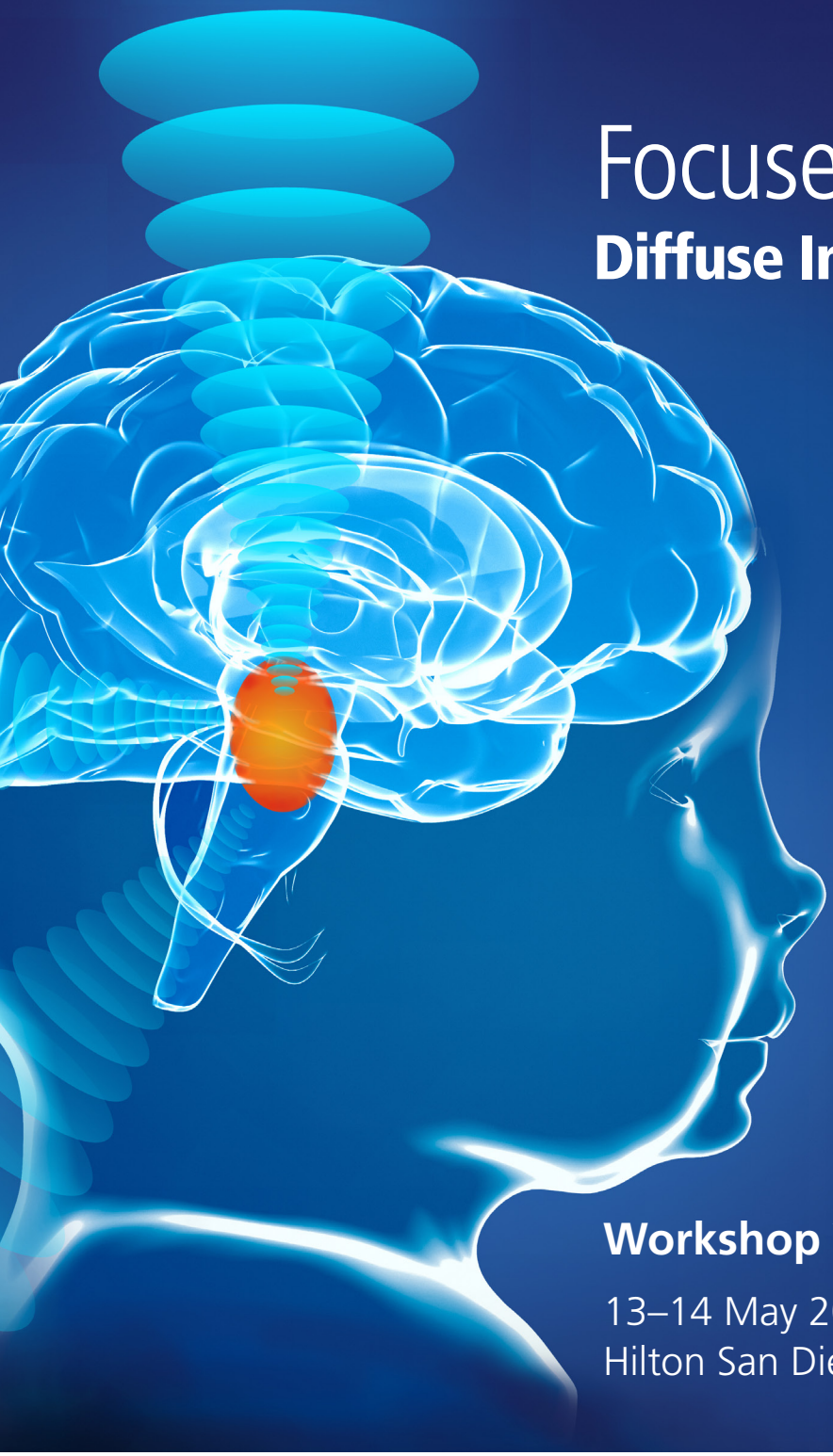

Focused Ultrasound for
Diffuse Intrinsic Pontine Glioma

Workshop White Paper

13–14 May 2025
Hilton San Diego Bayfront, San Diego CA

Sponsored by

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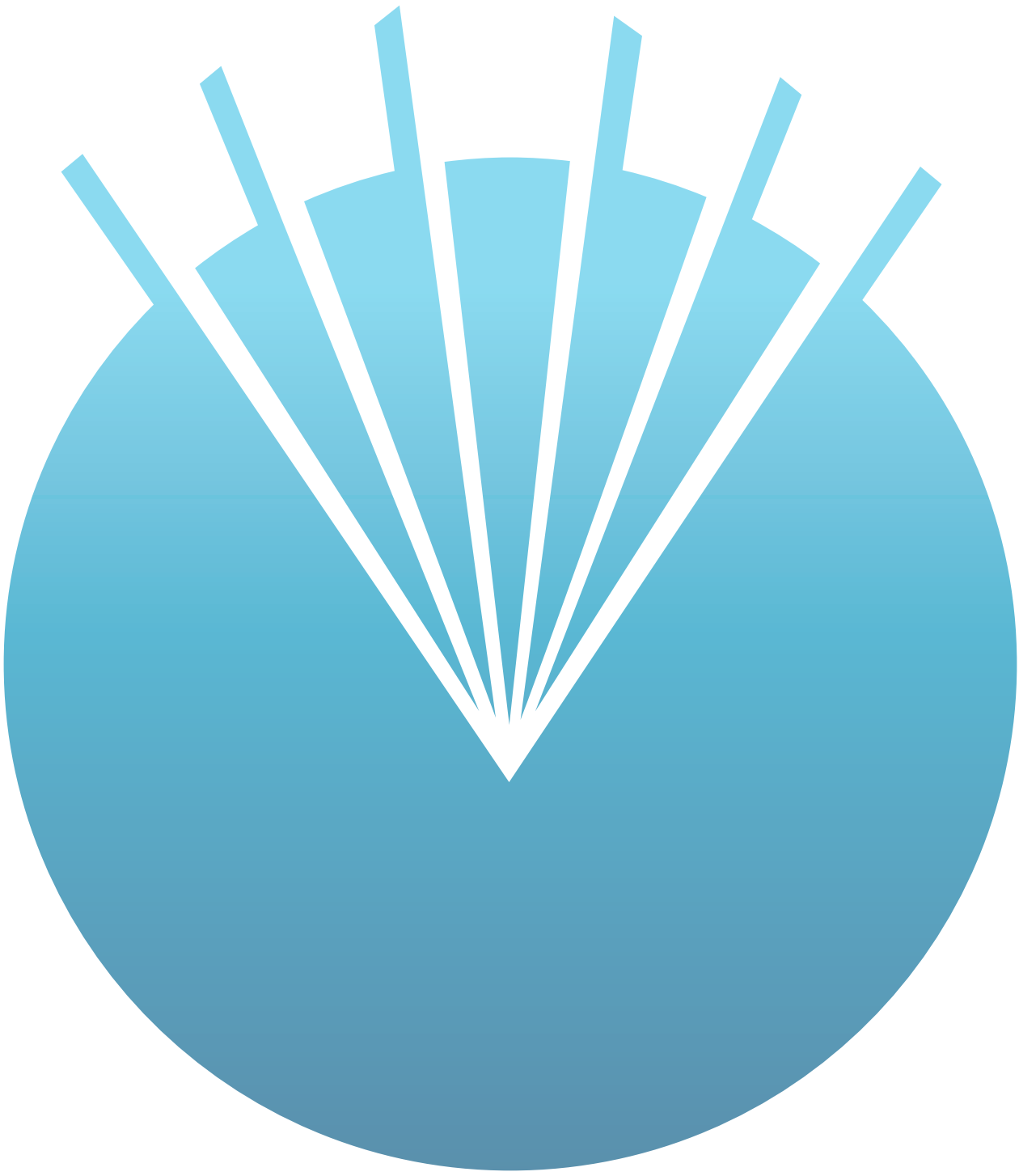

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Executive Summary

This white paper summarizes a 1.5-day workshop, "Focused Ultrasound for Diffuse Intrinsic Pontine Glioma (DIPG)," which was organized by the Focused Ultrasound Foundation. It was held in San Diego, California, as a satellite meeting before the Society for Neuro-Oncology's 2025 Pediatric Neuro-Oncology Conference.

The purpose of the workshop was to convene the DIPG focused ultrasound community to discuss the progress made, existing challenges, and novel opportunities for using focused ultrasound to diagnose and treat life-threatening pediatric brain tumors. The Focused Ultrasound Foundation funded or co-funded most of the research studies that were presented during the workshop.

During the first day, experts presented the latest preclinical and clinical research related to the practice of combining microbubbles with focused ultrasound to open the blood-brain barrier (BBB) for treating DIPG. Day two was centered on emerging focused ultrasound approaches for addressing DIPG, including sonobiopsy, immunomodulation and immunotherapy, and sonodynamic therapy. The presentations were focused on the state of the field and clinical trials, and robust discussion ensued after completion of the talks.

The primary goal of the discussions was to answer a set of burning questions aimed at moving the field forward in each respective discipline. Specifically, questions around optimal design of preclinical research and clinical trials, technologic considerations for the pediatric brain tumor patient, standardized workflow and reporting protocols, and expanding indications were also discussed.

During the roadmap development session, attendees suggested the formation of collaborative, multi-institution DIPG/diffuse midline glioma (DMG) focused ultrasound working groups to continue the important discussions that were initiated during the workshop and to formulate plans for additional research. Specific areas of research that were identified as a priority included a comparison of liquid biopsy platforms, preclinical studies to further characterize the pharmacokinetics of drugs within brain tissue post-BBB opening, and clinical trials evaluating combination therapies with immune modulating agents.

The Focused Ultrasound Foundation intends to continue to develop and implement the ideas suggested in the roadmap, as well as to fund preclinical and translational studies and expanded clinical trials to advance focused ultrasound for the treatment of DIPG, DMG, and other pediatric brain tumors.

Summary Tables

Table 1 Workshop Themes, Presentations, and Discussion Topics

Theme	Presentations	Discussion Topics
Microbubble-Based Focused Ultrasound Blood-Brain Barrier (MB-FUS BBB) Opening		
Optimization of MB-FUS BBB opening for therapeutic delivery: balancing safety, efficacy, and translation	• Preclinical drug delivery studies (ONC201, panobinostat, napabucasin, olaparib) • GB-13 immunotoxin delivery • Clinical trial updates (Children's National, SickKids/Sunnybrook)	Trial platforms, devices, therapeutic agents, workflow, and treatment monitoring
Liquid Biopsy & Sonobiopsy		
Developing minimally invasive diagnostic and monitoring tools to assess tumor biology and treatment response	• Overview of liquid biopsy for DIPG and clinical experiences • Sonobiopsy preclinical development and early feasibility in humans	Biomarkers, methodologies, biospecimen collection, and standardization across sites
Immunotherapy		
Leveraging focused ultrasound to enhance delivery and efficacy of cellular and immune-modulating therapies for DIPG/DMG	• Overview of immunotherapy strategies (CAR-T, TAA-T, vaccines, checkpoint inhibitors) • Preclinical evidence for focused ultrasound plus immunotherapy in DIPG/medulloblastoma • Clinical trial protocol for combining MB-FUS BBB opening with multi-antigen T-cell infusion	Safety, timing, biomarkers, and preclinical model needs
Sonodynamic Therapy (SDT)		
Using ultrasound-activated drugs (e.g., 5-ALA) to selectively target tumors in eloquent brain regions	• Overview of SDT mechanisms and translational preclinical work • Children's National SDT201 clinical trial update	Technical challenges (head frame, acoustic coverage), imaging correlates, biologic mechanisms, and future research opportunities

Table 2 Challenges Identified

Microbubble-Based Focused Ultrasound Blood-Brain Barrier (MB-FUS BBB) Opening

- Successful BBB opening does not always translate into survival benefit in preclinical models
- Drug selection and timing of administration remain uncertain
- Strict eligibility criteria can slow enrollment
- Technical workflow/logistics (MRI suite, anesthesia, infusion timing) of patient treatments
- Device variability and lack of pediatric-specific systems

Liquid Biopsy & Sonobiopsy

- No consensus on best analytic platform (ddPCR, Nanopore, WGS)
- Lack of standardized timing and biospecimen collection
- Few CLIA-certified labs for pediatric neuro-oncology
- Risk/hesitancy of adding CSF procedures in fragile patients

Immunotherapy

- Cold, heterogeneous tumor microenvironment
- Variability in patient responses to CAR-T and TAA-T
- Potential toxicity (neuroinflammation, pseudoprogression)
- Limited translation from preclinical models to children
- Challenges accessing pharmaceutics for combination trials

Sonodynamic Therapy (SDT)

- Technical issues (head frame, hypothermia, acoustic coverage)
- Inconsistent imaging biomarkers post-treatment
- Biologic mechanisms of 5-ALA SDT not fully understood
- Risk of premature reporting affecting trial enrollment

Table 3 Priority Action Items

Microbubble-Based Focused Ultrasound Blood-Brain Barrier (MB-FUS BBB) Opening

- Standardize dosing schemes and parameter reporting across devices
- Develop tailored MB or MB protocols for specific therapies
- Broaden eligibility criteria for trials (e.g., allow necrosis)
- Incorporate PK/PD expertise for drug timing
- Explore pediatric-specific and portable devices

Liquid Biopsy & Sonobiopsy

- Standardize biospecimen collection and timing across trials
- Perform head-to-head platform comparisons
- Embed liquid biopsy or sonobiopsy in all focused ultrasound clinical trials
- Encourage Ommaya use for CSF collection
- Centralize analysis in high-volume labs

Immunotherapy

- Design focused ultrasound plus immunotherapy combination trials with robust biomarker integration
- Use pediatric-specific, immunocompetent preclinical models
- Sequence therapies carefully (radiation, focused ultrasound, T-cells, antibodies)
- Monitor safety/toxicity with standardized immune correlates
- Create multi-arm, multi-institution trial platforms

Sonodynamic Therapy (SDT)

- Optimize acoustic and drug dosing parameters
- Investigate mitochondrial/iron metabolism links
- Incorporate advanced imaging and biopsy correlates
- Explore SDT combinations with other treatments
- Standardize focused ultrasound parameter reporting across sites

Table 4 Roadmap Discussion

Technical and Preclinical Studies

- Create a device-agnostic and standardized dosing scheme to use for the administration of MB-FUS
- Standardize the terminology used for harmonic dose, cavitation dose, passive cavitation dose, and ultrasound cavitation dose
- Characterize the harmonics and subharmonics of each commercially available MB contrast agent
- Outline the biological effects of varying MB parameters for clinical translation
- Disseminate the MB consensus paper
- Develop a monodispersed MB that has been designed for therapeutic use
- Continue to identify and test therapeutics that could be translated into clinical use
- Incorporate AI into discovery studies (e.g., to reduce gadolinium dose, predict drugs to target specific subpopulations in heterogeneous tumors)
- Design preclinical studies that match the standard for human treatment (e.g., once a day MB-FUS in the laboratory does not translate well with once-a-month panobinostat administration)

Clinical Trial Development

- Continue to collaborate to align clinical trial parameters across institutions
- Continue to include preclinical researchers in the design, review, and data monitoring of clinical trials (e.g., different drugs require different ultrasound parameters and administration routes)
- Develop a more formal structure for peer protocol review and comment
- Work with device manufacturers to develop and use standardized guidelines for reporting treatment parameters
- Specify the MVD and MB administration parameter windows to be used in clinical trial protocols (and whether any modifications are allowed)
- Develop a clinical trial protocol for expanding MB-FUS BBB opening into the treatment of other types of brain tumors (e.g., medulloblastomas, ependymomas) using drug sensitivity testing to choose the therapeutic agents for each type of tumor

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About Diffuse Intrinsic Pontine Glioma

Pediatric diffuse intrinsic pontine glioma (DIPG), a biological subset of diffuse midline glioma (DMG), is a rare and fatal brain tumor that forms in the pons, a neuroanatomical part of the brainstem. DIPGs is most often diagnosed in children between the ages of 5–10 years; however, it can occur across the pediatric, adolescent, and young adult age ranges. DIPG and DMG are incredibly challenging to treat, in part because of their location in this sensitive brain location with a relatively intact blood-brain barrier (BBB). Median survival time is nine months, and only 10% of patients live longer than two years.[1,2] A major hurdle in the effective treatment of brain tumors has been the low penetration of most therapeutics. The BBB is a protective cellular and membranous system that tightly regulates molecular trafficking into the brain parenchyma. Focused ultrasound is emerging as a platform capable of transient opening of the BBB; it is thus being used to address this unmet clinical need.

