the tumor periphery. Cancer cells were present in the microbubble-treated rats but were absent in PSNEs, indicating complete ablation using nanodroplets as cavitation nuclei. The percentage of ablated volume in the PSNE treated group was 80-90% compared with 20% with microbubbles. Tumor volume also decreased after ablation. In both the control and the study group using cavitation nucleated by PSNE, the remaining cancer cells continued to grow after successful tumor ablation by ultrasound and PSNE at day 8.

The use of PFB PSNE enabled tighter spatial control of cavitation in the brain than ultrasound contrast agents. Ablation was highly localized and correlated spatially with transducer focus when PSNE was used as cavitation nuclei, whereas pre-focal ablation was observed when ultrasound contrast agent was used as cavitation nuclei. There was an insignificant increase in animal survival after cavitation-mediated non-thermal ablation of tumors, due to incomplete ablation of cancer cells. Adjuvant treatment is most likely needed to eradicate surviving cancer cells and improve survival rates of preclinical studies.

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Ablation by Sonodynamic Therapy

Sonodynamic Therapy
Concept, Mechanisms, and Application to Brain Cancer

Francesco Prada discussed sensitizer-mediated therapies for the treatment of brain tumors. Sonodynamic therapy (SDT) refers to low intensity, low frequency treatment. The sonosensitizer must selectively accumulate in the targeted tissue. Sensitization of a target tissue aims to provide a non-toxic sensitizing agent that is activated through ultrasound energy, thus exposing the sensitized tissue to a form of energy. This technique has already been applied

using light in photodynamic therapy, with the use of 5-aminolevulenic acid (5-ALA) in melanoma. However, light does not penetrate deeply within tissues, whereas sonodynamic therapy, a low-intensity ultrasound energy, penetrates more effectively the tissues and skull, and can be used in GBM treatment. The main mechanism of action of SDT occurs through the interaction between ultrasound and sensitizing agent and the resulting generation of reactive oxygen species (ROS) via exciting the sensitizing agents. Ultrasound mechanical effects also induce other tissue-specific effects. Generation of ROS, the main biologic effect, occurs by pyrolysis and sonoluminescence. Microcavitations generate light deep in the tissue. Activation of photosensitive agents and mechanical effects induces anti-angiogenetic mechanisms and modulates the immune system within the tumor microenvironment (TME), leading to expression of tumor antigens and reversing the tumor inhibition targeted at the immune system.

Different sensitizers have been tested in SDT such as porphyrin-based molecules for xanthene dyes. These accumulate selectively in the tissues sparing the surrounding structures and are only activated when they interact with ultrasound. Prada reviewed sensitizers used in clinical settings such as 5-ALA, indocyanine green, and sodium fluorescein. 5-ALA is a protoporphyrin precursor that accumulates in and selectively sensitizes malignant glioma cells (due to their higher metabolism) and converts to protoporphyrin IX. Tumor cells can be highlighted intraoperatively through certain wavelength of light under the microscope, guiding the surgery for GBM. Ohmura and colleagues showed that 5-ALA mediated SDT was effective in reducing tumor size in glioma, and results were confirmed by another study testing SDT both in vitro and in vivo. More recently, 5-ALA was demonstrated to be effective in killing glioblastoma cells in vivo.

Sodium fluorescein is a dye used during surgical guidance. Fluorescein's mechanism of selective accumulation in tumors is different than other sensitizers in that it extravasates through the BBB into the brain tumor. The fluorescence of this agent is very high. Sodium fluorescein penetrates normal brain tissue readily and crosses the BBB and cerebrospinal fluid (CSF) barrier. It is extensively used in medicine, particularly in the setting of various brain tumors including GBM, which expands the potential applications of SDT. A preclinical study showed that fluorescein mediated SDT in a rat glioma model was effective in killing glioma cells. SDT significantly inhibited outgrowth of ectopic glioma cells across all 3 FUS exposure conditions tested, demonstrating that SDT could shrink tumor size. Another safety study in a swine model testing potential effects of SDT with 5-ALA to the brain was conducted. A clinical dose of 5-ALA and fluorescein were given prior to sonication and 2 different brain areas were sonicated. There were no signs of early or late damage on MR imaging or histopathology of healthy brain tissues. Two clinical trials are ongoing and will evaluate this technique in de novo GBM prior to resection (NCT04845919 and NCT04559685).

