



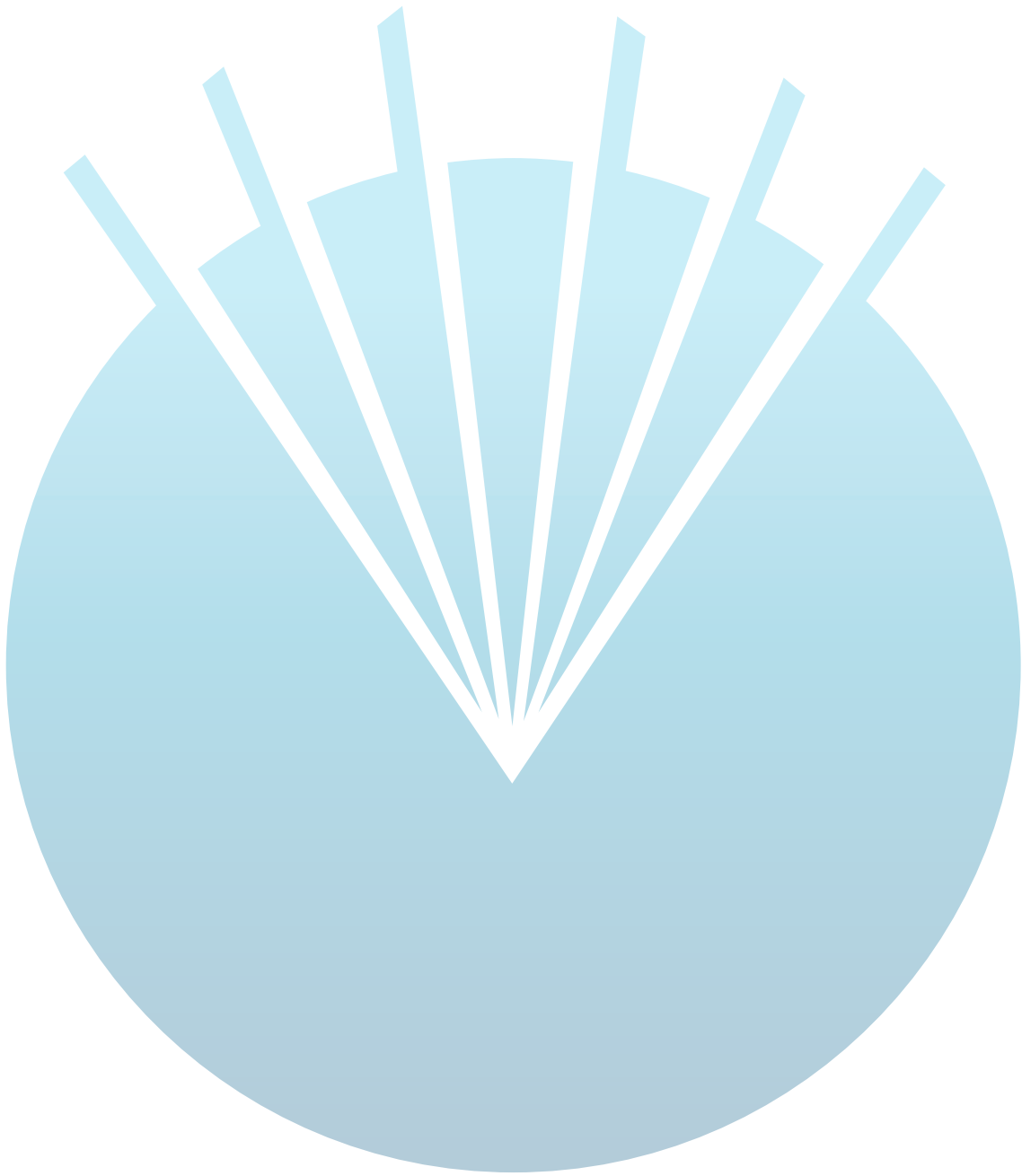
Focused Ultrasound for Diffuse Intrinsic Pontine Glioma (DIPG)

A Focused Ultrasound Foundation Workshop

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Executive Summary

This white paper summarizes a half-day virtual workshop, “Focused Ultrasound for Diffuse Intrinsic Pontine Glioma (DIPG),” organized by the Focused Ultrasound Foundation. During the workshop, experts led panel discussions on preclinical research, focused ultrasound technology, clinical study opportunities, and outcome assessments for DIPG. In advance of the workshop, attendees viewed nine recorded presentations that not only provided background information in each of these areas but also set the stage for the interactive panel discussions.

The presentations on preclinical research and the technology concentrated on the focused ultrasound mechanisms of action that hold promise for treating DIPG, which are focused ultrasound blood-brain barrier (BBB) opening combined with radiotherapy, focused ultrasound-enhanced drug delivery, and sonodynamic therapy (SDT). The associated panel discussions centered on in vivo models, potential drug therapies, and pathologic considerations.

The presentations on clinical studies and outcome assessment covered focused ultrasound with oral panobinostat in children, immunotherapy, SDT, and liquid biopsy. During the panel discussion, attendees asked about treating the primary tumor volume versus the area surrounding the tumor, the technological advances needed to increase the number of clinical trials, and the sharing of data and resources among studies that use the various focused ultrasound modalities.

During the roadmap session, attendees suggested developing a data repository to catalog neuropathologic findings in preclinical DIPG specimens after focused ultrasound (i.e., a centralized histopathology resource). A new preclinical working group will share data, biospecimen tissue, and experiences while seeking to form collaborations. Future studies could address the heterogeneity of DIPG and diffuse midline glioma (DMG) tumors, determine the degree to which the BBB must be opened, devise ways to expand the treatment envelope, test the pharmacokinetics of various agents to improve the translation of preclinical studies to effective clinical trials, develop microanalyses for assessing drug penetrates, and correlate preclinical data with clinical studies.

Specific technological advances that could improve pediatric focused ultrasound treatments in the brain include developing frameless systems that do not require full immobilization

of patients, eliminating the need for head shaving, incorporating spot control for BBB opening, expanding the treatment envelope, introducing a radiolabeled drug with focal BBB opening, and innovating treatments that could be conducted outside the magnetic resonance imaging (MRI) environment so they would be less costly, easier to use, and usable worldwide.

Clinical goals for the development of focused ultrasound to treat DMG and DIPG include developing collaborations with neuropharmacologists, ensuring that all patients have access to clinical trials, improving measures to determine the specificity and sensitivity of drugs that are delivered across the BBB, and harmonizing clinical trial protocols.