Welcome

Lauren Powlovich, MD, MBA, welcomed attendees to the Foundation's second DIPG Workshop. It has been four years since the Foundation's first DIPG Workshop; however, because of that workshop, the landscape for treating DIPG with focused ultrasound has changed with the start of three new clinical trials and the publication of a wealth of preclinical data. Several publications were distributed to attendees in advance of the workshop.[3–8] Dr. Powlovich thanked **Roger Packer**, MD, **Lindsay Kilburn**, MD, **Cheng-Chia "Fred" Wu**, MD, PhD, and **Sibo Zhao**, MD, for serving on the workshop's steering committee. She then provided an overview of the meeting agenda, and attendees introduced themselves.

Session 1 – Optimization of Microbubble-Based Focused Ultrasound Blood-Brain Barrier Opening for Therapeutic Delivery

This session featured two preclinical and three clinical presentations centered on the practice of combining microbubbles with focused ultrasound (MB-FUS) to open the blood-brain barrier (BBB) for the treatment of DIPG. It concluded with a panel discussion of a multi-institutional DIPG clinical trial platform, focused ultrasound brain devices, therapeutic agents for delivery, logistics/workflow, and treatment monitoring.

Preclinical Studies

MB-FUS BBB Opening for DIPG: Preclinical Summary

Cheng-Chia "Fred" Wu, MD, PhD, presented a summary on the state of preclinical research findings related to MB-FUS BBB opening in DIPG models. He made the following points during his presentation:

- Currently, radiotherapy is the only effective treatment for DIPG. A new drug called ONC201 (dordaviprone) is a dopamine receptor D2 antagonist that is in Phase III randomized controlled clinical trials for non-brainstem DMG (NCT05580562).
- Using focused ultrasound to open the BBB in the brainstem has been shown to be safe in a murine model of DIPG[9,10] and to increase the concentration of panobinostat within targeted areas for therapeutic benefit (Wu et al., accepted for publication).[11] These studies speak to the potential of combining focused ultrasound with therapeutics to enhance drug efficacy in DIPG/DMG.
- There was early evidence generation of tumor control but no survival benefit,[12,13] so it is not as easy as opening the BBB and choosing a drug. Opening the BBB does not automatically lead to enhanced delivery with a survival advantage. There are more questions than answers at this time.
- Dr. Wu's research team tried to predict different drugs that may be beneficial for DIPG. With one of the drugs, a small molecule called napabucasin, focused ultrasound did not increase drug concentration but convection-enhanced delivery (CED) increased efficacy.[14]
- Dr. Wu's group also conducted extensive research on the safety and feasibility of opening the BBB during radiation. They found that MB-FUS BBB opening in the brainstem one month after radiation, one week after radiation, and during radiotherapy all appear to be safe in preclinical models.[15] Similar findings were shown in the setting of thalamic DMG.[16] Furthermore, different radiotherapy fractionations (e.g., 39 greys in 13 fractions, the clinical dosage frequently used in Europe) and stereotactic doses in the cortical regions were also safe.[17]
- The complexity of choosing the right drug target with focused ultrasound warrants further consideration. For example, Dr. van Vuurden's group conducted studies to deliver olaparib with focused ultrasound and radiotherapy. Although they were able to detect higher levels of olaparib with that approach, the efficacy was not as robust as anticipated.[18]

- Dr. Wu's group successfully increased the delivery of an optically labeled anti-PD1 antibody (programmed cell death protein 1) with some local control and survival benefit.[19] Whether the benefit was a factor of enhanced drug delivery or immunomodulatory effects of focused ultrasound remains to be determined (e.g., single-cell RNA sequencing data have suggested an immune modulatory component). Combining focused ultrasound with cellular therapy is also under investigation.
- There are also potential uses of theranostics with focused ultrasound. For example, Hong Chen's group used focused ultrasound to transiently open the blood-brain-tumor barrier and deliver radiolabeled nanoclusters (64Cu-CuNCs) to tumors for positron emission tomography (PET) imaging and quantification in a mouse model of DIPG.[20]

Group Discussion

Attendees asked Dr. Wu about the focused ultrasound parameters being used in preclinical studies. He said that the majority of early studies used a single peak negative pressure of 0.6 megapascals (MPa) to prolong BBB opening to 48 hours and allow for repeated dosing. When moving toward immunotherapy, his team stayed with 0.6 MPa. Future work will involve characterizing parameters for each type of commercially approved MB and creating tailored MBs: one for BBB opening without inflammation, and one for delivering immunotherapy such as checkpoint inhibitors or cellular therapy. When asked about translating larger volumes of BBB opening into human clinical trials, Dr. Wu said that preliminary experience from both the Children's National Group and the SickKids group have shown that the BBB opening to the entire brainstem can be safely performed, so this should be the goal for future clinical trials. Dr. Wu also commented about using larger animal models at Virginia Tech, saying that the team will use the Insightec device with companion animals starting with naturally occurring canine gliomas. The first safety and feasibility studies will deliver a generic, standard of care medicine and then test the delivery of chimeric antigen receptor T-cell (CAR-T) therapy. He added that reviewing MRI scans revealed that the skull density ratio in canines should be amenable for the study and noted that brain tumors are more common in mid-size dogs, and their skulls are not as thick as what is found in larger animals.

MB-FUS Opening for GB-13 Delivery

Mark Borden, PhD, presented the design of an early-stage, collaborative preclinical research project involving MB-FUS combined with a novel therapeutic called GB-13 that he is conducting with **Randy Schrecengost**, PhD, and **Adam Green**, MD. Dr. Borden made the following points during his presentation:

- GB-13, developed by Targepeutics, is an immunotoxin drug that binds to cancer cells and causes cell death. It targets a clinically validated, tumor-restricted biomarker, and the project will investigate its effectiveness in several different DIPG cell lines.
- The specific aims of the study, which will be performed in a DIPG mouse model, are to determine whether (1) MB-FUS BBB opening is an effective delivery method for GB-13 in the pons, and (2) weekly treatments of MB-FUS plus GB-13 improve survival in a xenograft model of DMG.
- The MBs will be monodispersed at about three microns (μm) in diameter; this choice was made based on pharmacokinetic experiments.
- MB-FUS BBB opening in the mouse brainstem will be achieved by using the FUS Instruments RK-50 portable, stereotactic-guided focused ultrasound system to apply

five minutes of sonication at 1 megahertz (MHz) frequency with 10-millisecond-long bursts at a 1% duty cycle.

- The study parameters were optimized by analyzing sterile inflammatory responses (SIRs) and degree of BBB opening at various MB volume doses (MVDs) and ultrasound pressures. The parameters tested included MVDs of 0.1, 1, 10, 40 µL/kg and mechanical indexes (MIs) of 0.2, 0.4, 0.6 MPa per square root of MHz. MVD, the volume of gas bubbles injected, is typically written as microliters per kilogram (µL/kg) and calculated as the injected fluid volume dose times the gas volume fraction.
- The ideal therapeutic window for the study was determined to be an MVD of 40 µL/kg at an MI of 0.2. MPa to achieve opening without moderate SIR.
- To obtain quantitative uptake data, the GB-13 will be radiolabeled with zirconium-89, which is visible on PET. Previous PET studies at the Mayo Clinic have shown that GB-13 stays in the tumor tissue for 48 hours when delivered by CED (Mukesh Pandey, unpublished data).

Group Discussion

The presentation stimulated a discussion on how to best identify which tumor model to use. Dr. Nazarian said that each model has pros and cons. For example, models that quickly develop DIPG tumors kill mice too early. Initiating the treatment too early can also affect the injected cells if a genetically engineered model is not used. Models that provide a lifespan of three to four months or less (e.g., HSJD-DIPG007 cell lines) are a good choice. Another option is to use an immunocompetent model to study the effect of the drug on T-cells or other immune cells. **Timothy Phoenix,** PhD, associate professor of pharmaceutical sciences at the University of Cincinnati College of Pharmacy, has developed good models that represent patient biology. Developing consistent cell lines in DIPG models takes time. Dr. Borden said that his studies required a cell line with the right receptor expression for GB-13. Testing both immunocompetent and immune incompetent lines was recommended because immune activation itself sometimes prohibits drug efficacy. It would be especially important to test a drug with an immune-activating component in an immunocompetent model. MB-FUS BBB opening also has the potential to activate the immune system, so it is important to understand this effect in immune competent models. To make data from a preclinical study translatable to human clinical trials, cell lines that have been successfully translated with other therapies should be used.

Attendees discussed the delivery of GB-13, which is also being tested with CED (an invasive procedure). A comparison of MB-FUS delivery of GB-13 and CED would be interesting and create an opportunity to measure tumor concentration of the drug and correlate drug concentrations with efficacy. It is unknown whether GB-13, as a fusion drug, would remain stable when exposed to low-intensity focused ultrasound (LIFU). In Dr. Borden's studies, the drug is injected after MB-FUS is applied, so it is not affected by the cavitation itself; however, the timing of drug injection with respect to the MBs is an open question. Co-injection is an option after considering the pharmacokinetics of the drug (i.e., how quickly it is cleared). The best timing to convect the drug into the brain tissue may be during initial BBB opening. The team will use PET imaging to quantify how much of the injected dose reaches the tumor.

Regarding the size of the MBs, Dr. Borden's team looked at different bubble sizes but did not see a large difference in terms of BBB opening when matched with MVD. When studying the pharmacokinetics of the different size MBs, they achieved the best area under the curve (AUC) with 3-µm MBs. The smaller MBs were not as stable, and the larger MBs seemed to be getting trapped in filtering organs (i.e., lung, liver, spleen). They looked at MBs as a function of time for the pharmacokinetic studies and noted that the larger bubbles became trapped in the liver and spleen. The 3-µm MBs provided optimum stability and were not filtered as readily as the larger bubbles.

Clinical Studies

MR-Guided Focused Ultrasound (MRgFUS) BBB Opening with Doxorubicin: United States

Lindsay Kilburn, MD, presented an update on the ongoing Children's National Hospital MB-FUS BBB opening plus doxorubicin clinical trial that is funded by the Focused Ultrasound Foundation. She made the following points during her presentation:

- This clinical trial is based on a parallel safety study by Dr. Meng at Sunnybrook Health Sciences Centre in Toronto. As one difference between the two protocols, the U.S. Food and Drug Administration (FDA) required Children's National to administer a corticosteroid drug (i.e., dexamethasone) at the time of treatment. After the first participant was treated, however, Children's National was able to decrease the dexamethasone dosing and hopes to eliminate the dosing as the study progresses because of concern that it may defeat the purpose of disrupting the BBB.
- DIPG was chosen as the initial target for these BBB opening studies because it proliferates in a confined space and usually has a defined volume with an intact BBB. Some data also suggest that the intact BBB contributes to DIPG's poor treatment outcomes.
- While planning this pediatric study, the team used data from an adult clinical trial as the proof-of-concept for combining MB-FUS BBB opening with the delivery of a drug.[21] Preclinical work, along with the long safety history of administering doxorubicin to pediatric patients, provided the data to support the choice of doxorubicin for this research study.[12]
- The study aims to evaluate the safety and feasibility of BBB opening in combination with doxorubicin using the Insightec Exablate 4000 Type 2 system in pediatric patients diagnosed with DIPG.
- The study's primary endpoints are safety (i.e., adverse events) and feasibility (i.e., MRI assessments immediately after and 24 hours after the BBB opening procedure show contrast enhancement on T1-gadolinium MRI sequences). As safety is being established and the trial continues, the timing of the doxorubicin administration has been moved forward—toward immediately after BBB opening in the MRI suite. (It was initially given within two hours after the participant had completed the BBB procedure when the patient had been moved to the intensive care unit for observation).
- The trial is enrolling patients who have received radiation therapy and have not had any subsequent therapy or tumor progression. The size of the pontine tumor is restricted to 10 to 30 cubic centimeters. Patients with ventricular-peritoneal shunts were initially excluded, but the protocol was subsequently amended to allow non-metallic shunts if the shunt would not interfere with the treatment. Patients with any necrosis in the tumor, which is common in post-radiation DIPG tumors, were also initially excluded, but enrollment challenges prompted another protocol amendment to allow some necrosis as long as that area can be avoided during treatment.
- With safety in mind, the study was designed with escalating sonication volumes in the first patients. The first three participants receive sonication to 50% and then 75% and 100% of the tumor volumes over the three cycles of treatment. If this is tolerated, subsequent participants will receive treatment to 100% of the tumor volume for all three treatments.