Therapeutic Uses of the Heme Metabolic Pathway from Photodynamic Therapy to Sonodynamic Therapy

Stuart Marcus, MD discussed photodynamic therapy (PDT) and its evolution to SDT. 5-ALA is part of the heme metabolic pathway, important for the production of heme and synthesis of cytochromes. 5-ALA is the first molecule of the heme pathway, it accumulates as protoporphyrin IX (PpIX) which fluoresces in pink. The pink fluorescence is the result of light activation in cells that accumulates PpIX and the subsequent release of energy. It is used as a visual aid during tumor resection and has potential as a therapeutic agent via PDT. Instead of the fluorescence, higher energies can be used to create a photodynamic effect: PDT is the blue light that produces higher energy from fluorescence and activates PpIX, which allows transfer to molecular oxygen causing cell apoptosis.

A topical form of 5-ALA is available. It provides selective fluorescence at the dermal and epidermal junction for skin cancer and precancer cells, which can be sustained for 24 hours. Fluorescence has the potential to treat skin cancers and precancer through light activation of PpIX using 5-ALA as a precursor. This technique was used in Gorlin syndrome (or basal cell nevus syndrome, caused by a patch gene mutation leading to the development of hundreds of basal cell carcinomas over a patient's lifetime). Using topical 5-ALA in monotherapy in children with Gorlin syndrome showed complete healing without scarring at the area of the cancer with a very good cosmetic effect. The response is durable due to the immune effect of PTD.

GBM cells selectively absorb large quantities of 5-ALA and convert it to PpIX, showing glowing tumors in patients taking ALA. An oral 5-ALA formulation is FDA approved as a visual aid for neurosurgical resection of GBM. The treatment of GBM with PTD has been achieved in the laboratory, but the technique is not commercially viable. The procedure requires laser, craniotomy, stereotype, and porphyrin measurement before and after treatment. SDT does not use microbubbles; it only uses 220 KHz frequency and targeting and delivers higher energy than those used for focused ultrasound BBBO.

A preclinical study in mice with glioma showed that 5-ALA and SDT significantly extended survival. Multipoint sonications are used in human tumors. The mechanism through which FUS activates the photodynamic effect is likely to be sonoluminescence. Sonoluminescence has a spectrum that activates the entire molecule of proto-porphyrin and sources of oxygen. Blue light used for topical 5-ALA and PDT drug-device combinations has achieved good results.

The combination of ALA-SDT is being tested in clinical trials in rGBM in a phase 0/2 clinical trial design (in phase 0, the tumor is removed after treatment, phase 2 is a dose ranging study) for ultimate submission for FDA approval. This study is using a proprietary intravenous formulation of ALA that received orphan drug designation in the US (IV ALA bypasses side effects of the oral ALA). The objective is to evaluate dose-limiting toxicities of SDT, assess any inflammatory response leading to cerebral edema and damage to non-targeted areas of the brain. Previous PDT safety studies showed efficacy even at the lowest targeted energy. Once safety is established in this phase 0/2 study, the energy dose will be doubled for the 2nd cohort, and again for cohort 3 (will have 4 times the energy dose of cohort 1). The company plans to initiate a phase 2b multicenter, multinational study beginning in 2022 as well as to advance the FDA approval process for SDT.

Treatment Monitoring

Focused Ultrasound Enabled Liquid Biopsy of Brain Tumors

Ying Meng, MD PhD discussed the clinical applications of FUS in the brain and presented the results of a liquid biopsy study funded by InSightec. Liquid biopsy is a promising application of FUS and is less invasive than traditional biopsy. Liquid biopsy allows sampling of tumor-related biomarkers in the bloodstream and can be performed along the patient care pathway to monitor disease, treatment response and recurrence, and predict the recurrence of symptoms. The abundance of other biomarkers (proteins, DNA, etc.) in circulation makes it harder to detect the relatively smaller number of tumor-related biomarkers and this poses a challenge to liquid biopsy. Liquid biopsy in brain tumors poses additional challenges because circulating tumor DNA (ctDNA) in patients with gliomas is considerably lower than systemic cancers. This is partially due to the fact that the BBB limits the entry of compounds into the brain as well as the shedding of biomarkers from the brain tumor into the bloodstream. Another hurdle in glioblastoma is the spatial heterogeneity of these tumors.