The Foundation intends to convene a DIPG working group, continue to develop and implement the ideas suggested in the roadmap, and fund clinical trials to advance focused ultrasound for the treatment of this devastating disease.

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The link to view the workshop and the recorded presentations can be found on the Foundation's [YouTube channel](#).

Welcome and Background Information

Javad Nazarian, PhD, welcomed attendees and stated the purpose of the workshop was to convene the DIPG community and discuss challenges and opportunities for translating preclinical focused ultrasound research to clinical trials.

Lauren Powlovich, MD, thanked Kullervo Hynynen, PhD, Sabine Mueller, MD, PhD, Javad Nazarian, PhD, Stergios Zacharoulis, MD, and Memhet Ozdas, PhD, for serving on the workshop’s steering committee. She then provided an overview of the workshop schedule and logistical information for asking questions during the panel discussions.

Pediatric DIPG is a rare, fast-growing, and fatal brain tumor that forms in the glial cells of the pons—a part of the brainstem. DIPG occurs in a vulnerable patient population: It is most often found in children aged seven and younger. DIPG tumors are incredibly difficult to treat because of their location and rapid spread in a critical part of the brain. Median survival time is nine months, and only 10% of patients live longer than two years.^{1,2} Focused ultrasound could address this unmet clinical need and help young patients suffering from DIPG.

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Preclinical Opportunities

Three recorded presentations set the stage for the preclinical panel discussion by providing background information on the focused ultrasound mechanisms of action that hold promise for treating DIPG, which are focused ultrasound BBB opening combined with radiotherapy, focused ultrasound-enhanced drug delivery, and SDT. The associated panel discussion centered on in vivo models, potential drug therapies, and pathologic considerations.

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Focused Ultrasound BBB Opening Combined with Radiotherapy



Watch the recording
11 minutes

Cheng-Chia Wu, MD, PhD, said that radiotherapy is the current standard of care for children with DIPG; therefore, the combination of radiation with focused ultrasound could be a logical next step for the future clinical treatment of DIPG. He made the following points during his presentation:

- Radiation of DIPGs temporarily improves survival by about six months, and re-irradiation is commonly used. There are no effective systemic therapies to date.
- Advances in pathology have shown that 80% of DIPGs carry the H3K27M mutation, leading to their classification as diffuse midline glioma H3K27M mutants. Therefore, it is important to develop a mouse model that carries this mutation and other genetic, histologic, and radiographic characteristics of human DMG. In addition, xenograph tumor growth time must be decreased from multiple months (the current length of time) to three or four weeks. An ideal mouse model would also be immune intact to assess safety, feasibility, and potential efficacy.
- The Oren Becher mouse model and other existing immune competent models may be ideal for research involving focused ultrasound plus radiotherapy.³ Oren's model in particular is syngeneic for DIPG, carries the H3K27M mutation, grows the tumor within three to four weeks after implantation in the brainstem, and has similar radiographic and histological characteristics as human DIPG. Furthermore, RNA sequencing for this model shows high protein activity that mimics human DIPG; therefore, computational strategies can be employed to find novel drug combinations to use with focused ultrasound delivery.

- Using focused ultrasound to open the BBB in the brainstem was shown to be safe in a murine model of DIPG.⁴
- Administering 30 greys (Gy) of radiation in five fractions did not affect the ability of focused ultrasound to open the BBB, or its permeability, one month after the radiation.⁵
- The Columbia group has developed a small animal radiation research platform to test a range of radiation dosages with promising results when administering 39 Gy of fractionated radiotherapy followed by focused ultrasound—induced BBB opening as a combination therapy (unpublished data).
- The next steps are evaluating tumor progression and studying adjuvant focused ultrasound with chemotherapy after radiation or concurrent radiation with focused ultrasound drug delivery.

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Focused Ultrasound– Enhanced Drug Delivery for DIPG



Watch the recording
10 minutes

Hong Chen, PhD, described her preclinical research using focused ultrasound to induce BBB opening and enhance drug delivery for the treatment of DIPG. She highlighted the following points:

- The safe use of focused ultrasound as a noninvasive technique for temporarily opening the BBB has been established in adults with glioblastoma (GBM), amyotrophic lateral sclerosis, Alzheimer’s disease, and Parkinson’s disease, but such clinical trials have not yet been conducted with pediatric patients.^{6–9}
- Focused ultrasound BBB opening for precise drug delivery to the brainstem (i.e., the region of the brain where DIPGs are found) must be optimized, proven safe, and translated to the clinical setting.
- In 2018, preclinical studies using focused ultrasound BBB opening to deliver radiolabeled gold nanoparticles to the brainstem confirmed that focused ultrasound significantly enhanced delivery efficiency.¹⁰ Positron emission tomography (PET) was used to quantify the in vivo delivery of the nanoparticles.¹¹
- Dr. Chen’s team has developed a genetically-engineered mouse model of DIPG.¹²
- Data show that the microbubble cavitation that is needed for BBB opening is reduced by the static magnetic field that exists in the MRI-guided focused ultrasound brain systems.¹³
- Passive cavitation imaging (PCI) is an ultrasound technique that can be used to monitor and predict focused ultrasound–induced BBB opening. Cavitation

dose painting shows the pixel-by-pixel correlation between PCI and PET, confirming the precise delivery and amount of a drug delivered to an exact location in the brainstem.¹⁴

- Focused ultrasound–induced BBB opening can achieve noninvasive, localized, and precise drug delivery to the brainstem. It shows promise for the clinical treatment of DIPG.

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Current State of the Field

Preclinical Focused Ultrasound DIPG Research



Watch the recording
9 minutes

Javad Nazarian, PhD, and **Mehmet Ozdas, PhD**, presented an overview of recent preclinical work investigating the use of focused ultrasound for the treatment of DMGs and DIPGs. They made the following points:

- There are six subtypes of gliomas, and the subtype that affects the midline structures (i.e., the pons, spinal cord, and thalamus) has mutations affecting core histone (H3) protein (H3K27M).
- Focused ultrasound could be useful in treating midline gliomas by opening the BBB or by reaching the tumor cells in the brain parenchyma while sparing the healthy cells.
- Tumor necropsy and histology show mutational variation in the cancer cells as they migrate through the pons. This means that treatments may need to vary based on the genetic signature of the targeted region.
- A Toronto research team used focused ultrasound to open the BBB at the brainstem in healthy rats.¹⁵ The application did not cause adverse physiological, behavioral, or histological changes in the rats. After further studies revealed that doxorubicin could be delivered to the brainstem via BBB opening, the team conducted a study in a murine model of DIPG.¹⁶ The researchers found that focused ultrasound–induced BBB opening created a four-fold increase in the uptake of doxorubicin and significantly suppressed the volumetric tumor growth rate in some of the tumors. However, there was no survival benefit in the focused ultrasound BBB opening group compared to the controls.
- In New York, researchers based at Columbia University found a five-fold increase in the delivery of etoposide when using focused ultrasound–induced BBB opening in a murine model of pontine glioma.⁴ Normal cardiorespiratory and motor functions were preserved during these experiments, and the treatment did not affect body weight or survival. Although this study also proved that increased concentrations of etoposide were being delivered after BBB opening, there was again no survival benefit in this study.