- Two participants have been enrolled to date, and preliminary experience supports the safety of the procedure.
- Early clinical data are showing that the procedure is safe. Technical challenges have included pairing a chemotherapy agent with anesthesia for a prolonged, sedated procedure and challenges of infusing chemotherapy in the MR suite with an MR-compatible infusion pump. The technology is advancing, and frameless technology may improve feasibility—especially in pediatric patients.
- Collecting these important safety and feasibility data combining MB-FUS and a drug with extensive safety experience in pediatric patients with DIPG is the first step toward expansion into other treatment areas. MB-FUS BBB opening will likely have multiple therapeutic applications, such as drug delivery and immunomodulation in DIPG as well as other tumors.

MRgFUS BBB Opening with Doxorubicin: Canada

Ying Meng, MD, PhD, presented an update on the ongoing SickKids/Sunnybrook Health Sciences Centre MB-FUS BBB opening plus doxorubicin clinical trial that is funded by the Focused Ultrasound Foundation. She made the following points during her presentation:

- The study design is a Phase I, prospective, single-arm, nonrandomized study to evaluate the safety and feasibility of BBB disruption using the Exablate 4000 Type 2 focused ultrasound system combined with doxorubicin in children with DIPG.
- The Exablate BBB disruption is performed in combination with standard doxorubicin chemotherapy cycles, as scheduled for the treatment of pediatric DIPG. Doxorubicin is administrated within two hours or at the same time as the Exablate BBB disruption procedure. The total duration of treatment is up to three cycles or per the planned chemotherapy treatment protocol.
- The target enrollment is 10 participants, and the study follow-up period is nine months after the last procedure. Of 11 patients screened, 3 have been enrolled to date (ages 5, 11, and 13).
- Because it is a safety study, the dosage escalation design is such that the first three participants received sonication to 50% and 75% and 100% of the tumor volumes over the first three treatments. The expectation is that the fourth participant and beyond will receive sonication over 100% of the tumor volume for all three cycles.
- With experience, treatment times have shortened while treatment volumes have increased. Gadolinium enhancement images show good degrees of BBB opening with no T2* changes concerning for toxicity.
- All treatment- or procedure-related adverse events were transient and found to be mostly related to doxorubicin. Some participants had some mild worsening of their preexisting deficit after the procedure.
- The clinical trial is now undergoing its scheduled Data and Safety Monitoring Board review, and some of the data are currently undergoing quantitative analysis of the MRI contrast enhancement. The research team is also reviewing the tumor biomarkers that were collected from the plasma or blood samples from the participants.

Group Discussion

Attendees discussed the reasons for screen failures in these clinical trials, which included post-radiation areas of necrosis and a competing focused ultrasound–related sonodynamic therapy (SDT) trial at Children's National Hospital that offered patients more than three treatment cycles, which was often more attractive to patients. The decision to initially exclude tumor necrosis created a substantial barrier; participants with small areas of necrosis can now be enrolled, but some of those necrotic areas are still too large. Some atypical DIPGs were excluded because they did not fit within the study parameters (i.e., either a size limitation or extension outside of the pons). Regarding tumor volume, some tumors extend too far out of the brainstem—into the medulla or midbrain. Those areas can now be covered with focused ultrasound. One potential participant had a tumor that was too small. Attendees recommended removing the lower limit on tumor volume.

The decision to initially exclude tumor necrosis created a substantial barrier for the BBB opening trial that did not exist for the SDT clinical trial. When offered a choice between the BBB opening and SDT trial, many families chose the SDT trial. It was discussed that the DIPG community is quite small, and families often communicate through social media channels and get excited about certain trials in different ways. The use of steroids has become unfavorably viewed in the DIPG community, which may have played a role in slow enrollment at Children's National where the approved protocol requires that patients receive a "reasonable dose" of steroids to prevent inflammation. Although the original plan was to have identical protocols in the US and Canada, differences in regulatory reviews led to minor differences between the two protocols.

When discussing treatment parameters, attendees felt that BBB opening was more successful in the participants who received lower doses of steroids. It was also discussed that the goal cavitation dose was more difficult to achieve in some areas of the brainstem than others and that the pressure needed to reach 0.3 MPa within the tissue was higher, possibly because of the tumor characteristics (e.g., density, location, membrane folds). Attendees acknowledged that tumor perfusion may also affect cavitation dose. It was discussed that differences in target area or administration of MBs (bolus versus infusion) may also affect the cavitation dose, and MB concentration can be increased to improve dosage. One recent ex vivo study indicated that MBs can squeeze into the parenchyma at 1 MHz and 0.5 MPa, but this has not yet been observed in vivo.[22] Increasing the pressure changed the behavior of the MBs in that study. Conversely, increasing MB volume dose increases the number of bubbles, but they still oscillate with small oscillations; therefore, more MBs with smaller oscillations is felt to be better than a few MBs that are violently cavitating. The characteristics of live tissues cannot be ignored: The tissue acts on the MB—not the other way around. Radiation changes the stiffness of the brain and therefore changes perfusion, elasticity, and MB behavior. Inflammation in the brain also makes it more vulnerable to damage. It may be possible to design a pump that adjusts MB dosages because there is currently no good way to control MB concentration.

In the discussed studies, it takes a few hours to open the BBB throughout the whole tumor. The BBB is not opened throughout the entire brainstem at the same time. The BBB remains open for less than 24 hours: when follow-up scans are performed 24 hours after treatment, the BBB is closed based on changes in enhancement as a surrogate but exactly when it closed is unknown.

Logistical challenges for conducting these clinical trials included the physical distances (within the hospital system) between the focused ultrasound, radiation therapy, and oncology departments, patient transportation, and the timing of chemotherapy administration when it cannot be administered in the focused ultrasound area. These are compounded in young patients by need for sedation.

The group briefly discussed a recommendation from the 2021 DIPG Workshop to conduct pharmacodynamic studies on drugs delivered across the BBB; however, it can be challenging to find experts in this area. Some pharmaceutical companies may have metabolism data, but they do not have data on drug uptake by tumor cells, especially in the setting of a disrupted BBB. Translation could be improved through a collaboration with Elizabeth (Liesbeth) de Lange, PhD, a neuropharmacologist at Leiden University who created the Leiden Computational Network Science model (a pharmacokinetics [PK]/pharmacodynamics [PD] model of the brain) for PD back-modelling. Trying to mimic human PK in mouse models before adding focused ultrasound is another idea.

Regarding doxorubicin infusion, Dr. van Vuurden said that doxorubicin has a half closure time of 6–10 hours (based on its molecular weight). If it has enough time to cross into the tumor tissue, it may also have enough time to move out of the tumor tissue. Children's National infused doxorubicin during the course of one hour when the clinical trial began but shortened the infusion time to 15 minutes after it became apparent that the BBB was closing quickly, and this matched the Toronto group's doxorubicin infusion time of 15 minutes. If this trial's results are negative, it will not necessarily mean that doxorubicin does not work with MB-FUS BBB opening. The infusion regimen may need to be changed to infuse the doxorubicin over a longer time period, perhaps at a lower dose. The doxorubicin molecule is quite large, so it may need more than 15 minutes to cross into the tumor tissue. Neuropharmacologists could help design dosage and infusion time with regard to focused ultrasound. Biodistribution questions could be answered with the appropriate radio-labeling strategy. It may also be possible to use microdialysis catheters in the not-too-distant future, especially in glioblastomas, to measure the amount of drug that crosses with focused ultrasound. When asked about doxorubicin toxicities, the teams reported one mild case of fever neutropenia and decreased blood counts (as expected), but no unexpected side effects. However, true toxicity cannot be measured in areas of the brain that cannot be biopsied.

In the future, thalamic DMGs and ependymomas may also be good targets for treatment with MB-FUS BBB opening and may provide increased opportunity for treatment prior to resection, which would provide important pharmacodynamic data to inform future work. There is more to learn about BBB opening in tumors in other locations. And like DMG and other high-grade gliomas, recurrent ependymomas could be a good target because they lack effective therapies.

Delsona BBB Opening with Panobinostat or Etoposide

Stergios Zacharoulis, MD, and **Elisa Konofagou**, PhD, presented an update on Columbia University's MB-FUS BBB opening plus panobinostat (NCT04804709) or etoposide (NCT05762419) clinical trial in children with progressive DMG. The current trial is co-funded by the Focused Ultrasound Foundation and supported by Hope and Heroes and the Fegel Family Foundation. Drs. Zacharoulis and Konofagou made the following points during the presentation:

- Dr. Konofagou and her team designed the portable Delsona focused ultrasound system with a 250 kHz, single-element transducer containing an imaging probe for real-time guidance. The system includes a focused ultrasound transducer tracker, position sensor, and passive cavitation detector, and uses neuronavigation guidance, among other features.[23–27] Delsona is being used in clinical trials in patients with Parkinson's disease, Alzheimer's disease, and DIPG/DMG.
- At Columbia University, treatment preparation includes trajectory planning, skull property extraction via CT imaging, and acoustic simulation and attenuation

estimating. During treatment, the patient is immobilized while sitting slightly bent forward and face down but awake in a treatment chair. The children are allowed to play games on an iPad during treatment.

- To open the BBB, focused ultrasound is applied with 0.2 MPa of peak-negative pressure at a frequency of 250 kHz and a pulse length of 10 milliseconds for a duration of two minutes in each target location during infusion of Definity® MBs.

Panobinostat

- The research team chose panobinostat as the therapeutic agent because it has been demonstrated to be a promising histone deacetylase (or HDAC) inhibitor (i.e., biological modifier) for treating DMG. Furthermore, it is potentially active, has minimal penetration into the cerebrospinal fluid (CSF), and its dosing regimen (i.e., three days per week) works well with MB-FUS BBB opening.[28,29]
- The study's primary objective was to evaluate the safety and feasibility of using focused ultrasound to open the BBB near one, two, or three tumor sites in children with progressive DMG. Its secondary objectives were to determine BBB/tumor imaging changes after using focused ultrasound in children with progressive DMG and to evaluate six-month progression-free survival and six-month overall survival.
- The treatment plan followed a three plus three escalation design for the number of tumor sites. The goal was to perform the MB-FUS BBB opening and then administer oral panobinostat over six cycles, with each cycle lasting three weeks: two weeks of MB-FUS followed by panobinostat on Monday, Wednesday, and Friday and then one week off. Gadolinium-enhanced MRIs were used to confirm BBB opening and closing. The entire protocol lasted 18 weeks. The MRI scans showed the BBB opening volume to be quite variable.
- Treatments were performed in the radiation oncology outpatient setting over a period of 30 minutes. The patients experienced no pain other than the discomfort of the stabilization. No anesthesia was administered.
- A total of three participants were enrolled before panobinostat was removed from the US market. The team opened the BBB in all three patients and opened the BBB in two different tumor sites in two of the patients. In one patient, it took one week to confirm BBB closure because the contrast enhancement was unclear.
- The MB-FUS BBB opening procedure was well tolerated. Two participants showed clinical improvement. Some panobinostat-related toxicity occurred. One participant unexpectedly passed away five days after a treatment; the death was caused by a pulmonary embolism and was unlikely related to the clinical trial.

Etoposide

- The panobinostat study was closed, and a similar protocol was adopted with etoposide. It follows the same escalation design, but a new algorithm was employed for confirming BBB closure. Etoposide is administered daily for 21 days followed by one week off.
- Three participants have been enrolled to date. The first patient had rapid tumor progression that continued within days and came off study as non-evaluable. The second patient had unsuccessful BBB opening despite multiple attempts and withdrew after two cycles. The third patient successfully completed four cycles and BBB opening was confirmed. This patient also showed transient clinical improvement and stable disease after the first two cycles.

Combined Data

- All six patients (three in the panobinostat group, three in the etoposide group) have now passed away from the disease.
- From a technical perspective, the BBB opening simulations assume that the entire transducer has contact with the skull (i.e., the contact length), but this is not necessarily what happens clinically. By analyzing the contact length, echogenicity of the skull, passive acoustic mapping signal, and incidence angle, the team found a correlation between the contact length and the volume of BBB opening.[30] The real-time imaging capabilities of the Delsona system allow the treatment team to visualize contact length, so now the parameters can be corrected to ensure optimal contact length and thus optimal BBB opening.

Group Discussion

Attendees discussed the fact that in these studies, it appeared that BBB opening occurred in the cerebellum rather than in the pons or brainstem. From a safety standpoint, the team tried to work its way in, starting with the cerebellum. They never reached the brainstem. This protocol uses only one transducer for accessibility, flexibility, and cost. Switching to a new, multi-element transducer could allow the team to reach different depths.

The observed clinical improvement cannot yet be explained. One patient with stable disease stopped treatments early because of familial reasons then experienced rapid progression upon return home. Clinical improvements were dramatic and occurred in both studies irrelevant of the drug. Participants experienced motion (i.e., hip flexion improvement), motor, and speech improvements and improved quality of life. When asked whether the improvements in motor behavior may have been caused by neuromodulation, Dr. Konofagou said that she did not think so. The pressure from the infiltrating disease makes the vessels not work properly. If anything, the improvements may be due to sonoporation increasing blood flow and perfusion in the region. The treatment may be improving cerebellum function by decreasing pressure and inflammation. We will now attempt to determine whether cerebral blood volume in the region can be correlated with ease of locomotor activity.

The neuronavigation system is co-registered with the patient's MRI and CT images. The team aligns the skull seen on the ultrasound image with the MRI and CT images, so they know in real time which slice of the skull they are approaching. This feature allows the team to determine the exact location of the transducer, because neuronavigation is error prone and can be centimeters off. If the child moves the headband during the procedure, the system tracks the head using skull coordinates and notifies the team about the level of motion. When children move a lot, there is a larger region of BBB opening, so the team is seeking better ways to restrain the patient.

Panel Discussion

Moderator
Lauren Powlovich, MD, MBA

Panelists
Mark Borden, PhD, **Lindsay Kilburn**, MD, **Elisa Konofagou**, PhD, **Ying Meng**, MD, PhD, **Sabine Mueller**, MD, **Roger Packer**, MD, **Hasan Syed**, MD, **Stergios Zacharoulis**, MD

Panelists and attendees discussed the creation of a multi-institutional DMG clinical trial platform, focused ultrasound brain treatment systems, which therapeutic agents to deliver, logistics/workflow issues, treatment monitoring, and future directions.

A Multi-Institutional DMG Clinical Trial Platform

The University of California–San Francisco (UCSF) participated in the SonALAsense SDT clinical trial with Children's National Hospital. Dr. Mueller said that she is excited about focused ultrasound's potential, especially LIFU, for the treatment of her patients. UCSF is collaborating with Children's National Hospital (Dr. Packer and Dr. Kilburn) and Princess Maxima Center for Pediatric Oncology (Dr. van Vuurden and Dr. Mario Ries) to advance the technology by creating a multi-institutional DMG clinical trial platform that can quickly introduce (and test) new therapeutic agents that have been chosen based on preclinical experiments and pharmacology studies.