FUS with injected MBs causes BBB disruption, increases BBB permeability, and induces a greater release of biomarkers (exosomes, DNA, RNA, cells) that become more readily available for detection by blood collection. The first study on the feasibility of this approach was done in an implanted rat glioma model. Several devices are in clinical trials to investigate liquid biopsy. In this study, the Insightec system was used in patients with GBMs. FUS was combined with adjuvant temozolomide (TMZ), and patients underwent FUS BBBO with each dose of TMZ. Blood samples were collected. A significant increase in cell-free DNA (cfDNA) was detected in post-treatment samples compared with pre-treatment. BBBO volume caused an increased release of biomarkers with a larger opening; but there was no difference in cfDNA amounts as a function of time elapsed from the last sonication and the blood collection. There was also a significant increase in other biomarkers such as neuron-derived extracellular vesicles, an interesting observation that warrants further research.

Imaging Modalities Used for GBM Monitoring

Benjamin Ellingson, PhD discussed the basic response assessment pre- and post-contrast T1-weighted MR imaging. The presentation reviewed the below imaging techniques used in FUS studies:

- Dynamic contrast-enhanced dynamic contrast enhancement (DCE)- MR imaging
  - Vascular permeability changes
- Contrast-enhanced T1 weighted digital subtraction
  - Steady state vascular permeability
- Dynamic susceptibility contrast (DSC) MR imaging
  - Blood volume, flow, and vessel size

The MacDonald criteria for response assessment in malignant gliomas used contrast enhancement and bidirectional measurements; these criteria were considered the standard for 20 years. Recently, the RANO working group improved the MacDonald criteria.

RANO provides definitions of minimal residual disease and response categories based on MR imaging and clinical features. The modified RANO (mRANO) criteria is adapted to bucket trials and incorporates immunotherapy and angiogenic therapies. The method was tested in a phase II trial of rGBM. The use of the mRANO criteria allowed patients to stay longer in the study, and these patients lived for 480 days, much longer than expected in rGBM. PFS was correlated with OS, indicating this method may be a good surrogate of early efficacy in GBM.

Exceptions to the contrast enhancement effect as a surrogate of treatment response may happen in several situations:

- Alterations in steroid doses may alter vascular permeability independent of changes in tumor burden
- Anti-VEGF agents target tumor vasculature and decrease vascular permeability and may mask contrast enhancement independent of tumor burden
- Early changes in contrast enhancement may be due to break down of cells or pseudo-progression

Clinical Trial Design

What Evidence Is Required for Reimbursement?

Stephanie Kennan reviewed the basics of Medicare coverage and reimbursement for devices. Once a treatment is approved by the FDA, commercial use can start immediately. Companies need to seek coverage and reimbursement from the Medicare program to get paid claims. Until then, only a small number of patients will be able to afford these new treatments and procedures.

As a first step, device companies must obtain a CPT code from the AMA to ensure providers get uniform information about the device. Initially, several new devices get a category 3 CPT reserved for experimental devices, a classification that limits coverage, since some insurers do not cover experimental devices (such as TriCare). The company can later get the device recategorized when it is no longer experimental. Getting a CPT code helps to familiarize physicians with the device and initiates discussions about the product. The next step is to get national coverage or seek alternative coverage though individual Medicare Administrative Contractors (MACs). MACs may take diverging decisions; some may be "early adopters" of a new technology and are targeted by many device companies. It takes approximately one year to get a national coverage decision (NCD) (a negative coverage decision, if issued, is very hard to reverse) while the process for a local coverage determination takes 4 years. Many MACs are related to private commercial insurers, which means that once Medicare covers a device, private insurers will likely follow. Keenan recommended against using the argument that a device saved money or reduced costs, a claim frequently used by many device companies in their Medicare discussions. She said Medicare did not factor this in their consideration for coverage; instead, they assessed the value of a new device to the patient.

With regards to reimbursement, Center for Medicare and Medicaid Services (CMS) tended to compare new devices to older technologies and calculate similar payment amounts. Yet many times, breakthrough technologies had significant advantage over older devices and were more costly, worthy of higher reimbursement. After reimbursement, CMS will continue to check the volume of claims and the reimbursement amounts for a new device. Medicare reimbursement rules are published in late spring and summer. It is important that device companies comment during the public comment period so that CMS addresses the company's concerns. In addition, when negotiating a higher reimbursement with Medicare or the change of a rule, device companies must propose a solution to CMS, so that the request is taken into consideration and discussed jointly.