- In Switzerland, Dr. Nazarian’s team is conducting in vitro research for SDT using patient biopsy cell cultures. His group is investigating whether advanced in vitro models can be used to screen novel sonosensitizing agents for DIPG.
- Focused ultrasound DIPG research is still in a very early stage. Although increased drug concentrations were achieved using focused ultrasound BBB opening in combination with etoposide and doxorubicin in animal models of DIPG, the increased drug concentrations did not lead to increased survival. It is unclear whether this is due to the tumor microenvironment or the lack of drug efficacy against DIPG.

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Panel Discussion

Dr. Nazarian and **Dr. Ozdas** led the preclinical panel discussion, which included Cynthia Hawkins, MD, PhD, FRCPC, Timothy Phoenix, PhD, Dannis van Vuurden, MD, PhD, and Cheng-Chia “Fred” Wu, MD, PhD. After presenting summary slides on the focused ultrasound preclinical milestones reached at the University of Toronto (i.e., BBB opening and drug delivery to the brainstem), Columbia University (radiotherapy sensitization), Sunnybrook Research Institute (i.e., SDT), and Washington University in St. Louis (i.e., gold particle delivery and quantification in the brainstem, magnetic field cavitation effects), Dr. Nazarian opened the panel with introductions and then led a Q&A discussion.

Dr. Wu shared that beyond the radiotherapy sensitization studies that he described in his recorded presentation, Columbia University researchers tested the use of concurrent, once weekly panobinostat with focused ultrasound in an animal model and obtained local control (a four-fold decrease in tumor volume) plus a survival benefit that was unfortunately eliminated by the drug’s gastrointestinal toxicity. More optimization is needed, including additional weekly treatments in such a fast-growing animal tumor model. Dr. Wu said that developing an effective preclinical model is essential for DIPG research.

Dr. van Vuurden introduced his work in pediatric neurosurgery at the Princess Maxima Center in Utrecht. Beyond patient care, Dr. van Vuurden conducts preclinical research using focused ultrasound and other modalities. His center is installing an Insightec ExAblate Neuro clinical focused ultrasound system that is compatible with its Philips MRI scanner. It will be used to treat patients with brain tumors, Alzheimer’s disease, and more. Dr. van Vuurden said that his preclinical experiments confirmed that it was possible to use focused ultrasound to deliver doxorubicin to the brainstem, but the protocol did not result in improved survival. The pharmacokinetics (fast clearance of doxorubicin) in the mouse model is problematic.

As a neuropathologist at SickKids, **Dr. Hawkins** said that the morphology of the brainstem after focused ultrasound treatments in preclinical models is not exactly the same as in healthy tissue, but it appears to be safe enough to proceed with caution in human clinical trials. She highlighted the importance of remembering that local tumor control is

what can be expected with BBB opening and that combination treatments will be needed to target cells that have migrated outside the margins of the visualized tumor.

Dr. Phoenix introduced his research on tumor vascular interactions and BBB function at the University of Cincinnati and Cincinnati Children's Hospital. His team is building preclinical models for DIPG and high-grade glioma. Dr. Phoenix said that DIPGs are more infiltrative and diffuse and maintain the BBB better than other types of pediatric brain tumors.

Dr. Ozdas explained that during his doctoral project, he developed a local drug delivery technology for psychiatric disorders. For his postdoctoral project, he is developing in vitro systems for screening, testing, and synergizing focused ultrasound parameters and drug concentrations. He has tested 5-aminolevulinic acid (5-ALA), an endogenous non-proteinogenic amino acid that is used as a biofluorescent tumor marker.

Q. What are good in vivo models for studying DIPG?

- Because it is important to determine the proper time point to begin treating the mice, the tumor models must have standardized measurements at each time point after injection.
- Time points for starting and applying focused ultrasound must be harmonized across models (e.g., in the DIPG7 model, the cells have spread too far to treat with ultrasound by day 21 or 28).
- The model cannot be so aggressive that the mice die in two or three weeks, because the drug may not work that quickly and useful observations could be missed. The model also cannot be so conservative that it takes too long for the mice to die. The ideal survival window may be two to three months.
- The best models mimic the morphology of DIPG (rather than forming a ball of tumor cells).
- For BBB opening with focused ultrasound, the model must have blood vessels that stay intact throughout the course of the disease. Tumor vascularity can be quite variable in each patient with DIPG. About 75% of patients have vascular proliferation with improperly formed BBB areas, but all patients have areas where the vasculature appears to be normal.
- Needed is a model that can be used to study the effect of focused ultrasound on pathological vasculature that has thin, improperly formed walls.
- The best syngeneic models will also have intact or competent immune systems. BBB opening causes local inflammation, which presents opportunities for cancer immunotherapy solutions.
- The best models will carry the H3K27M mutation.
- Researchers at Virginia Tech are developing large animal models for focused ultrasound.

Q. Is it possible to develop immunocompetent models of DIPG?

- The Oren Becher mouse model is syngeneic for DIPG, carries the H3K27M mutation, grows the tumor within three to four weeks after implantation in the brainstem, and has similar radiographic and histological characteristics as human DIPG.³ A study in microglia and microphage activation of the model with radiation has begun, and a subpopulation has been characterized.
- The mouse models developed at SickKids and two other mouse models (RCAS/TVS, electroporation) are immunocompetent. Each model has benefits and limitations.

Q. What therapeutics are currently being used to treat DIPG? Should they be tested in focused ultrasound BBB opening preclinical studies?