The platform design will include dose escalation, target selection, and patient monitoring. Some potential therapeutic agents for testing include large molecules, monoclonal antibodies, oncolytic viruses, and other cellular therapies. The type(s) of focused ultrasound devices that will be used for the platform is under discussion.

Focused Ultrasound Brain Treatment Systems

Dr. Mueller noted that one patient treatment at UCSF with the Insightec system required at least 30 support staff, including neurosurgery and neuroradiology, but the team is gaining experience with the device. Broadening its use to other centers is not easy because the Insightec system is expensive and cumbersome. Dr. van Vuurden said that Insightec acknowledges the cumbersome nature of its brain system and will likely develop a LIFU device that can be used outside of the MRI.

Dr. Konofagou and Dr. Zacharoulis raised the topic of scalability of the various types of focused ultrasound brain treatment systems. Dr. Konofagou said that the Focused Ultrasound Foundation has been pivotal in getting systems into community hospitals or in hospitals without a focused ultrasound–dedicated MRI. The Delsona system can be tailored to a specific drug or anatomic site. Each treatment system has its own benefits and challenges with regard to accuracy, precision, cost, portability, anesthesia, workflows, space, and staffing requirements.

Dr. Syed said that although Children's National uses the Insightec device, its level of accuracy/precision may not be needed for LIFU. Other brain systems to consider include NaviFUS, Alpheus, and the implantable Carthera SonoCloud device. The logistics for pediatric treatments can be challenging; anesthesia, MRI/radiology, neurosurgery, neuro-oncology, and others are all needed. Adding more focused ultrasound systems to the clinical trial platform could introduce more levels of variability and flexibility for different treatment goals.

Dr. Wu raised the possibility that the right device for children has not yet been invented. Some lessons can be learned from radiotherapy, where there is 2D radiotherapy and the gamma

knife, but nothing in between. Intensity-modulated ultrasound (IMUS) could provide the in between—with multi-element steering or acoustic holograms. Needed are collaborations to work on a pediatric-specific device.

Dr. Syed explained that the benefits of the NaviFUS device include neuronavigation (no MRI), the ability to target the brainstem or pons, and a real-time feedback system. Dr. Wu added that the NaviFUS system's absolute volume per treatment is not as large as that of the Insightec system. Dr. Syed noted that it would take the NaviFUS system four to six treatments at two to three minutes each to cover the pons, which may be feasible to be done in an awake patient.

Dr. Powlovich reminded attendees that the Focused Ultrasound Foundation is device agnostic and supports all devices and all stages of development. Earlier stage companies must be prepared to enter the US market with the resources, manufacturing capacity, and relationships needed to distribute devices.

Therapeutic Agents

Dr. Packer noted that the therapeutic agents selected also present variability (e.g., some chemotherapies have a short half-life). Those with a short half-life may need to be delivered with a focused ultrasound system that can be used more frequently. Targeting might be more, or less, important with gene and cell therapies or immunotherapy drugs. However, several other delivery methods, such as CED or tumor rim injections, never resulted in the hoped-for "bystander effect." If the preclinical models do not show proof before translation to a clinical trial, then the risk of more limited treatments may be too high for patients. Dr. Kilburn agreed, adding that selection of the device used to deliver a therapeutic agent depends on the agent and whether the entire tumor must be treated. Some agents require more precise delivery than others (e.g., SDT). An ideal approach is to consider more than one device, identify the advantages and disadvantages of each, and match the device to the therapeutic.

Logistics and Workflow Challenges

Dr. Kilburn stated that the logistics for scheduling MRI-guided treatments with sedation and a neurosurgery team has limited the frequency of the procedures.

Dr. Konofagou said that some focused ultrasound systems are not MRI-guided, so confirmation of BBB opening must occur after the procedure in a separate step. FDA has questioned whether gadolinium could cause toxicity when it enters the parenchyma after BBB opening. The cavitation dose may be a safer and more therapeutically interesting metric to use to quantify opening. Cavitation can also have therapeutic effects, but this aspect of cavitation is currently not used in clinical settings. Dr. Meng added that her team relies heavily on acoustic feedback that is incorporated into the ExAblate system because the MRI does not provide real-time information during sonication.

Dr. Syed and Dr. Meng discussed the possibility of MRI T2* changes necessitating plan or treatment changes. Dr. Meng said that T2* images during treatment help to finetune the dosage for each patient. Dr. Syed said that having T2* images enhances MRI-guided focused ultrasound treatments.

Dr. Wu suggested that artificial intelligence (AI) could potentially be used to create algorithms for inverse planning for IMUS to create devices that can logistically offer more frequent BBB opening.

Treatment Monitoring

Dr. Powlovich said that cavitation dose mapping is now being used instead of thermometry mapping and has been incorporated into clinical focused ultrasound systems. Dr. Borden added that a preclinical study investigated varying MI and MB volume dosages. Cavitation was the factor most highly correlated with BBB opening.

Dr. Packer asked whether any preclinical models showed the level at which focused ultrasound caused toxicity, Dr. Wu responded that acoustic pressure studies showed that 0.4 to 0.7 MPa produced inflammation and that above 0.7 MPa caused hemorrhaging and necrosis. The acoustic parameters for all of the clinical devices are within the non-inflammatory range, but there is room to escalate the pressures without causing necrosis. (For example, as Dr. Borden explained earlier, the MB volume dose, rather than the acoustic parameters, can be modified.)

Dr. Konofagou added that an MI links intensity, pressure, and frequency. Animal studies have shown short-term damage that is reversible within three days. Increasing pressures offers some therapeutic advantage. Optimizing therapeutic ultrasound based on the parameters set for contrast-enhanced ultrasound imaging is not possible.

Dr. Chen commented that a correlation exists among stable cavitation dose, BBB opening volume, and drug delivery, as well as between inertial cavitation dose and hemorrhage. These cavitation monitoring–based doses can be used for both BBB opening and for preventing hemorrhage.

Dr. Wu said that the implanted SonoCloud device opens large areas of the BBB before drug delivery and has been shown to be safe and well-tolerated without cavitation monitoring. The SonoCloud device has not been used in the brainstem, however, and the group felt that some level of monitoring is needed for the brainstem.

Dr. Powlovich stressed the importance of reporting all dosing parameters in publications (e.g., harmonic dose, cavitation dose, gadolinium enhancement, MRI findings). The group briefly discussed the fact that more than 20 years of safety data for focused ultrasound exists, whether the safety parameters will move beyond the lowest safest range clinically, and whether therapeutics delivered with focused ultrasound might become more toxic at higher doses.

Future Directions

Dr. van Vuurden said that many avenues remain to be explored, including different modalities, different MBs (monodispersed versus polydisperse), and even histotripsy (e.g., with immune checkpoint inhibitors, which is currently being tested in neuroblastoma).

Dr. Wu suggested testing new ultrasound parameters in veterinary clinical studies, especially because canines develop de novo tumors. For example, Virginia Tech just started a pilot histotripsy study in canines. When considering gliomas, high-intensity focused ultrasound (HIFU) and LIFU intersect at the necrotic core, where vascularity for drug delivery and/or MB delivery is poor. To address this issue, he suggested heating the glioma to above 40 degrees for one hour to create radiosensitivity. Another option is to apply histotripsy to the necrotic core while avoiding intact brain and then further stimulate via LIFU or BBB opening. Dr. Mueller agreed with the idea of moving forward in larger animal models.

Dr. Packer added that treating cortical tumors before brainstem tumors might have been a better approach, so that studies of adults could inform studies of children. Unfortunately, children with fatal brain tumors do not have the luxury of time; yet, paradoxically, moving too quickly or being too aggressive early on risks the trial being prematurely closed secondary to complications. Dr. Wu suggested conducting parallel studies on resectable cortical tumors. Dr. Mueller disagreed, noting that assessing toxicity in a non-brainstem midline tumor will not inform toxicity in the midline. Clinicians must assess toxicity in the disease that they want to cure, and other technologies (e.g., CED) have shown that data from cortical tumors cannot be extrapolated to eloquent areas (such as the brainstem). Toxicity levels and patients' tolerance are what must be considered, especially if combined with immunotherapy. No clinical setting is truly the same. Dr. Packer said that the team has delivered 52 brainstem treatments over the past 18 months and wants to continue to push the envelope. However, moving forward will be challenging without enough data.

When asked whether mice and humans have the same size capillaries in the brainstem (to accommodate MB diameters), Dr. Konofagou said that her team wrote a paper showing that both capillaries and arterioles are affected by BBB opening and that the MBs do go through the capillaries (Noel et al. 2026; in revision).[31] Human and mice capillaries are both 8–10 microns, and MBs are ~3 µm in diameter. However, the tumors may have smaller vasculature.

Burning Questions

Q: What volume of BBB opening is needed?

The current thinking is that treatment volume should ideally cover the entire tumor and extend past the radiographic tumor border to cover microscopic spread. The opening volume should also allow delivery of the desired quantity of a drug to a specific location. There is no direct evidence at this time, however, that larger volumes have led to better outcomes.

Q: What drugs or therapeutics should be used in conjunction with MB-FUS BBB opening?

Delivering drugs that have been tested with CED may be a good option, especially using efficacy data collected through that work. Another idea is to investigate delivering immunotherapy drugs right after radiation therapy. Creative strategies for collaborating with pharmaceutical companies are needed. For example, the Foundation has talked with more than 30 drug companies to find a partner for a sarcoma clinical trial.

Q: What is the optimal timing for clinical trial enrollment?

The Columbia University team suggested beginning treatment within three months of diagnosis and enrolling participants immediately after radiation therapy rather than waiting for tumor progression.

Q: What has been learned about the timing of drug delivery related to MB-FUS, and is the timing optimal and consistent?

The timing of drug delivery after MB-FUS differs for each drug (i.e., panobinostat, etoposide, doxorubicin) based on its half-life. How the drug is administered (oral versus intravenously) is another consideration, and more preclinical studies are needed to finetune each method of administration. A drug's plasma level must remain high until the BBB is open. For some drugs (e.g., methotrexate, gemcitabine), longer exposure to focused ultrasound is needed to increase brain penetration. Longer infusions, both during and after focused ultrasound, may be needed. Importantly, radiotherapy studies have shown that some drugs (e.g., methotrexate) can reach toxic levels when delivered after radiotherapy, but they are safe when delivered before radiotherapy. Similar "windows of administration" studies may be needed for focused ultrasound.

Q: How can we measure and validate drug delivery and response post-FUS?

Suggestions included radiolabeling the drug or microdialysis.

Q: How can results be harmonized for optimal interpretation?

Researchers can adhere to the reporting guidelines developed by the Focused Ultrasound Foundation. Making data regarding treatment parameters used in clinical trials with Insightec devices more widely available was another suggestion.

Session 2 – Expanding Applications: Liquid Biopsy, Immunotherapy, and Sonodynamic Therapy

This session featured two presentations on liquid biopsy, a review of immunotherapy for DIPG, and an update on the SDT201 clinical trial in patients with DIPG.

Liquid Biopsy Presentations

This segment of Session 2 provided an overview of liquid biopsy for DIPG and an update on preclinical and clinical sonobiopsy work for DIPG and GBM. A panel discussion on liquid biopsy resulted in a comprehensive list of suggestions for collecting, analyzing, and storing liquid biopsy specimens while standardizing these processes across the field.

Overview of Liquid Biopsy for DIPG: Shared Experiences

Javad Nazarian, PhD, presented an overview of focused ultrasound–assisted liquid biopsy for DIPG. He made the following points during his presentation:

- MB-FUS BBB opening may allow liquid biopsy to more easily and accurately capture tumor biology, tumor evolution, and treatment response. The Pediatric Neuro-Oncology Consortium (PNOC), an international consortium dedicated to bringing new therapies to children and young adults with brain tumors, recently met and discussed the topic of repeated biopsies in children with DIPG.
- BBB opening could provide access to larger quantities of biological material that has been shed by the tumor. CSF and blood plasma are both used for liquid biopsy, but CSF is more enriched in tumor-associated biomolecules. Liquid biopsy may be able to capture the significant clonal evolution that DMG tumors undergo during the disease course. These tumor changes cannot be seen on MRI; therefore, liquid biopsy of CSF could be used to detect tumor recurrence before an MRI shows the recurrence.
- Many different types of liquid biopsy assays exist, including single nucleic acid monitoring, gene panel profiling, shallow whole genome sequencing, protein profiling, metabolome assessment, immune profiling, and methylation.
  - The companies that provide single nucleic acid monitoring use either digital droplet polymerase chain reaction (ddPCR) (e.g., RainDance Technologies, Bio-Rad) or nanopore sequencing (e.g., the Oxford Nanopore Technologies).
  - These are good and sensitive platforms for monitoring tumor response, and several researchers have validated them against imaging studies.
- An abundance of circulating tumor DNA (ctDNA) at diagnosis is predictive of clinical response in DMGs. Higher ctDNA levels correspond with a higher tumor burden. The hypothesis is that higher plasma and CSF ctDNA levels may predict the openness of the BBB, so patients with an abundance of ctDNA will have longer progression-free survival (PFS) because the drug penetrates the tumor much better.[32]
- The Oxford Nanopore MinION sequencer is an efficient system that captures DNA and also provides a methylation profile.[33]
  - The Children's National team has successfully used this system to perform liquid biopsy with CSF. The team found that whole genome sequencing of CSF closely

follows tumor DNA genomic aberration.[32,34–36] CSF captures tumor biology better than blood plasma, however.

- Importantly, the MinION system concurrently captures methylation data and nucleic acid data, eliminating the need to re-run the sequencing.
- The quality of MinION data is comparable to that of Illumina EpicArray data (the gold standard), but the MinION system required only 15 ng of cell-free DNA (cfDNA) compared to 250 ng for Illumina.