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Abbreviations

AE	Adverse events
AMA	American Medical Association
BBB	Blood-brain barrier
CAR	Chimeric antigen receptor
CBER	Center for Biologics Evaluation and Research
CDER	Center for Drug Evaluation Research
CDRH	Center for Devices and Radiological Health
CNS	Central nervous system
CPT	Current Procedural Terminology
CSF	Cerebrospinal fluid
DAMP	Damage-associated molecular pathogens
DCE	Dynamic contrast enhancement
DIPG	Diffuse intrinsic pontine glioma
DLT	Dose-limiting toxicity
DMG	Diffuse midline glioma
DSC	Dynamic susceptibility contrast
DTI	Diffusion tensor imaging
EBRT	External beam radiation therapy
FUS	Focused ultrasound
GRE	Gradient recalled echo
HCPCS	Healthcare Common Procedure Coding System
HIFU	High intensity focused ultrasound
HT	Hyperthermia
JHU	Johns Hopkins University
LCD	Local coverage determination
MAC	Medicare Administrative Contractors
MDDT	Medical Device Development Tools
MDSC	Myeloid derived suppressor cells
MR	Magnetic resonance imaging
MRgFUS	MR-guided focused ultrasound
NK	Natural killer
OCE	Oncology Center of Excellence

OR	Operating room
OS	Overall survival
OSEL	Office of Science and Engineering Laboratories
PCD	Passive cavitation detection
PFS	Progression-free survival
RANO	Response Assessment in Neuro-Oncology
ROS	Reactive oxygen species
SNR	Signal-to-noise ratio
TAM	Target addressable markets
TMZ	Temozolomide

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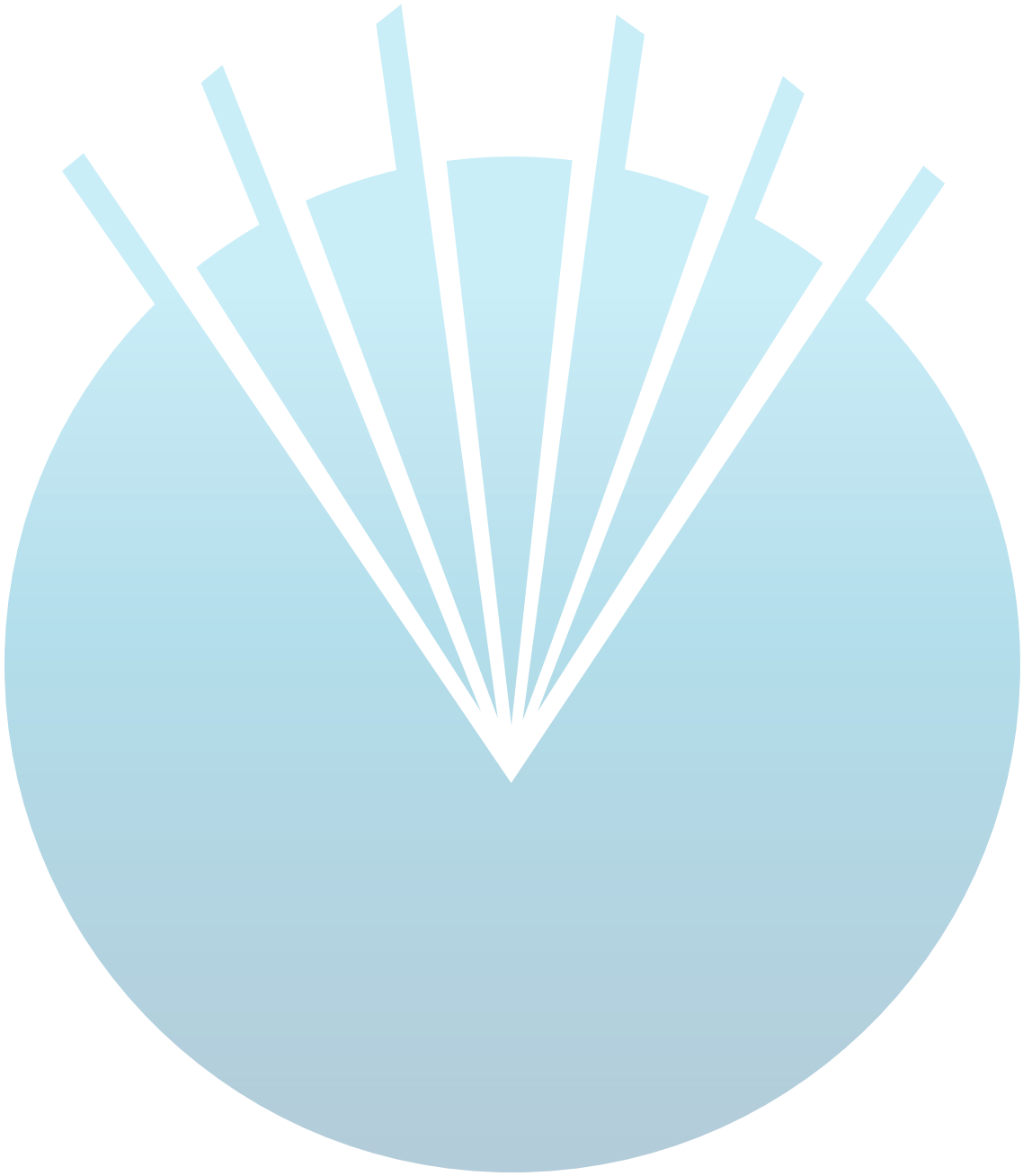