- Princess Maxima has a drug library for agents that do and do not cross the BBB. Researchers there have tested several drugs for use with focused ultrasound.
- The fast-eliminating pharmacokinetics of mice models cause problems for testing various drugs. Rat models usually metabolize drugs more slowly than mice.
- Liposomal formulations may help address pharmacokinetic issues.
- *MDM2* oncogene inhibitors work differently with human and mouse proteins. They are less potent in mouse models.
- Researchers should consider what is known about each drug and to review its phase I adult studies to determine the dosing levels that can be achieved. The Princess Maxima Center has a microfluidic system that can mimic the plasma levels of specific agents in various tissues.
- The tumor microenvironment in DIPG can also affect drug diffusion. It may be valuable to test biopsy tissue with various drugs to determine their diffusion properties. This would be a Phase 0 study, but it would be difficult to conduct because of the limited time to treat patients with DIPG and the incredibly small amount of tissue collected in the biopsy process. This study may be more possible for patients with midline thalamic gliomas, where larger biopsies are possible. Alternately, additional biopsies for target validation can sometimes be taken after a patient undergoes radiation therapy. Studies have determined the number of cores that can be taken safely during biopsies of DIPG and DMG.
- PET can also be used to measure drug delivery uptake levels. Other possible measurement modalities are radiolabeling and mass spectrometry.
- New biomarkers may become available for measuring drug effectiveness and therapeutic effects. The lack of identified biomarkers is also being investigated for GBM.

Q. Can larger animals be used for preclinical DIPG studies?

- A porcine model can be used for BBB opening studies; however, there are no tumor models in pigs. Immunodeficient pig models may be useful.
- Dogs may spontaneously develop gliomas.
- Large animal studies could provide substantial value for the practical clinical delivery of therapies. For example, in BBB opening, a current challenge is to open a large enough volume of the BBB at the brainstem to include the entire tumor area.

Q. What vascular changes are observed in focused ultrasound–treated biospecimens? Is the pathologist who is reading the slides blinded to which specimens have been treated?

- The pathologist is blinded, and the vessels appear slightly distended with adjacent small hemorrhages. After reviewing several slides, the focused ultrasound pathology becomes recognizable to the pathologist.
- Blood vessel integrity and vascular density after focused ultrasound would be important to determine.

Q. Are there other neuropathologic considerations?

- There is cellular and radiographic variability from patient to patient and with various stages of tumor development.
- At the time of autopsy, DIPGs usually have several components, including
 - A highly vascularized central tumor with proliferative vessels (such as in GBM)
 - Vascular changes from radiation
 - Other tumor areas with normal vasculature (like a diffuse astrocytoma) and infiltrative tumor cells
- High-resolution imaging could be useful for measuring vascular changes before and after a treatment in clinical trials.

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Focused Ultrasound Technology

To provide an overview of the topic and set the stage for the panel discussion, the workshop included two recorded presentations on focused ultrasound technology. The topics included technology requirements for treating pediatric patients and for using SDT.

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Focused Ultrasound Technology Requirements for Pediatric Patients

Elisa Konofagou, PhD, described technological considerations that must be included in the designs of focused ultrasound clinical trials for pediatric patients. Under her leadership, Columbia University is conducting a clinical trial ([NCT04804709](#)) for pediatric patients with progressive DMG.



Watch the recording
11 minutes

- The study is using a new focused ultrasound system designed at Columbia to open the BBB.¹⁷ It is not integrated with MRI; instead, it is guided by neuronavigation and real-time cavitation monitoring.
- The patient sits upright and immobilized in a treatment chair during the procedure, which lasts approximately 30 minutes. MRI assessment is conducted after the treatment. The system's 0.25-MHz focused ultrasound transducer is controlled by a robotic arm and includes a water cone for cooling.
- Treatment planning includes the use of numerical simulations to determine and correct the focal shift caused by the skull.¹⁷
- The team uses real-time transcranial cavitation monitoring for safety to track the stable and inertial cavitation of the microbubbles in the treatment region of the brain.
- Columbia is conducting a parallel clinical trial ([NCT04118764](#)) using the device for BBB opening in patients with Alzheimer's disease. The opening volume in these patients is approximately 4.3 cubic centimeters.
- For the DMG trial, the focused ultrasound transducer is aimed at the lowest part of the suboccipital skull—right above the neck. The skull is thicker in this region. Coupling gel is applied to the head at the target area, and the hair is shaved from that area.
- At the time of the workshop, two patients had been treated in the pediatric DMG clinical trial. Both treatments reached the targeted areas, which was confirmed with transcranial cavitation passive acoustic mapping of the brainstem.

- Going forward, the team is designing a new treatment chair with more cushioning and better immobilization of the head. It is also developing faster and real-time treatment mapping and reducing the treatment time to closer to 15 minutes. It is also cumbersome to immobilize children in the MRI three times per week for the post-treatment assessment. The parents appreciate that no anesthesia is used for the MRI scans.

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Sonodynamic Therapy Technology



Watch the recording
7 minutes

Sheng-Kai Wu, PhD, described his work using 5-ALA-mediated SDT in various rat brain tumor models. He made the following points:

- SDT originated from a similar anti-cancer therapy called photodynamic therapy (PDT). The energy source for PDT is light, and it works for superficial tumors but does not reach deep-seated tumors. The energy source for SDT is ultrasound, which can travel deeper into the body than light. SDT can therefore reach deeper tumors, such as those in the brain.
- When injected into the body, sonosensitizing agents are absorbed by tumor tissue. When ultrasound is used to activate the agent, it becomes toxic to the tumor tissue. The proposed mechanisms behind SDT are cavitation and microstreaming. When these mechanisms activate the sonosensitizing agent, the agent forms a reactive oxygen species or free radicals that induce cell death (apoptosis).^{18,19}
- 5-ALA hydrochloride is an imaging agent that is used for real-time visualization of tumor tissue during surgery. The drug is given approximately two hours before the ultrasound exposure, and it accumulates in the brain tumor. In the tumor cells, the prodrug 5-ALA forms fluorescent protoporphyrin IX (PpIX). PpIX is activated to have a therapeutic effect when the entire tumor is exposed to the ultrasound. PpIX peak intensity is reached after about six hours in vitro.
- The two routes for 5-ALA administration are oral or intravenous injection. The temporal kinetics of both routes are similar, but a higher oral dose is required to achieve the same tissue concentration of PpIX.²⁰ The SDT treatment time window is four to eight hours after 5-ALA administration. In animal models, the peak intensity of fluorescence occurs at six hours.²¹
- A study in a rat model of glioma using one SDT treatment during continuous ultrasound exposure showed a slight temperature elevation and elucidated the possible role of the mechanical effects of focused ultrasound in SDT.²²
- A new study in a different rodent brain tumor model is under way. In these brain tumors, multiple targets are sonicated with three different burst lengths and at a lower frequency and higher pressure than the previous study. The

treatments are given on a weekly cadence for three weeks at a time point that is six hours after intravenous 5-ALA administration. Early data show 100 percent survival with destruction of all tumor tissue at three weeks in all animals. The burst length of 86 ms showed the best therapeutic outcome.