- The team Children's National has a companion correlative study and has collected and run liquid biopsy assays on plasma and urine from patients enrolled on the focused ultrasound studies there (mostly the SDT). These liquid biopsies are performed via Meso Scale Discovery (MSD) for cytokine analysis, metabolomics, proteomics, and sequencing. These tests require only small amounts of the biospecimens. The correlative trial enrolled 11 participants and collected 155 longitudinal plasma samples, 152 longitudinal peripheral blood mononuclear cell samples, and 109 longitudinal urine samples. Urine can be collected during focused ultrasound delivery. Analyses are still ongoing; however, preliminary data suggest that:
  - The first SDT treatment caused a spike in inflammatory cytokines that was not seen after the second SDT treatment.
  - Pro-inflammatory cytokine levels fell to baseline after the third week post treatment.
  - Anti-inflammatory cytokine (interleuken-10) levels increased at two weeks post treatment.
  - Whether the analytes were systemic or tumor-related remains to be determined.
  - Analysis of the plasma samples showed elevated amino acids associated with impaired mitochondrial oxidative metabolism. The beta alanine and hydroxylysine spikes were especially pronounced after the first SDT treatment.
  - A poster (number DMG-39) that displays proteomic changes in urine samples was presented during the Society for Neuro-Oncology's 2025 Pediatric Neuro-Oncology Conference. The urine proteomics revealed elevated markers of mitochondrial dysfunction, apoptosis, immunity, heme metabolism, and oxidative stress. These markers are all associated with 5-aminolevulinic acid (5-ALA) pathway activation.
- Now is an ideal time to build liquid biopsy into both preclinical studies and patient care. The data support the utility of integrating liquid biopsy assays for real-time monitoring of disease progression and therapy response.

CSF ctDNA Analysis of DMG and Pediatric High-Grade Gliomas: Sick Kids Experience

On behalf of the research and clinical teams at SickKids, **Anirban Das**, MBBS, MD, DM, presented an overview of the SickKids approach to liquid biopsy testing on CSF. He made the following points during his presentation:

- Finely tuned treatment decision-making based on ctDNA liquid biopsy analyses is now occurring for many types of cancer care. Clinical applications include early diagnosis and prognosis, molecular profiling, treatment selection, monitoring of therapy response and tumor evolution, and residual disease detection.

- One method of ctDNA analysis, ddPCR, is a good tool to use with H3K27 mutant DIPGs. Full-depth whole genome sequencing provides much more DNA information, but the cost is not practically feasible. For the DIPG clinical trial at SickKids, the team is running a targeted hybrid-capture panel plus low pass whole genome sequencing.
- To show the clinical utility of CSF liquid biopsy in pediatric brain tumors, SickKids collected 142 samples from 115 patients and 32 controls from 2017 to 2024. The pediatric high-grade glioma samples produced an 80% yield, and the low-grade gliomas produced a 50% yield, with the detection of both targetable and additional alterations.
  - A total of 40 known H3.3K27M samples were tested with the ddPCR platform, which showed 100% sensitivity from CSF but only 14% sensitivity from plasma. Both CSF and plasma were 100% specific. In patients who refused or could not undergo stereotactic needle biopsy, ddPCR was able to detect K27M in CSF in both radiologically typical and atypical tumors.
  - Low pass whole genome sequencing, however, can be used to detect the oncogenic PDGFRA gene amplification on the fourth chromosome. This information can then be used to choose targeted therapies after radiation has failed. For this reason, SickKids has moved from ddPCR to the combination of the DNA panel and the low pass whole genome sequencing for DMGs and most brain tumors. The low pass whole genome sequencing provides actionable clinical data and is cost effective for clinical practice.
  - Serial monitoring of DMGs with liquid biopsy of CSF ctDNA can be clinically useful for confirming diagnosis and tumor progression. With DMG, clonal evolution and tumor dissemination should be conceptualized, especially when considering targeted therapies.
  - Liquid biopsy of CSF ctDNA also helps to exclude the presence of K27 and to find additional drivers that ddPCR cannot find. The panel and the copy number profile can be used to make alternative diagnoses.
- Liquid biopsy of CSF ctDNA is also useful when treating mismatch repair high-grade gliomas. These tumors arise from a germline genomic defect that leads not only to accumulation of mutations, but also to microsatellite regions with increased numbers of insertions and deletions (INDELS).
  - Running low pass whole genome sequencing and then counting the number of INDELS in the microsatellites is a sensitive tool for detecting and quantifying microsatellite instability and tumor mutational burden. These data can then be used to create a mismatch repair burden DNA score and are useful for treating tumors that quickly evolve.
  - CSF ctDNA can help clinicians determine whether tumor progression is due to a new tumor variant or an evolved tumor under therapeutic pressure. This information directly affects the therapy or treatment decision-making process.
  - For mismatch repair high-grade gliomas, analysis of CSF ctDNA can be used for early detection of evolving lesions on surveillance, monitoring treatment response, and stopping treatment and serial surveillance.
- In summary, clinically validated CSF liquid biopsy panels to diagnose, monitor, treat, and change the course of treatment for pediatric brain tumors currently exist. MB-FUS BBB opening is the next frontier and may enhance liquid biopsy by increasing the yield of cfDNA in the CSF and blood.

Group Discussion

Attendees discussed the workflow for using CSF liquid biopsy to serially monitor pediatric patients. At SickKids, the team has collected CSF from the lumbar spine every six months and collected blood every three months. Going forward, they plan to collect blood more frequently—at 15 days, one month, and then every month. The amount of DNA that is needed to combine ddPCR with low pass whole genome sequencing to obtain clinically relevant information is significantly low: this workflow only requires 5 to 10 milliliters of CSF depending on the age and the size of the child. The amount is minimal for clinical needs, so the rest of the CSF can be used for proteomic and metabolomic analyses.

When asked when liquid biopsy might become available at more hospitals, Dr. Das said that liquid biopsy was already moving into the clinical realm. Canada has centralized national tumor boards, so SickKids receives samples from across the country and around the world. They have found that using next generation sequencing plus low pass whole genome sequencing covers the most common diseases. At SickKids, the medical team is already using liquid biopsy results to make clinical decisions for the treatment of some types of tumors (e.g., germ cell tumors, which have radiology limitations). There are glimpses of medical utility for DMG liquid biopsy results, but those tests have not yet been clinically validated. Dr. Das commented on his graph showing patients with highly proliferative, genomically unstable tumors who are being treated with immunotherapies: if higher ctDNA does not clear with treatment, then the treatment is not working; if the ctDNA clears with treatment, then the treatment is working. The graph shows better outcomes as the ctDNA clears.

Several liquid biopsy platforms are now Clinical Laboratory Improvement Amendments (CLIA) certified, but there are challenges in achieving this certification so very few US centers have CLIA-certified systems that can be used for clinical decision making. Europe has an entirely different system. A pathology department must have an adequate patient volume to justify the purchase of liquid biopsy equipment, and interpreting liquid biopsy laboratory reports can be challenging. For example, if a positive result is found in a sample from a patient on therapy, does that mean therapy should be changed? If a patient did not have a biopsy and does not have a diagnosis, then it might be good to send out a sample for analysis. Despite these challenges, there are increasingly sufficient data to support obtaining CSF from patients with DMG and high-grade gliomas.

Liquid biopsy is currently performed mostly in the context of clinical trials. Some of the tests used in clinical trials, however, are not yet in use in the clinic, so data from ongoing studies (e.g., PNOC022) will support their clinical translation. The PNOC022 clinical trial has 120 participants, so more than 500 longitudinal pediatric CSF and plasma specimens have been collected and stored in its biorepository. The data from this cohort might allow pathologists to certify a platform that is useful for the pediatric community, but no consensus has been reached on which platform to use.

Attendees discussed which liquid biopsy platform might be best for multi-site focused ultrasound clinical trials. Using liquid biopsy may provide a benefit for earlier enrollment of patients in clinical trials; it could also serve as a proper biomarker of BBB opening and disease monitoring. However, each platform has different capabilities and different strengths and weaknesses. Dr. Das said that the two platforms that were presented are complimentary, not competing. A head-to-head comparison of the platforms might help to determine the error rates for a lack of tumor detection, but each platform has its own limitations, so head-to-head comparison may not be possible. The first platform that is clinically validated may be the most used option. Clinicians need to learn the capabilities and uses of each platform. Although some centers have used liquid biopsy platforms for five to seven years, there is still no routine or easy way to use the technology. After it is standardized, the Oxford Nanopore platform could

be used for all analyses. Notably, some commercial liquid biopsy entities do not know which mutations are relevant to pediatric neuro-oncologists. Two companies are geared toward adult cancer: Foundation Medicine and CDx Diagnostics.

Children's National had not been collecting CSF for its two focused ultrasound DMG clinical trials because they were hesitant to add a procedure with any level of risk. Dr. Kilburn said they would now be willing to add CSF collection before and after the MB-FUS BBB opening procedure, and CSF collection is already included in the protocol as optional. For patients with DMGs, adding an Ommaya device could provide an added safety measure and be clinically useful. If Ommaya insertion was added to a protocol where it was felt that it would serve to add a safety measure if a patient developed acute hydrocephalus or tumor edema, this could also be used to increase the frequency of CSF testing. Ommaya insertion is already mandatory for several immunotherapy trials but not for PNOC22. It took a slight cultural shift to obtain CSF via lumbar puncture as part of staging and disease assessment in patients with DMG, but now providers rarely request omission. Most neurosurgeons would be amenable to placing an Ommaya reservoir unless a patient had a contradictory mechanical reason (e.g., small ventricle, shunt in place). However, it was also discussed that there can be challenges when there remains debate on the question of whether a procedure should be classified as a research procedure versus a procedure for supportive care/safety. Additionally, placing Ommaya reservoirs could be challenging for a hospital that is not a part of PNOC or the National Cancer Institute's Pediatric Brain Tumor Consortium (PBTC). For example, even with a high neuro-oncology patient volume, the Cook Children's Hospital team would find it difficult to place Ommayas unless required to do so for a clinical trial, mainly because of limited resources. ctDNA could be analyzed on specimens collected from an Ommaya to help answer the question of how long the BBB stays open, and finding an alternative to gadolinium for BBB opening confirmation (e.g., liquid biopsy biomarkers) would be useful.

Some members of the group wondered whether the Focused Ultrasound Foundation would support studies to test liquid biopsy platforms. One idea was to analyze a subset of the PNOC022 samples on two liquid biopsy platforms. The PNOC022 cohort has several long-term survivors. Others suggested systematically embedding liquid biopsy into focused ultrasound clinical trials and then comparing outcomes. Obtaining a set of samples that are shared by two institutions and run on two platforms could also be used to compare platforms. Dr. Mueller said that UCSF could collaborate by sharing samples and their associated imaging and outcomes data. The experts agreed that building comparative analyses into clinical trials and sharing samples across platforms could move the field forward.

Attendees discussed analytes for liquid biopsy analysis, including methionine, thiamine, and MSD panels. CSF data can be used to track mutations, tumor evolution, clonal changes, and tumor heterogeneity. Genome sequencing would be complimentary. The SickKids panel includes genes that are relevant for pediatric neuro-oncologists—from the more common to the rarer ones (e.g., mismatch, which have a secondary polymerase hit). The SickKids panel was diligently developed by neuropathologist Cynthia Hawkins, MD, PhD, FRCPC. Dr. Hawkins presented "CSF Liquid Biopsy for CNS Cancer Diagnosis and Disease Monitoring" at the 2025 Pediatric SNO meeting liquid biopsy session. She could be invited to collaborate with the focused ultrasound community.

The group agreed on the importance of as much standardization as possible, including the timing of the sample collection. They noted that some standardization is happening in Europe. One attendee suggested writing a white paper that describes the needed standardization and includes context from PNOC studies.

Sonobiopsy for DIPG

Hong Chen, PhD, described her preclinical research developing sonobiopsy. She made the following points:

- Sonobiopsy uses ultrasound to enrich circulating biomarkers from ultrasound-targeted disease regions, enabling sensitive and spatially targeted molecular diagnosis through liquid biopsy. It can be applied to CSF, blood, urine, or other targeted tissues. Ultrasound can be applied through various mechanisms, including MB-FUS BBB opening or histotripsy. Focused ultrasound enables researchers to collect spatially targeted, pinpoint information from a specific part of the brain, or a specific part of a brain tumor, to make a molecular diagnosis.
- The first sonobiopsy paper was published by the Stanford group in 2009.[37] In 2018, the Washington University in St. Louis (WUSTL) group was the first to preclinically use MB-FUS BBB opening to capture brain tumor–specific biomarker release from the brain to the blood.38 More recently, the field has been expanding beyond brain tumors into Alzheimer's disease, gene therapy, and heart transplantation.[25,39,40] Other applications currently under preclinical investigation include fibrosarcoma, prostate cancer, and colon cancer.
- Over the past seven years, the WUSTL team has been advancing sonobiopsy for brain tumors from preclinical mouse models through porcine studies into human clinical trials.[41–43] In 2023, its patent was licensed to Cordance Medical to commercialize the technology, and Cordance was granted breakthrough device designation from FDA.
- To test the concept in humans, the WUSTL team designed a single-transducer, handheld focused ultrasound device and coupled it with the Medtronic neuronavigation probe.
  - In a safety study, five patients who were newly diagnosed with glioblastoma (GBM) and were scheduled to undergo tumor resection underwent sonobiopsy before the resection.
  - Blood samples were collected 5 minutes before sonobiopsy and then 5, 10, and 30 minutes after the ultrasound of the GBM tumor. The ultrasound pressures used for the sonobiopsy were quite low.
  - Blood plasma and corresponding tumor samples were sent to Invitae genetic testing for blinded ctDNA analysis. WUSTL also ran in-house ddPCR analysis of cfDNA on the plasma samples.
  - Biomarker enrichment after sonobiopsy was found in some, but not all, of the patients, possibly due to the low ultrasound pressures applied. Pathology analysis of the tumor samples revealed that no damage was caused by the ultrasound.
- To apply the concept to DIPG, preclinical studies were conducted in a mouse model, and the Focused Ultrasound Foundation funded this work. Dr. Nazarian provided the cell lines for these investigations. The cell lines did not have the H3K27 mutation but did express green fluorescent protein (GFP) and firefly luciferase (fLUC).
  - After validating the tumor model, the WUSTL team used MB-FUS to open the BBB over the DIPG tumor area and then collected blood plasma and CSF samples.
  - After validating the ddPCR assay validation for GFP and fLUC, the team used ddPCR to quantify plasma concentrations of ctDNA, ctRNA, cfDNA, and cfRNA and CSF concentrations of ctDNA and ctRNA.

  - For plasma, there were no significant changes in DNA concentrations before and after BBB opening, but there was significant enrichment of RNA, especially for GFP.
  - The baseline DNA level was higher in the CSF, which is consistent with the clinical data. Applying ultrasound BBB opening enriched ctDNA levels in the CSF, along with GFP and fLUC expression. RNA concentrations in the CSF were much lower than in the plasma, and they were not significantly enhanced by the ultrasound.
  - Applying sonobiopsy to the brainstem did not cause hemorrhage. The procedure was safe and feasible in this mouse model of DIPG.
  - The enrichment ratio on circulating biomarkers was dependent on the source (plasma vs. CSF), analyte (ctDNA vs. ctRNA), and individual marker (GFP vs. fLUC).
- The data from these recent studies have been submitted for publication.