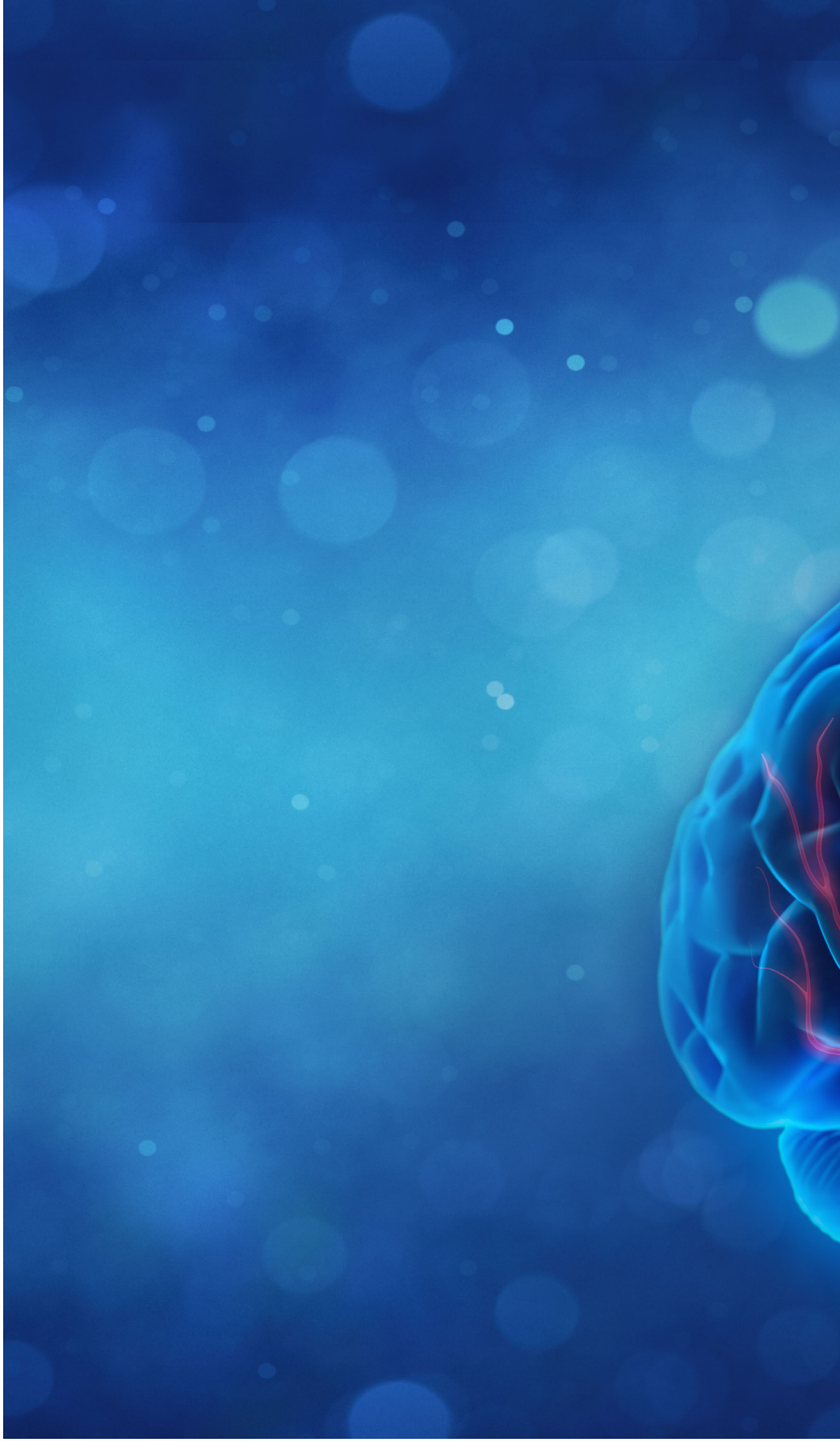

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