- In summary, multiple SDT treatments showed a promising therapeutic outcome in preclinical studies. Therapeutic efficacy of SDT seems to be proportioned to burst length. A longer burst produces a greater therapeutic effect, but a shorter burst is ideal for larger volume exposures. A tradeoff exists in this parameter. Finally, use of a phased array focused ultrasound system may provide an option for clinical translation because it would shorten the treatment time.

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Panel Discussion

Kullervo Hynynen, PhD, led the panel discussion on focused ultrasound technology, which included Hong Chen, PhD, Gregory Czarnota, MD, PhD, and Elisa Konofagou, PhD. Dr. Chen has been using focused ultrasound for drug delivery to the brainstem. Dr. Czarnota is a radiation oncologist who is using focused ultrasound to enhance the effects of radiotherapy treatments. Dr. Konofagou has been developing focused ultrasound for BBB opening in animal models and human clinical trials.

Before beginning the Q&A discussion, Dr. Hynynen presented slides to introduce the topic. He made the following points:

- A technical problem that might arise with the use of focused ultrasound ablation to treat DIPG is the tumor's location near bones that can absorb standing sound waves.²³ A study found that a curved focused ultrasound aperture could reduce the formation of standing waves.
- The acoustic characteristics of skull bones in pediatric patients differ from those in adults, and more data are needed in this area.²⁴
- DIPGs are in the central part of the brain, which is a good location for focused ultrasound penetration.
- Two focused ultrasound clinical systems are currently in use for pediatric transcranial tumor applications (i.e., Columbia and Insightec). Each has a different technical configuration, but both are being used to open the BBB.
- Potential focused ultrasound therapies for treating DIPG include BBB opening for drug delivery (if an effective drug can be found), SDT, and radiation therapy enhancement with the combination of bubbles and focused ultrasound.²⁵
- Dr. Hynynen's laboratory has evaluated the effect of various sonication parameters on SDT.

Q. How can focused ultrasound be used to enhance radiation therapy?

- Low-powered focused ultrasound and microbubbles can augment radiotherapy by creating a 40- to 60-fold improvement in its radiation effect in multiple tumor types. Human clinical trials are under way for breast cancer and head and neck tumors.
- The mechanism of action might include induction of ceramide-related endothelial cell apoptosis, which then leads to vascular disruption.²⁶
- Beyond focused ultrasound, no other techniques to enhance radiotherapy are currently in use or in development. Therefore, this type of biophysical mechanism is promising and enticing to this field.
- Important factors to address include calibration, dosage, and what tumor stage(s) to apply the therapy. The timing and order of radiotherapy and sensitization treatments are also important considerations.
- In carefully controlled animal experiments, it does not seem to matter whether the radiation or focused ultrasound is given first. Researchers have found that the maximum treatment effect occurs when the modalities are given six hours apart.
- The mechanism for radiation sensitization is inherently linked to the vascularization of the target tumor. In preclinical research, hyper-vascular tumors have required more treatment than those with less vasculature.
- Inertial cavitation may be the most promising focused ultrasound bioeffect for enhancing radiation.

Q. How much work is still needed to be able to use focused ultrasound–induced BBB opening for the treatment of DIPG?

- The results using the Columbia approach have been good thus far, and there may be a benefit to using the treatment to reduce disease. The team safely conducted a total of 24 sonications for BBB opening to the posterior fossa of two patients at an encouraging pace of two treatments per week.
- Treating children who have been taking steroid medications necessitates more planning and simulations because the system is sonicating through the increased fat on the posterior inferior region of the skull.
- The monitoring system has detected cavitation in the muscle and skin, so additional studies are still needed.
- In some large animal studies, researchers have observed hemorrhages in the brainstem after opening the BBB. These hemorrhages may be due to the strong reflection of the sound waves off the proximal skull in that region. More optimization of the technical parameters is still needed. Such optimization might include using suboccipital targeting and pretreatment mapping of the focus area.

- Additional research is needed to eliminate the needs for head shaving and immobilization of the patient's head.
- Additional studies in humans will determine whether a larger volume of the pons can be safely treated with each sonication in clinical trials.

Q. What are the existing hurdles for using SDT to treat DIPG?

- The molecular mechanism in and around the tumor must be determined (i.e., what reaction does the SDT initiate on a molecular and cellular level). Sometimes multiple agents with multiple mechanisms achieve synergistic effects.

Q. Which of the treatments discussed is the most promising?

- For a disease such as DIPG, all of these approaches are needed and should be further advanced. In the future, it may even be possible and more effective to combine the treatments or perform them in series.
- Tumor response (e.g., vasculature and elasticity mapping) must be monitored in every type of treatment.

Q. At Sunnybrook, how is the current SDT study different from the 2019 study? Does it use the 5-ALA 10- to 15-mg per kilogram clinical dosing recommendation? Have any abnormal physiology responses been observed in the animals?

- The drug dose stayed the same (60 mg/kg), but the treatment was repeated three times. The frequency was 0.5 hertz lower.
- There has been no attempt to change the drug dose, but that might be possible based on the treatment reaction.
- There are no abnormal physiology responses in the animals. At such high dosage, the drug can be quite irritating to the veins. It must be injected slowly over a period of one minute.

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Clinical Studies and Outcome Assessment

To provide an overview of the topic and set the stage for the panel discussion, the workshop included three recorded presentations on clinical studies and one on outcome assessment. The associated panel discussion covered several outstanding questions for the translation of the technology in current and future clinical trials.