Group Discussion

An attendee said that this study design was ideal for the neurosurgery community and suggested using sonobiopsy with other tumor types near the brainstem (e.g., medulloblastoma). The group discussed the model used in this study (a mouse cell line—the Becher RCAS model) and the fact that there was no cell death because the mice did not receive any other treatment. Dr. Nazarian said that giving the mice radiation therapy would produce a large spike in ctDNA and ctRNA. Attendees hypothesized why the images showed a strong signal in the spine. Responses ranged from CSF leaking into the spinal cord (the pons is close to the fourth ventricle), the heterogenous cell line, and false signals in animal models.

Liquid Biopsy Panel Discussion

Panelists
Hong Chen, PhD, **Anirban Das**, MD, and **Javad Nazarian**, PhD.

Panelists and attendees addressed which biomarkers to monitor, the best methodologies for liquid biopsy, whether to collect CSF, blood plasma, or both, and additional considerations.

Questions

Q: What biomarkers or "windows of response" should be monitored via liquid biopsy for DIPG?

- Measuring H3K27M gene expression in blood plasma can be used to diagnose DIPG and to monitor its progression. Analysis of ctDNA can be used to measure changes in tumor volume.
- Biomarkers can be used to measure the openness of the BBB.
- Biomarkers can be measured for each specific treatment (e.g., immunotherapy).
  - If the treatment targets mitochondria, then mitochondrial markers can be analyzed.
  - If the agent targets metabolism, then metabolic markers cane be analyzed.
  - Proteomics can be used if the treatment target is a specific protein or with CAR-T cell therapies. Proteomic changes occur in DIPG.
  - The MSD platform could be used for focused ultrasound plus immunotherapy trials. B7H3 that is coming from the tumor can be detected in both plasma and CSF (and other cells do not typically have high levels of B7H3).
- Biomarkers may differ for each type of biosample—CSF or blood plasma.

Q: What is the best methodology for liquid biopsy?

- Standardized specimen collection methods at standard time points for each type of biosample.
  - Standardize CSF collection procedures based on collection method (e.g., Ommaya port, lumbar puncture). Use caution when interpreting data from CSF that is collected from a constantly draining shunt.
  - Document or map the spatially targeted sonication location on the tumor(s) or tissues. Save a new trajectory based on the final location of the ultrasound probe.
  - Collect biospecimens at agreed upon time points for diagnosis, pre-treatment, mid-treatment, post-treatment, post-radiation, and at the time of radiological progression, for longitudinal sampling, and any other time points that may be of interest.
  - Obtain multiple samples at agreed upon time points before (baseline) and after MB-FUS BBB opening. Be aware of hospital limitations on the amount of blood that can be collected within a 24-hour or 3-day time period. Coordinate with PK or PD collections if a drug is administered.
  - Document the time of each collection relative to the focused ultrasound procedure.

  - Continue to conduct experiments to determine the optimal timepoints for the collection of each type of biosample. Additional questions include the amount of time it takes the biomarker to reach circulation and the CSF after sonication of the brain or other tissue.
  - Determine the best methods to store and bank biosamples for future assays and re-analysis as the technology advances.
- Correlate liquid biopsy results with imaging data.
- Run comparative studies to determine which liquid biopsy platforms would best meet the needs of the focused ultrasound community.
  - Use the PNOC022 cohort to conduct a standardization comparison between the two liquid biopsy systems.
- Use the Oxford Nanopore Technology nanopore sequencing device for fast analysis of cfDNA (when speed is needed).
  - The nanopore device can be used in the operating room.
  - The nanopore device can be used for molecular testing and to analyze methylation and tumor subtypes before pathology results are returned.
- Further develop focused ultrasound devices to enrich liquid biopsy.
  - Determine whether sonobiopsy could be used to re-biopsy tumors that are otherwise ineligible for re-biopsy.
  - Incorporate physician perspective into future device design (i.e., diseases to study, target volume, early diagnosis, drug treatment monitoring, recurrence detection).
  - Work together as a community to develop a platform technology, especially for patients with DMG.
  - Continue to pursue FDA approval of sonobiopsy, Delsona, and other platforms, such as the Cordance device.
  - Initiate more clinical trials in pediatric patients, especially children with DIPG.

Q: Should CSF, blood, or both be collected and analyzed?

- Whether CSF, blood, or both should be collected depends on the treatment (e.g., immunotherapy).
- Add urine collection and analysis, especially for proteomics.
- Consider developing a timeline for longitudinal liquid biopsy collection in patients with DIPG.
- Encourage investigators to consider incorporating the insertion of an Ommaya reservoir where clinically appropriate into focused ultrasound clinical trials which will also facilitate collecting CSF for liquid biopsy.
- Design clinical trials to include collection of biospecimen samples as close as possible to the time the focused ultrasound is administered, both before and after.
- Consider centralizing biospecimen collection and analysis at the best laboratory or platform (similar to what is being done in the PNOC studies).

Additional Considerations

- **Suzanne LeBlang**, MD, the Focused Ultrasound Foundation's director of Clinical Relations, is leading the Foundation's liquid biopsy efforts in adults. Dr. Chen is on the Foundation's liquid biopsy scientific advisory board.
- BLOODPAC, a consortium managed by the Center for Computational Science Research, Inc. in Illinois, seeks to accelerate the development, validation, and accessibility of liquid biopsy assays to improve outcomes for patients with cancer. The BLOODPAC Brain Tumor consortium group recently published liquid biopsy guidelines that should be distributed to DIPG workshop attendees.[44] Similar guidelines could be written for the pediatric population. Attendees suggested nominating a pediatric representative to serve in the BLOODPAC consortium.
- The liquid biopsy aspect of clinical trial protocols should be standardized across multi-institution studies. For example, Children's National has a separate liquid biopsy protocol that is considered an add-on study to both the SDT and MB-FUS BBB opening clinical trials. Some research questions could be answered across multiple safety studies that have low enrollment numbers or via preclinical studies.
- A standard template for ultrasound-based protocols that is independent of the exact treatment modality should be developed. SickKids is creating a template for immunotherapy with specific checkpoint inhibitors that is agnostic of tumor type. The same biomarker protocol could be followed for focused ultrasound clinical trials.

Immunotherapy

The immunotherapy segment of Session 2 featured two background presentations, the presentation of a protocol for a future immunotherapy clinical trial, and two discussions.

Overview of Immunotherapy for DIPG

Anirban Das, MBBS, MD, DM, presented his experience studying immunotherapy for mismatch repair hypermutant tumors with the hope that common principles can be applied to DIPGs and DMG. He made the following points:

- Radiation therapy and radiation retreatment (re-RT) are the current standard of care for patients with DMGs. With this care, median overall survival is 9 to 11 months, and after progression, life can be extended up to another 7 months with re-RT. The time from diagnosis to death can also be extended from 14 to 23 months with re-RT. These are the current treatment benchmarks.
- Patients with non-pontine disease, older age, and some co-mutations within the DMGs are known to have better survival than others.
- DMGs have a "cold" tumor microenvironment, but each individual tumor has its own nuances, and each tumor changes over time, especially in response to various treatments.[45,46]
- There are four general immunotherapy approaches for treating DMG: two are cell therapies (i.e., CAR-T cells, autologous cell transfers), and two modulate the tumor microenvironment (i.e., cancer/antigen vaccines, antagonistic or agonistic antibodies,). These approaches are currently being delivered via three different techniques (i.e., CED, intra-arterial therapy, and focused ultrasound).[4]
  - Cell therapies, or CAR-T cell therapies, have been delivered to patients of various ages with various types and locations of DMG, sometimes in combination with radiation therapy.[47–50] Results have varied, but some patients have shown a real benefit from the treatment. Multi-antigen T-cell therapy uses T-cells with specific tumor-associated antigens, and an ongoing Phase 1 dose escalation trial at Children's National Hospital (NCT03652545) has enrolled and is following 33 participants.
  - To modulate the tumor microenvironment and turn a "cold" tumor into a "hot" tumor, clinical trials have tested Oncolytic DNX-2401 and H3K27M-targeted vaccines.[51–53] Some patients have notable responses, and liquid biopsy analysis may help tease out the responders.[54] At least five clinical trials are currently enrolling participants for studies that modulate the tumor microenvironment with or without a combination therapy, and promising preclinical work in this area is also ongoing.[55] The other type of therapy that could modulate the tumor microenvironment is novel monoclonal antibodies. Preclinical studies are successfully promoting tumor regression, antitumor immune memory, and immune surveillance as the tumor develops.[56,57] Accessing pharmaceutics for combination clinical trials is also a challenge. Combinations must be based on good clinical data, but consultation with preclinical scientists who do timing experiments (e.g., What is the best tumor stage to apply the treatment?) provides important insights for clinical trial design.
- Several exciting immunotherapy concepts could be considered in the context of MB-FUS BBB opening.[58,59] Considerations include the tumor microenvironment, the chosen agent, and the timing of the treatment with MB-FUS BBB opening. Patient selection is

another important consideration, because many patients are pre-treated with other therapies that can confound the treatment effect.

- Dr. Meng and researchers at Sunnybrook Health Sciences Center in Toronto have conducted MB-FUS BBB opening clinical trials for immunotherapy delivery.[60] This initial study found evidence of enhanced brain penetration of trastuzumab in patients with Her2-positive breast cancer brain metastases (NCT03714243).
- In conclusion, immune strategies are promising for DMG/DIPG, but they must be based on robust preclinical data and mechanistic understandings because technological innovations and manipulations continue to change the field. Both peripheral and local effects are important, so delivery will likely include both intravenous and intraventricular methods. Clinical trials should incorporate biomarkers for identifying response versus resistance or escape. Liquid biopsy must be an integral part of disease monitoring, along with biopsies and autopsies to study the tumor microenvironment and mechanisms of immune escape. Ultimately, synergistic and combinatorial approaches are probably needed to extend life for patients with DMGs and DIPGs.

Focused Ultrasound Plus Immunotherapy for Pediatric Brain Tumors: Preclinical Evidence

Natasha Sheybani, PhD, presented some of the preclinical work that has been done in DIPG/DMG research and then shared additional data from her laboratory's pediatric medulloblastoma studies. She made the following points:

- Cancer immunotherapies represent a powerful tool in the armamentarium for pediatric brain tumors, and they come with a great deal of versatility.
- One promising frontier for DMGs is in the context of focused ultrasound plus PD1 blockade. Several recent abstracts have demonstrated the ability of MB-FUS BBB opening to improve antibody access within mouse models, and some of the early evidence is showing efficacy.[19,61,62]
- Within the constraints of preclinical research systems, the questions for using immunotherapies to treat another type of pediatric brain tumors, medulloblastomas, involve understanding whether the cerebellum can be safely targeted, determining whether the tumors can be rendered permissive to macromolecular therapeutics (i.e., immunotherapies), and investigating whether currently ineffective cellular therapies can be potentiated in this context.
- The team has been working to establish the safety and feasibility of cerebellar MB-FUS BBB opening in a naive mouse model by administering MBs as a bolus just before sonication with conservative acoustic exposure conditions (unpublished data). The safety profile, based on imaging and histology data plus physiological monitoring and motor testing, is favorable. Next steps involve using the same acoustic exposure conditions to improve penetrance and set the foundation for pursuing monoclonal antibody–based immunotherapy treatments. Thus far, the peak negative pressures tested have yielded superlative improvements in reporter antibody access. Translation of this initial work into a syngeneic mouse model of high-risk medulloblastoma, group 3, has begun.
- This group is also interested in leveraging MB-FUS BBB opening with the immunomodulatory effects of focused ultrasound plus cellular therapies. The immunomodulatory effects of focused ultrasound included BBB permeabilization–

mediated therapeutic delivery, tumor immunomodulation, tumor destruction, and sonogenetic control. In immunotherapy, focused ultrasound can help overcome barriers by heightening vascular activation with its mechanical effects, by modulating cytokine gradients to potentially change the biochemical milieu that would draw T-cells into a tumor, and by alleviating exhaustion via targeted checkpoint blockade. In the context of pediatric brain tumors, sterile inflammation caused by focused ultrasound is not yet thoroughly understood, especially in sensitive structures such as the cerebellum or brainstem. Next steps involve investigating the immunomodulatory effects of focused ultrasound to potentiate adoptive T-cell transfer.

- Time and more data will reveal when it might be safe to begin extrapolating knowledge from other brain tumor contexts over into pediatric brain tumors. Immunotherapies are complex, and focused ultrasound is a powerful but multiparametric tool. There are a lot of knobs to turn and many ways that those various knobs might impact research outcomes for basic discovery and translation. Time and resources are needed to systematically understand safety- and outcome-driven factors.
- To answer the questions "Can I get my immunotherapy to go where it needs to go effectively? And if I do that, how long does it stick around?" the following challenges facing BBB opening monitoring must be addressed:
  - Conventional MR contrast agents poorly recapitulate complex biologics (e.g., gadolinium-based contrast agents routinely used for MR contrast are 140- to 150-times smaller than an IgG molecule).
  - Current real-time feedback approaches may not account for spatial heterogeneity and the shear complexity of MBs inside an endogenous tumor environment.[63]
  - Molecular imaging may offer a tool for performing empirical assessments.[64] For example, in one preclinical study, clear enhancement on MRI was not corroborated on PET when timing of antibody delivery was altered; these findings were used to inform the design of an anti-CD47 delivery paradigm, which showed improved survival responses and held critical implications for system dose titration (e.g., 18-fold decrease in antibody administration with focused ultrasound). These dosing implications could be particularly important for pediatric applications.
- Finally, a note about animal models: "All models are wrong, but some are useful." The goal should be to identify key clinical bottlenecks and then perform model selection on the basis of how well the specific bottleneck is recapitulated. Whether the issue is tackling the BBB versus the myeloid compartment, certain animal models stand to shine and, at scale, could help answer key questions through empirical means.
- Ancillary variables (e.g., onboard anesthesia), treatment dosing, route of administration, and sequencing—and their relevance to clinically feasible workflows—along with MBs and acoustic parameters for cross-system benchmarks are all critical considerations going forward.[65–67] These factors can be difficult to reconcile as preclinical studies are translated into human clinical trials. Cross-system benchmarks and acoustic exposure conditions must be mapped across these spaces.
- The field is now in a foundational position to be incredibly diligent about reporting, harmonizing, and standardizing practices for all correlatives and analyses. Published guidelines on immunological analyses after focused ultrasound treatment are relevant to both preclinical and clinical work, so hopefully the community will continue to adopt and use these guidelines.[68]

Group Discussion

When asked about the timing of focused ultrasound administration relative to pre- and post-CD47 blockade, Dr. Sheybani said that the pre-therapy was given immediately before sonication and the post-therapy was administered about 15 minutes after sonication. Her other studies have extended that window of time. She added that the timing of drug administration relative to focused ultrasound currently varies across ongoing and published clinical trials.