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Focused Ultrasound with Oral Panobinostat in Children with Progressive DMG



Watch the recording
12 minutes

Stergios Zacharoulis, MD, presented Columbia University's early Phase I data from the clinical trial ([NCT04804709](#)) using focused ultrasound to open the BBB with concomitant oral panobinostat administration in children with progressive DMG. He reviewed how Dr. Konofagou and her team designed a novel focused ultrasound system with a 2.5 MHz transducer, transducer tracker, position sensor, passive cavitation detector, and neuronavigation guidance¹⁷ and then described their early results, as follows:

- Before the pediatric study began, Columbia's novel device was used successfully in the launch of a parallel adult clinical trial in patients with Alzheimer's disease ([NCT04118764](#)).
- The design for the pediatric DMG study is to enroll 15 participants aged 4 to 21 years who will undergo six months of treatment followed by two years of follow-up. The primary endpoint is evaluating safety and feasibility for opening the BBB at one, two, or three tumor sites. Secondary endpoints are imaging changes in the tumor, progression-free survival (PFS), and overall survival (OS). Inclusion and exclusion criteria include a washout period and requirements for normal organ function.
- The research team chose panobinostat as the therapeutic agent because it has been demonstrated to be a promising chemotherapy for treating DMG, it is potentially active, it has minimal penetration into the cerebrospinal fluid (CSF), and its dosing regimen (i.e., three days per week) works well with focused ultrasound-mediated BBB opening.^{27,28}
- The main aim for the current clinical trial is to determine the feasibility of escalating the number of tumor sites when opening the BBB. Within a few minutes after the microbubbles and focused ultrasound are used to open the BBB, the oral panobinostat is administered to the patient. BBB opening is verified on MRI two to four hours after the chemotherapy administration.

BBB closure is confirmed on Day 3 with contrast-enhanced MRI. The procedure is performed three times in the first two weeks on Days 1, 3, and 5. The third week is an off week, which completes one three-week cycle. This schedule is then repeated for six cycles. The dose of the panobinostat remains the same, but the number of tumor sites for BBB opening is escalated after the first two cycles.

- The treatment occurs in the radiation oncology outpatient setting over a period of 30 minutes. The patients do not experience any pain other than the discomfort of the stabilization. No anesthesia is administered.
- To date, three patients (aged 10, 14, 18) have been treated. The patients were 12, 11, and 13 months postdiagnosis at the time of enrollment. All tumor biopsies were H3K27M positive. Two of the three patients had undergone re-irradiation after treatment. No severe adverse events have occurred. One patient progressed on treatment after the first cycle, and the other two patients showed clinical improvement after the second cycle. Follow-up time has reached 5, 4, and 3 months.

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Cancer Immunotherapy for DMG and DIPG



Watch the recording
10 minutes

Hideho Okada, MD, PhD, described current immune modulation and immunotherapy treatment for DMG and DIPG. He made the following points:

- The tumor microenvironment for DMG and DIPG may be even “colder” than it is for GBM. DIPG cells produce fewer cytokines and chemokines and are associated with fewer T cells and less inflammatory myeloid cells when compared with adult GBM.²⁹ DIPGs do not have increased macrophage or T cell infiltration relative to non-tumor brains, nor do they overexpress immunosuppressive factors, such as PD-L1.³⁰
- Researchers investigated BBB function and heterogeneity across various mouse models of pediatric high-grade gliomas and DMGs. They found that their pathological differences may be due to differences in vascular heterogeneity and their responses to alterations in developmental BBB signals.³¹ The BBB is more intact in DMG models, and the DMG models are the most like a normal brain.
- Neuroinflammation affects immune cell trafficking across the BBB, especially across its second layer.³² The administration of poly-ICLC can enhance infiltration of antigen-specific T cells and the therapeutic efficacy of peptide or incomplete Freund’s adjuvant vaccines in a mouse model.³³
- A multicenter clinical trial that enrolled and treated 29 patients with 3K27M DMGs used mass cytometry to characterize the tumors and predict responses to specific vaccines.³⁴ OS and PFS were associated with the induction of a vaccine-specific CD8-positive response. After undergoing six weeks of the standard of care

radiation therapy, the 19 participants with DMG and 10 participants with DMG who were vaccinated every three weeks with a peptide were matched to the tumor plus poly-ICLC. This process was repeated eight times. In general, clinical trials of cancer vaccines produce immunological response data that are not always associated with clinical outcomes; however, in this study, the expansion of CD8-positive cells was associated with OS and PFS (but exhausted CD8-positive cells were not). The study also reported the effects of dexamethasone treatment on the vaccine response and the possible roles of myeloid cells as predictive biomarkers.

- In summary, the tumor microenvironment of DMG and DIPG is characterized by fewer T cells and less inflammatory myeloid cells when compared with adult GBM. The BBB appears to be intact in DMG and DIPG models. Better understanding of T cell homing across the BBB should help formulate improved strategies. Expansion, but not exhaustion, of CD8-positive effector memory CD8-positive cells is associated with better OS and PFS.

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Clinical Sonodynamic Therapy



Watch the recording
10 minutes

Stuart Marcus, MD, PhD, described the first SDT clinical trial that is being designed for treating DIPG. He made the following points:

- The first-in-human SDT clinical trial for GBM is taking place at the Ivy Brain Tumor Center at the Barrow Neurological Institute in Phoenix, Arizona. It is a Phase 0/2 energy dose-ranging study using a fixed dose of SONALA-001, SonALAsense's sterile intravenous formulation of 5-ALA. The principal investigator and investigational new drug (IND) application holder is Nader Sanai, MD.
- 5-ALA is the first committed molecule in the heme biosynthetic pathway. When an excess of 5-ALA is provided to gliomas, the penultimate compound in the heme pathway, PpIX, accumulates, and it can function as a fluorescent marker or as a therapeutic agent.
- In PDT, high energies of visible light are used to activate PpIX. Researchers developed a 5-ALA PDT clinical trial protocol, and more than 60 patients with recurrent GBM were treated in Germany. However, the PDT protocol necessitated the use of invasive procedures (i.e., craniotomy and interstitial fiber optic insertion) to deliver laser light to activate the PpIX, and complications varied greatly based on the skill of the surgical team. Therefore, SonALAsense made the decision not to commercialize PDT technology.
- Ultrasound is a noninvasive tool that can also provide the cavitation energy needed to activate PpIX. Preclinical studies conducted in 2018 in Japan proved the concept for SDT in a mouse model of glioma.³⁵ Another preclinical study in a rat model of glioma confirmed the Japanese results and optimized survival after SDT using a multipoint sonication method.²²

- For treating GBM, PpIX is activated by focused ultrasound, which produces light by sonoluminescence to activate the PDT process and to release reactive oxygen species (ROS) that cause immediate or programmed cell death within the tumor, as has been repeatedly shown in preclinical models.
- The multipoint sonication method is being tested in the first-in-human clinical trial now under way at the Ivy Brain Tumor Center. The first cohort of patients with recurrent GBM will receive 200 J of focused ultrasound to half of the tumor volume prior to resection. Cohort 2 will receive 400 J, and Cohort 3 will receive 800 J. Tumor resection occurs four days after the SDT. The trial is measuring safety, tolerability, oxidative stress response (cellular membrane disruption), and apoptosis (programmed cell death).
- SonALAsense is sponsoring a multicenter clinical trial for patients with DIPG that will be similar to the clinical trial for patients with recurrent GBM. The DIPG study will be a drug and energy dose—ranging safety study. The drug-ranging aspect will be used to recommend Phase II dosing. The IND application for the study was approved by the US Food and Drug Administration (FDA) in September 2021. The initial treatment sites will be Children’s National Hospital, University of California San Francisco, and Ivy Brain Tumor Center. Half of the pontine volume will be treated at baseline, and the second half will be treated 30 days later. The primary endpoints are safety, tolerability, and dosing data.

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Liquid Biopsy



Watch the recording
10 minutes

Carl Koschmann, MD, described how liquid biopsy can be used to monitor DIPG diagnosis and treatment. He made the following points:

- Although other cancers have molecular plasma tests that are used for detection and monitoring of clinical responses, MRI is the only approved diagnostic modality for brain tumors because serial brain biopsies carry significant risk. Brain tumors often increase in size early in the treatment course, which may be due to real progression or radiation-related swelling and necrosis, which is called pseudo-progression.
- Tumor DNA can be found in the CSF of patients with brain tumors, and treatment response in patients that do respond to treatment can be measured by monitoring the allele fraction of mutations in the cell-free tumor DNA.^{36,37}
- To create a prospective patient cohort for several clinical trials, investigators at the University of Michigan Brain Tumor Center wrote a protocol for collecting CSF and plasma tumor DNA for correlation with MRI and other clinical changes at specific treatment time points. They were able to optimize sensitivity for CSF and plasma and correlate the data with change in tumor area.

- The allele fraction change over time correlated with PFS, especially in CSF. Other patterns in both plasma and CSF, such as cell-free tumor DNA spikes, could be used to both predict tumor progression and differentiate pseudo-progression and pseudo-response to treatment with Avastin (bevacizumab).
- Nanopore electronic sequencing can be used to run new plasma assays. New techniques are in development using loop-mediated isothermal amplification to increase speed, amplify small amounts of liquid media, generate concatemeric product, and reduce the error rate.³⁸
- Another collaborative study is investigating the addition of antibodies to mutant nucleosomes to fluorescently label them and improve their detection in DIPG plasma samples. Early data show that even small amounts of plasma can be enriched and stained to reveal quantifiable results that correlate with diagnostic and therapeutic status.
- Digital droplet polymerase chain reaction is highly sensitive and specific for recurrent alterations in CSF and plasma cell-free tumor DNA. Nanopore sequencing compares favorably (e.g., lower input, faster, cheaper) to next-generation sequencing for analyzing CSF for multi-gene responses. Fluorescent microscopic quantification of mutant nucleosomes is feasible, and serial CSF collection is feasible and useful in the management of glioma. It can also be used to differentiate pseudo-progression and pseudo-response, so should likely become standard of care for patients with high-grade gliomas. All of these technologies can be used in parallel.

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Panel Discussion

Dr. Mueller and **Dr. Zacharoulis** led the panel discussion, which included panelists Tabitha Cooney, MD, Lindsay Kilburn, MD, Carl Koschmann, MD, Stuart Marcus, MD, PhD, and Cheng Chia “Fred” Wu, MD, PhD. After Dr. Mueller briefly summarized the recorded content, Dr. Zacharoulis provided an update on the current status of DIPG and DMG clinical trials. He made the following points and then opened the panel discussion:

- The **DIPG clinical trial** that began in July 2021 at Columbia University has thus far delivered 24 sonications in three participants. It has generally been well-tolerated with no grade III+ toxicities. Two new participants are scheduled to begin the trial in November 2021.
- Eleven clinical trials are currently testing targeted therapies using systemic administration. Some of the agents have specificity for DIPG, and some have central nervous system penetration. Six immunotherapy clinical trials to deliver systemic therapy are under way.

Q. Is there any preclinical evidence to support the use of SDT for DIPG?

- Preclinical studies in Dr. Nazarian’s laboratory show that 5-ALA accumulation is significantly higher in DIPG cells than in both C6 rat glioma and native rat brain cells. The tendency of the cells to retain 5-ALA and produce PpIX is also longer in these models.
- These preclinical data were used to support an FDA application to test SDT in a clinical trial for DIPG, and the FDA recently approved the application (“SDT201”). The safety trial includes a design to initially treat half of the patients with a second treatment to follow 30 days later.
- PDT has a 15-year history of treatment in GBM. As compared with BBB opening, PDT provides an immediate effect on the molecule in the tumor, and the effect occurs only in the tumor. It is a highly selective therapy (possibly the most highly selected chemotherapeutic agent), because 5-ALA has a 98 percent positive predictive value for GBM, which does not change with recurrence. Apoptosis and cell death are found within four days after treatment. The metabolism of the glioma cell is turned against itself.

Q. With local therapy, what are the differences between treating the primary tumor volume versus the area surrounding the tumor?

- The progression of the tumor is what causes death in these patients, so the area surrounding the tumor will be important to treat as therapies are advanced.
- The tumor volume treated, treatment timing, and leading edge as a target will also contribute to the success or failure of future local therapies after safety has been established.
- There is a large gap between what can currently be done with new technologies versus what is desired for their use. For example, microbubble administration is limited by its current FDA approval for the number of times a dose can be given in a day.

Q. What technology advances are needed to conduct new clinical trials?

- Microbubble delivery (e.g., continuous infusion) that is suitable for repeated BBB opening
- The ability to open a highly controlled and set volume of the BBB (using an implantable ultrasound transducer, a transducer with multiple arrays, or the use of an acoustic hologram)
- Ports and catheters for frequent CSF collection (such as an Ommaya reservoir) in patients with DIPG and DMG
- Novel panels for new CSF biomarkers

- The ability to amplify and analyze cell-free DNA in plasma
- Combination therapies with immunotherapy or radiation therapy
- Standardization and expansion (e.g., PET scanning) of imaging assessments, biomarkers, and liquid biopsy techniques
- Arterial spin-labeled imaging for liquid biopsy or measuring capillary permeability (e.g., K^{trans})
- The ability to predict (possibly with artificial intelligence) chemotherapy diffusion coefficient changes that will lead to positive results for DIPG or DMG (as has been done with GBM)
- Focused ultrasound devices and microbubbles for BBB opening that are unaffected by the MRI magnetic field

Q. Should liquid biopsy be incorporated into the recommended outcome assessment measures for future focused ultrasound clinical trials?