MB-FUS BBB Opening Combined with Immunotherapy Clinical Trial

Lindsay Kilburn, MD, and **Roger Packer**, MD, described a new clinical trial protocol that the Children's National Hospital team is developing to deliver a combination immunotherapy to children with DIPG. They made the following points:

- **Luca Szalontay**, MD, a neuro-oncologist at Children's National, is leading this research study, which is titled "A Safety and Feasibility Study to Evaluate Blood-Brain Barrier Disruption Using Exablate MR-Guided Focused Ultrasound in Combination with Multi-Antigen T-Cell Infusion in Patients with Newly Diagnosed Diffuse Midline Glioma." She previously worked with the Columbia University focused ultrasound group before taking the position at Children's National.
- Early clinical trials and some preclinical studies with cellular therapies have shown hints of efficacy in certain patient populations.47,49,69,70 However, not all patients respond, and the responses are generally not durable. The hypothesis is that MB-FUS BBB opening might enhance the delivery of the therapy and also modulate the tumor microenvironment. This trial was also designed to leverage the cellular therapy expertise at Children's National. To date, most CAR-T cell therapy studies for DIPG/DMG have used local infusion into the ventricular system.
- MB-FUS BBB opening will be used to deliver multi–tumor associated antigen specific T-cell (TAA-T) therapy. TAA-T targets multiple tumor-associated antigens (TAAs) in a single product. The rationale for choosing this immunologic product rather than something like CAR-T cells was based on the available clinical safety experience. Furthermore, TAA-T cells were infused intravenously as opposed to being locally injected into the ventricular system.
- The ReMIND dose-escalation study (NCT03652545) showed that "TAA-T had a favorable toxicity profile (4%) when compared with CAR-T therapy and may elicit anti-tumor immune responses that contribute to prolonged survival."71 TAA-T has also shown remarkable safety in the pediatric setting and an early signal of efficacy in pediatric brain tumor patients.
- The goal of the new study is to evaluate the combination of cellular therapy with the same TAA-T cells that were used in the ReMIND trial but to combine that therapy with MB-FUS BBB opening.
    - It will enroll participants with newly diagnosed DIPG (group A, n=10) or newly diagnosed thalamic DMG (group B, n=10) at Children's National.
    - The prospective, two-arm, nonrandomized study will evaluate the safety and feasibility of BBB disruption using the Exablate 4000 Type 2 focused ultrasound system in pediatric patients with newly diagnosed DMG undergoing treatment with TAA-T. A second primary objective is to describe the toxicities related to the combination therapy.

- The first patients enrolled in the study will receive MB-FUS as a reduced treatment volume in all treatment cycles (up to three). There will be no intrapatient volume escalation. Subsequent patients will receive full treatment volume if initial safety is established.
- The primary endpoints are to evaluate the safety and feasibility of a single treatment of TAA-T cells and BBB opening. Secondary objectives include evaluation of repeated treatments and preliminary efficacy. Exploratory objectives will include assessment of correlative studies in imaging, immune response, and quality of life.
- After the protocol is finalized and the Investigational New Drug (IND) submission has been prepared and approved, the full inclusion and exclusion criteria will be listed on clinicaltrials.gov.
- Insightec is partnering on this study to provide technical support, but Children's National will hold the IND for this clinical trial.

Group Discussion

During the discussion, an attendee asked whether any exhaustion markers or correlates were found in the ReMIND study. Dr. Kilburn said that some participants received PD1 after the cellular therapy, so the team is analyzing those data and trying to align the correlates. When asked about the plan for monitoring the antigens targeted in the ReMIND trial, Dr. Kilburn said that the team plans to use archival tissue. Because these same antigens have also been used in similar solid tumor programs and they are expressed across a variety of different cancers, Dr. Nazarian looked across different brain tumor types, including DIPG, to show that there was good expression of the targets before suggesting them for the ReMIND study. At this point, antigen studies have only been done on archival tissue, so whether there are other ways to look them was noted to be a good question.

Attendees discussed whether the MB-FUS BBB opening procedure might lead to too much uptake of immune cells into the brainstem. Toxicity is a risk, especially since broad toxicity (e.g., an inflammatory response) has been found with GD2 CAR-T cell therapy. A Seattle group found less toxicity with its single antigen study, but its quad CAR-T study is ongoing. The previous clinical trials–both at Children's National and at SickKids–have provided quite a bit of clinical experience with applying focused ultrasound to the brainstem, however the experience with BBB opening is still more limited. The ReMIND clinical trial provided safety data with using TAA-T in patients with DIPG, so this clinical trial is felt to be a reasonable next step. The Children's National team remains as cautious as possible while pioneering new treatments. Regarding a question of localized or systemic delivery for cellular therapy, Dr. Kilburn said that many CAR-T brain tumor studies are moving toward intraventricular administration; however, some immunologists still suggest that peripheral infusion may be a better approach for engaging the immune system. The optimal delivery may also differ depending on the product and tumor type.

All immunotherapy approaches in treating DIPG have raised concerns for potential tumor inflammation. This potential for an increased immune response is the reason for the staged volume approach. If pseudoprogression does occur in this study, bevacizumab administration may be given as a possible supportive care measure. The group discussed the use of bevacizumab in other clinical trials72 and in preclinical studies, where it is used to prime the tumor microenvironment for improved vascular activation or improved BBB opening.

MB-FUS BBB opening for a T-cell versus a chemotherapy or antibody was another topic of discussion. Data suggest that T-cells may not actively penetrate all areas of the tumor, so the rationale for the proposed trial is that MB-FUS BBB opening could improve tracking of the T-cells into the tumor and overcome an immunosuppressive tumor microenvironment.

It would also be helpful to look at these therapies in non-brainstem tumors. Preclinical and clinical research studies are being performed in parallel to address many of these outstanding questions, and attendees suggested submitting preclinical modeling with the IND application along with imaging data that tracks T-cells through the process. Immunocompetent DMG models or the humanized mouse models developed by Nicholas Vitanza, MD, could also be used for the preclinical work.

A final topic of discussion was a recent FDA guideline to move away from animal testing. Dr. Maruvada said that all clinical trials are evaluated on a case-by-case basis, and the conversation should be held with the lead reviewer and their team. Dr. Packer thanked FDA attendees for their continued guidance and encouragement to increase sonication volume in the brainstem to ensure potential therapeutic benefit.

Sonodynamic Therapy

This segment of Session 2 featured one talk followed by a discussion.

Overview and What Can be Shared from Clinical Trials

Hasan Syed, MD, described the SDT201 clinical trial at Children's National Hospital. He made the following points:

- SDT is a combination therapy that activates a sonosensitizing agent with focused acoustic energy. SONALA-001 is an investigational drug manufactured by SonALAsense. It contains aminolevulinic acid (ALA) hydrochloride, a pharmacologically inactive compound that is converted into a drug through a metabolic process (i.e., a prodrug), which readily crosses the BBB and accumulates in glioma cells. An oral form of ALA is FDA-approved as a visual aid for the surgical resection of high-grade gliomas. ALA's primary mode of action is activation to the metabolite, protoporphyrin IX (PpIX), via MR-guided focused ultrasound. SDT is similar to photodynamic therapy, but with SDT the photon energy is replaced with sound or acoustic energy.
- ALA is an organic compound in the body and a part of heme synthesis. After ALA's metabolite, PpIX, moves into the mitochondria, it becomes a fluorophore that can be visualized under light, and it becomes excited by higher wavelengths of light or sound energy. When PpIX becomes excited, its downstream effects result in apoptosis, lipid peroxidation, and formation of reactive oxygen species. These effects cause tumor cell apoptosis.73 In ALA SDT, activation of PpIX induces necrosis and apoptosis directly in the glioma tissue. Various preclinical studies have shown that SDT selectively slows the growth of gliomas and increases survival in rat models.[74–76]
- The first-in-human Phase 0/1 SDT clinical trial was conducted at the Ivy Brain Tumor Center in Phoenix, AZ, by Nader Sanai, MD, in patients with recurrent high-grade gliomas. The trial design included an intra-tumor control with 50% of the tumor treated with the focused ultrasound. Four days after treatment, tumors were resected to compare biomarkers between the treated half and the untreated half. From a safety standpoint, the ALA SDT was well-tolerated, and histology showed that reactive oxygen species caused cell death and triggered apoptosis with the same rapidity of action as seen in preclinical studies (i.e., the changes were seen within 96 hours).
- In DIPG-specific preclinical studies, Dr. Nazarian tested 5-ALA uptake, conversion to PpIX, and effect of ALA SDT on DIPG patient–derived primary cell viability. These DIPG cell lines accumulated ALA at a higher rate than C6 rat glioma cells and demonstrated that ALA SDT is a viable treatment in DIPG patient cells.[77] This important translational research was used to inform the first pediatric clinical trial.
- Children's National led the initiation of the SDT201 clinical trial (NCT05123534), which is titled "A Phase 2 Study of Sonodynamic Therapy Using SONALA-001 and Exablate 4000 Type 2.0 in Patients with DIPG." To date, more than 45 treatments have been completed.[6]
    - The Phase 1/2 study was designed to enroll 18 to 24 children older than 5 years with newly diagnosed DIPG at three different sites (Children's National, Nicklaus Children's Hospital, and UCSF). Patients with no tumor progression 4 to 24 weeks after standard radiotherapy were enrolled, but those with dissemination or progression and those who had undergone other experimental treatments were excluded.

  - Study design included escalating doses of SONALA-001 (from 5 mg/kg to 10 mg/kg) and acoustic energy.
  - Investigators learned that a head frame with an anterior crossbar, small pins that can be placed in the forehead versus the temporal regions, and an anterior shift kit to prevent the frame from hitting the table or frame locks is particularly useful in pediatric patients.
  - The treatments are performed in the MRI with real-time feedback. Some technical challenges the team had to overcome include the head frame issues mentioned above, hypothermia, treatment coverage for larger tumors, and acoustic membrane water leakage. The hypothermia was mitigated with modified warming techniques, and treatment coverage has been extended by offsetting the frame and adjusting the transducer. Insightec also introduced a new transducer, called the Sparse transducer, which creates a larger treatment envelope.
  - For safety reasons, only half of the pons was treated in the first patient in each cohort. With no adverse events, the team moved forward to treat the entire pons. After initial experience, the study was also amended to allow for monthly treatments, which explains the large number of treatments that have now been performed. One participant received about nine treatments in sequence.
  - The research team is analyzing all post-treatment imaging and clinical characteristics to better understand what happens in the tumor after SDT.
  - Enrollment on the trial is now closed, and analyses of the available data are ongoing.
- SDT201 completed ascending drug and focused ultrasound energy dose combinations while assessing safety and clinical/radiographic outcomes. Technical challenges were successfully addressed without delays in patient recruitment. The timing of anesthesia, total treatment time, and sonication duration decreased as the investigators gained more experience with the procedure. SDT appears to be an innovative approach for challenging pediatric brain tumors.
- Going forward, significant ongoing research is needed to optimize treatment parameters, establish safety in other locations of the brain, explore efficacy in other brain tumors, explore and optimize combination therapies, and further understand the biologic impact of SDT.

Group Discussion

Attendees discussed the potential for clinical trial participants to show signs of skin photosensitivity (as found with photodynamic therapy). Dr. Syed said that photosensitivity was not observed in this study, but such issues have been observed in patients who received high-intensity focused ultrasound. Study participants were advised to avoid light exposure for 48 hours after treatment. A photosensitivity caution is included on the oral 5-ALA package insert.

For participants in the clinical trial, decisions to continue treatment were based on disease assessment and made on a case-by-case basis. Interpretation of some of the imaging changes was challenging, even when the DIPG-DMG National Brain Tumor Board reviewed the images. Clinical symptoms did not always match radiographic findings. It has been interesting to follow these patients over time with repeated treatments, and the teams are working to understand differences seen in this trial compared to prior experience with other treatment approaches.

Some of the social media posts from families that participated in this research study described surprisingly good improvements in quality of life. Attendees asked whether the study procedure could have produced any neuromodulation effects. Dr. Narsinh reminded the group that the focused ultrasound parameters were escalated over the course of the study. Dr. Kilburn said that some protocol changes affected the focused ultrasound parameters, so maintaining consistency in the parameters across the study was challenging—but parameters were maintained within each cohort. An attendee suggested systematically reviewing the focused ultrasound parameters being used for the SDT research and comparing them with data from neuromodulation trials. Dr. van Vuurden said that the focused ultrasound parameters that were used might influence the neuronal and glioma interactions in an interesting way. He offered to send his DMG models for neuromodulation testing to transcranial ultrasound stimulation researchers in the Netherlands to learn how the parameters affect neuronal and glial interaction. The group further discussed refraining from reporting results after the first two or three participants have been treated, as early reporting may complicate clinical trial enrollment if patient communities direct families toward or away from small studies.

When asked why low- and high-grade gliomas respond differently to 5-ALA, Dr. Nazarian said that six different enzymes are involved in converting 5-ALA to PpIX, and the expression of these enzymes is quite varied in DMG cell lines. Low-grade cells that are not aggressively growing do not convert 5-ALA to PpIX. This lack of conversion in low-grade tumors is likely due to the expression of these enzymes in mitochondria. Some of the preliminary qualitative plasma samples from the study participants showed an increase in mitochondrial signaling; therefore, it would be valuable to look at the tumor genome from these patients, especially postmortem, and correlate mitochondrial signaling with the treatment response. Tumor heterogeneity and tumor density also play a role. An attendee suggested using T2*-weighted MRI sequences with non-gadolinium contrast agents for quantitative susceptibility mapping of iron accumulation because there is a relationship between the expression of these enzymes and iron metabolism. This type of imaging is being used in the study of ferroptosis, an alternate mechanism for inducing cell death. If done thoughtfully before and after treatment, T2*-weighted metabolism sequences might help answer some of these questions. The Focused Ultrasound Foundation may be interested in funding preclinical studies to investigate the mechanism behind 5-ALA–mediated SDT.

When asked whether medulloblastomas and ependymomas take up 5-ALA, Dr. Nazarian said that any cell line that grows quickly takes up 5-ALA and becomes damaged. He added that studying 5-ALA in vitro with focused ultrasound is complicated, because the cells become incredibly sensitive to light. Even the tiny amount of light in microscopes is enough to kill the cells. Determining the right amount of pressure, or sequence, for combining focused ultrasound with 5-ALA in dark—as well as finetuning the focused ultrasound pressure in the well—is not easy. Dr. Syed added that PpIX is metabolized by ferrochelatase and, in general, the enzyme concentration of ferrochelatase is lower in tumor cells.

Attendees briefly discussed MRI imaging for SDT studies. When asked whether there was a ring of enhancement around the tumor border on MRI susceptibility-weighted imaging sequences, Dr. Syed said that his team performed that sequence to look for blood and did not see any major changes, but that sequence is worth pursuing. Some literature describes changes in diffusion restriction after SDT, which might explain some of these changes. Dr. Narsinh added that tumor heterogeneity creates variable imaging results and that it is challenging to determine whether imaging changes are due to the residual effects of radiation versus the SDT itself.

Overall, SDT could be a promising strategy for treating DIPG due to its high safety profile. More research is needed to quantify the amount of 5-ALA that enters the brainstem. SDT plus

imaging with lights could be incorporated into pre- or post-treatment biopsies (which would require a paradigm change) to test for apoptosis. Patient tissue could also be used to study the pathways.

Dr. Narsinh commended the leadership of Drs. Syed, Kilburn, and Packer in this clinical trial with regard to communication between enrollment sites. He noted that although communication was challenging on multiple levels, ensuring shared treatment parameters across sites was critical.

Expanding Indications Panel Discussion

Panelists
Anirban Das, MBBS, MD, DM, **Lindsay Kilburn**, MD, **Natasha Sheybani**, PhD, **Hasan Syed**, MD

Panelists and attendees addressed optimizing and expediting immunotherapy and SDT clinical trials and combination therapies. The recommendations were based on patient selection, focused ultrasound parameters, drug selection, and timing of drug.

Patient Selection: Stage, Subtypes, etc.

- Present options to patients while considering patient fit (e.g., differences in eligibility, differences in the studies, newly diagnosed or recurrent disease, access to centers with focused ultrasound systems)
- Realize that families decide to participate based on follow-up schedule, perceived risk, potential side effects, access, and other aspects of enrollment
- Include patient advocates in clinical trial design and communications; address challenges with patients sharing information on social media
- Determine how to prioritize each therapy or treatment modality for certain patients based on:
  - Potential toxicities
  - Best first-line therapy determined by markers of resistance (e.g., P53, EGFR, or K20 mutations), biopsy data (interleukin-13 receptor alpha 2), and/or liquid biopsy data
  - Best recurrent disease therapy, including re-RT and possible combinations with re-RT
  - Patient gender
- Establish a potential recommended sequence for patients moving from one clinical trial to another and coordinate with other available clinical trials
- Although a rare subset, consider the histologically and molecularly defined DIPG subtypes that responded differently in chemotherapy and radiation therapy clinical trials (e.g., Ras/MAPK with K27)
- Consider changing the diagnostic paradigm to radiology plus ctDNA liquid biopsy; then perform the regular biopsy after treatment to determine whether the treatment worked and provide important tissue for biologic correlatives post treatment (e.g., T-cell uptake in the tumor, myeloid interaction, mitochondrial load, mitochondrial damage)
  - Regular biopsies are not being used to determine treatment plans.
  - Post-treatment biopsies would be much more informative.
  - Currently very limited biopsy data for post–radiation therapy DIPGs exist.
- Consider conducting HIFU, mechanical, or LIFU clinical trials in diseases outside of the brainstem (e.g., ependymoma, recurrent medulloblastoma) using novel approaches that treat a part of the tumor and then compare pre- and posttreatment biopsy data to

establish a control. An alternative to treating part of the tumor would be administering drugs or classes of drugs that do not penetrate the BBB on their own

- Create window of opportunity studies for focused ultrasound interventions that are based on solid preclinical data from DMG models and offer an advantage to the patient
- Consider categorizing the tumors based on anatomic site
- Use data from other studies (e.g., ONC201, PNOC022) to update patient selection criteria in new protocols
- Consider using theranostic agents for DIPG (e.g., Gallium-68– or Copper-64–based DOTATATE PET/CT scans for imaging somatostatin receptors to diagnose and treat neuroendocrine tumors and meningiomas)
- Consider closing clinical trials that have slow patient enrollment or that have met their primary endpoints early (e.g., the doxorubicin studies)
- Include ancillary studies (i.e., imaging changes, biopsy/autopsy data, liquid biopsy ctDNA monitoring) in future clinical trials
- Consider incorporating data from the Princess Maxima biome studies (i.e., MIMIC protocol) that predict responses to the ONC201 and ONC206 clinical trials when designing T-cell clinical trials

Focused Ultrasound Parameters

- Secure support from focused ultrasound device manufacturers until the devices are approved for each indication
- If new tumor types are included in trials, confirm that the focused ultrasound technology is capable of achieving the proper depth for treatment
- Consider priming the immune system with preclinical or clinical histotripsy or other mechanical ablation modalities
- Consider incorporating lessons learned from histotripsy ablation of other tumor types for brain tumor treatment

Drug Selection

- Try to define the strongest immune suppressive components of the tissue microenvironment (e.g., Tregs, innate myeloid compartment) so that pro-inflammatory cytokines released in the microenvironment can attract, but not exhaust, the CAR-T cells
- Consider using nanoparticles that change the innate immune system in the bone marrow and microglia to synergize with CAR-T therapy

Timing of Drug

- One argument for infusing T-cells prior to BBB opening may be increased time for systemic exposure
- Consider the stimulation cycle before administering T-cells; use data presented at the Foundation's Cell and Gene Therapy Roundtable to reach consensus on the timing of T-cell administration

- Optimize MB dose and type with ultrasound dose to control desired or unwanted inflammatory responses
- Look at the timing of BBB opening relative to key cellular or molecular (protein) signatures of innate vs. adaptive immune response

Roadmap Discussion

To conclude the workshop, attendees discussed each theme, identified the information needed to answer the questions that were raised, and offered potential solutions for moving the field forward.

Liquid Biopsy

- Evaluate and compare liquid biopsy platforms for CSF, ctDNA, and cfDNA analysis with banked samples from clinical trial cohorts
- Determine which platforms and assays could be standardized for use with focused ultrasound or sonobiopsy
- Standardize liquid biopsy collection and processing protocols across clinical trials to allow for collation of findings

Technical and Preclinical Studies

- Help create a device-agnostic and standardized MB dosing scheme to use for the administration of MB-FUS
  - Terms used—sometimes interchangeably—include harmonic dose, cavitation dose, passive cavitation dose, and ultrasound cavitation dose
- Characterize the harmonics and subharmonics of each commercially available MB contrast agent
- Outline the biological effects of varying MB parameters for clinical translation
- Disseminate the MB Consensus Paper that is being written, once finalized
- Support the development of a monodispersed MB that has been designed for therapeutic use
- Continue to identify and test therapeutics that could be translated into clinical use
  - Form and fund a working group or consortium to brainstorm disease applications, drugs, and/or drug combinations
  - Empower the working group to pursue innovative ways to partner with pharmaceutical companies to test the newly identified therapeutics
  - Empower the working group to develop a shared laboratory platform for testing its ideas (e.g., one group does in vitro testing, another does in vivo testing, another does liquid biopsy) as a type of development pipeline
  - Review Dr. Nazarian's list of 120 clinically relevant, FDA-approved therapeutics for use with MB-FUS BBB opening in preclinical in vivo studies with translation to clinical trials
  - Create a table of drugs with each drug's matched harmonic dose needed to cross the BBB
- Incorporate AI into discovery studies (e.g., the Wu lab uses AI to reduce gadolinium dose, other labs use algorithms to predict drugs to target specific subpopulations in heterogeneous tumors)

- Design preclinical studies that match the standard for human treatment (e.g., once a day MB-FUS in the laboratory does not translate well with once-a-month panobinostat administration)

Clinical Trial Development

- Continue to collaborate to align clinical trial parameters across institutions
- Continue to include preclinical researchers in the design, review, and data monitoring of clinical trials (e.g., different drugs require different ultrasound parameters and administration routes)
- Develop a more formal structure for peer protocol review and comment
- Work with device manufacturers to develop and use standardized guidelines for reporting treatment parameters
- Specify the MVD and MB administration parameter windows to be used in clinical trial protocols (and whether any modifications are allowed)
  - Justify MB administration route (i.e., continuous infusion versus bolus) while considering the half-life of the MB
  - Incorporate methods that avoid MVD dilution (e.g., remixing the MBs every few minutes to prevent them from rising to the top of the infusion bag)
  - Engineer an MR-compatible device to keep MBs mixed during infusion
- Develop a clinical trial protocol for expanding MB-FUS BBB opening into the treatment of other types of brain tumors (e.g., medulloblastomas, ependymomas) using drug sensitivity testing to choose the therapeutic agents for each type of tumor

Conclusion

Lauren Powlovich, MD, MBA said that the workshop's rich discussions were invaluable and thanked the steering committee, presenters, and all attendees for their meaningful contributions. She welcomed the submission of preclinical and translational studies, as well as clinical trial protocols, to the Focused Ultrasound Foundation for funding or expert/peer review, especially for immunotherapy and SDT research.

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Abbreviations

ALA	aminolevulinic acid
AUC	area under the curve
BBB	blood-brain barrier
CAR-T	chimeric antigen receptor T-cell
CED	convection-enhanced delivery
cfDNA	cell-free DNA
CLIA	Clinical Laboratory Improvement Amendments
CSF	cerebrospinal fluid
CT	computed tomography
ctDNA	circulating tumor DNA
ddPCR	digital droplet polymerase chain reaction
DIPG	diffuse intrinsic pontine glioma
DMG	diffuse midline glioma
FDA	United States Food and Drug Administration
fLUC	firefly luciferase
GB-13	an immunotoxin drug that binds to cancer cells and causes cell death
GBM	glioblastoma
GFP	green fluorescent protein
HIFU	high-intensity focused ultrasound
IMUS	intensity-modulated ultrasound
IND	investigational new drug
INDELS	insertions and deletions
IP	intraperitoneal
IRB	Investigational Review Board
LIFU	low-intensity focused ultrasound
MB	microbubble
MB-FUS	microbubbles plus focused ultrasound
MHz	megahertz
MI	mechanical index
MPa	megapascals
MRI	magnetic resonance imaging
MSD	Meso Scale Discovery
MVD	microbubble volume dose
PBTC	Pediatric Brain Tumor Consortium

PD	pharmacodynamics
PD1	programmed cell death protein 1
PET	positron emission tomography
PK	pharmacokinetics
PNOC	Pediatric Neuro-Oncology Consortium
PFS	progression-free survival
PpIX	protoporphyrin IX
Re-RT	re–radiation therapy
SDT	sonodynamic therapy
SIR	sterile inflammatory response
TAA	tumor-associated antigen
TAA-T	tumor associated antigen–specific T-cell
UCSF	University of California San Francisco
WUSTL	Washington University in St. Louis

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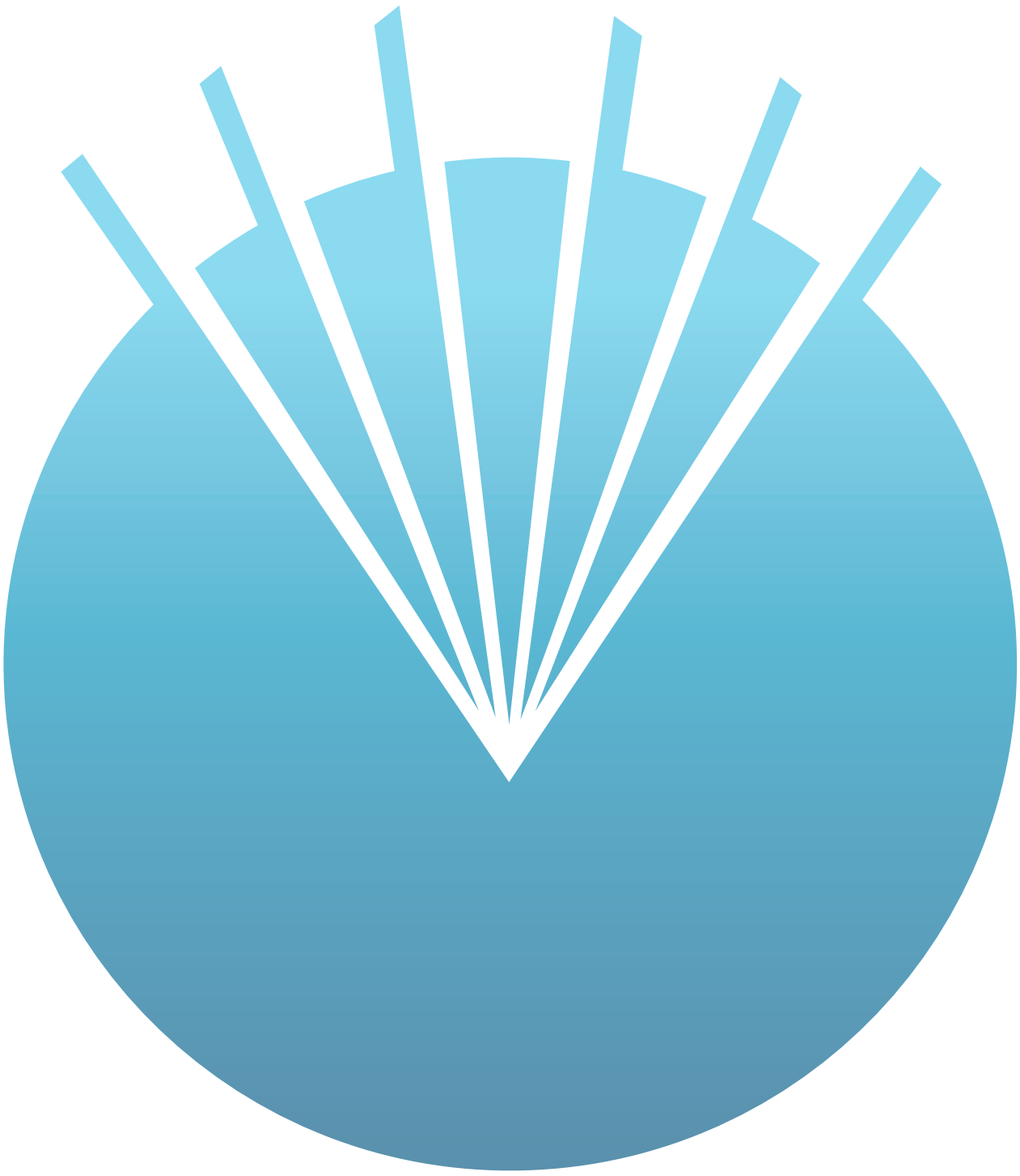

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