- Treatment monitoring with liquid biopsy can be used in all types of clinical trials. CSF and plasma collection and analyzation procedures could and should be standardized across all trials to provide additional diagnostic and treatment monitoring metrics. The timing of biosample collection around focused ultrasound treatments is important to consider when first beginning clinical trials. Ports and catheters for frequent CSF collection may be a future possibility.
- Focused ultrasound can also be used to amplify the amount of circulating cell-free DNA, which can improve biomarker measurements but may also increase the risk of metastatic disease. This risk may not be a factor with DIPG and DMG, because their cells require the brainstem microenvironment to grow, and they require a genetic change to convert into leptomeningeal cells. The amplification effect is also likely to be temporary and washed out by the continuous renewal of CSF.

Q. How can cell-free tumor DNA be found in CSF and plasma when the BBB has not been opened in patients with DIPG? Do the treatments allow the DNA to cross?

- Cell-free tumor DNA is more difficult to find in low-grade tumors, perhaps because high grade glioma treatments cause focal disruptions in the BBB, especially in their necrotic, hypervascular areas. That is probably enough to spill out the DNA from the dying cells.
- Patterns of change in H3K27M variant allele fraction over time demonstrate clinical utility for predicting progression and sustained response and possible differentiation of pseudo-progression and pseudo-response.^{39,40}

Q. How can focused ultrasound best be used with DIPG immunotherapy studies?

- The combination of focused ultrasound with immunotherapy has great potential. For example, CAR-T cell and antibody efficacy can be enhanced by combining focused ultrasound with immunotherapy.⁴¹
- Preclinical studies using focused ultrasound and immunotherapy in a model of DIPG are now underway. The potential exists for using focused ultrasound to increase T cell access to the tumor cells. Focused ultrasound may also be useful for combination therapy with cancer vaccines and other areas of immunotherapies (e.g., PD1 inhibitors, abscopal effects) or in combination with radiation therapy.
- Beyond DIPG, several publications describe the effect of focused ultrasound on the tumor microenvironment in other types of cancers.

Q. What are the steps for choosing the next most promising therapeutic agents to combine with focused ultrasound BBB opening in patients with DIPG and DMG? What is the best way to optimize drug selection for clinical trial purposes?

- The focused ultrasound community could create a multisite infrastructure for testing various therapeutics within a DMG platform trial. Patients could be enrolled at various disease stages (e.g., recently diagnosed, post radiation, or at the time of progression) to create different arms of the studies.
- A working group could be tasked with selecting which agents and technological parameters to test on the collaborative focused ultrasound platform.
- A working group could create consensus on outcome assessments for all clinical trials. Using the same outcome assessments would allow direct comparison of different studies.
- Researchers should consider drugs that have a longer half-life or those that accumulate in the tumor tissue to maximize the potential of combination therapies.

Q. What are the next steps for clinical trials that combine focused ultrasound with radiation therapy?

- Now that small animal studies have established the safety parameters for using a clinical dose for this type of combination therapy, the next goal is to develop a working model for radiation plus focused ultrasound plus systemic treatment. The therapeutic agent could be panobinostat or a different systemic therapy.
- Translational studies are the next step.

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Roadmap Session

The roadmap session created open discussion among all attendees.

The Foundation posed three questions to the group. Attendee responses are listed below:

1. What preclinical laboratory studies need to be done to continue to move the field forward?

- Develop resources for centralized pathology review and a data repository to catalog neuropathological findings in preclinical DIPG specimens after focused ultrasound (i.e., a centralized histopathology resource)
- Create a database of preclinical models for DMG and DIPG, agree on larger animal models to study, and develop immunocompetent animal models
- Organize a preclinical working group to share data, biospecimen tissue, and experiences while seeking to form collaborations
- Address the heterogeneity of DMG and DIPG tumors
- Determine to what degree the BBB needs to be opened
- Devise ways to expand the treatment envelope
- Test the pharmacokinetics of various agents to improve the translation of preclinical studies to effective clinical trials
- Develop microanalyses for assessing drug penetrates
- Correlate preclinical data with clinical studies

2. What technology advancements are needed?

- Develop frameless systems that do not require full immobilization of patients
- Eliminate the need for head shaving
- Incorporate spot control for BBB opening into human clinical systems
- Expand the treatment envelope
- Introduce a radiolabeled drug with focal BBB opening
- Allow treatments outside the MRI environment that are less costly and easier to use, enabling treatments worldwide
- Incorporate improvements in the development of the bubbles that are used with focused ultrasound

3. What are the next steps to ensure successful focused ultrasound clinical trials for the treatment of DIPG?

- Create a clinical working group for continued collaboration on focused ultrasound for DIPG preclinical and clinical studies
- Establish consensus on the clinical goals for the development of focused ultrasound devices to treat DMG and DIPG
- Develop close collaborations with neuropharmacologists
- Ensure that all patients have access to clinical trials
- Improve measures to determine the specificity and sensitivity of drugs that are delivered across the BBB
- Ensure adequate organization of clinical trials to avoid mistakes made in the development of other technologies
- Harmonize clinical trial protocols with standardized imaging and treatment parameters
- Add quality-of-life measures (for the patient and the caregiver) to future clinical trials
- Consider the development of larger, multisite comparative studies that use multiple devices
- Create a flow of information and feedback from clinical to preclinical researchers when identifying research problems or questions

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Conclusion

Dr. Powlovich and **Dr. LeBlang** thanked the steering committee, presenters, and panel moderators for their work in planning and conducting this workshop.

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Abbreviations

5-ALA	5-aminolevulinic acid
BBB	blood-brain barrier
CSF	cerebrospinal fluid
DIPG	diffuse intrinsic pontine glioma
DMG	diffuse midline glioma
FDA	U.S. Food and Drug Administration
GBM	glioblastoma
Gy	greys
IND	investigational new drug
OS	overall survival
PCI	passive cavitation imaging
PDT	photodynamic therapy
PET	positron emission tomography
PFS	progression-free survival
PpIX	protoporphyrin IX
ROS	reactive oxygen species
SDT	sonodynamic therapy

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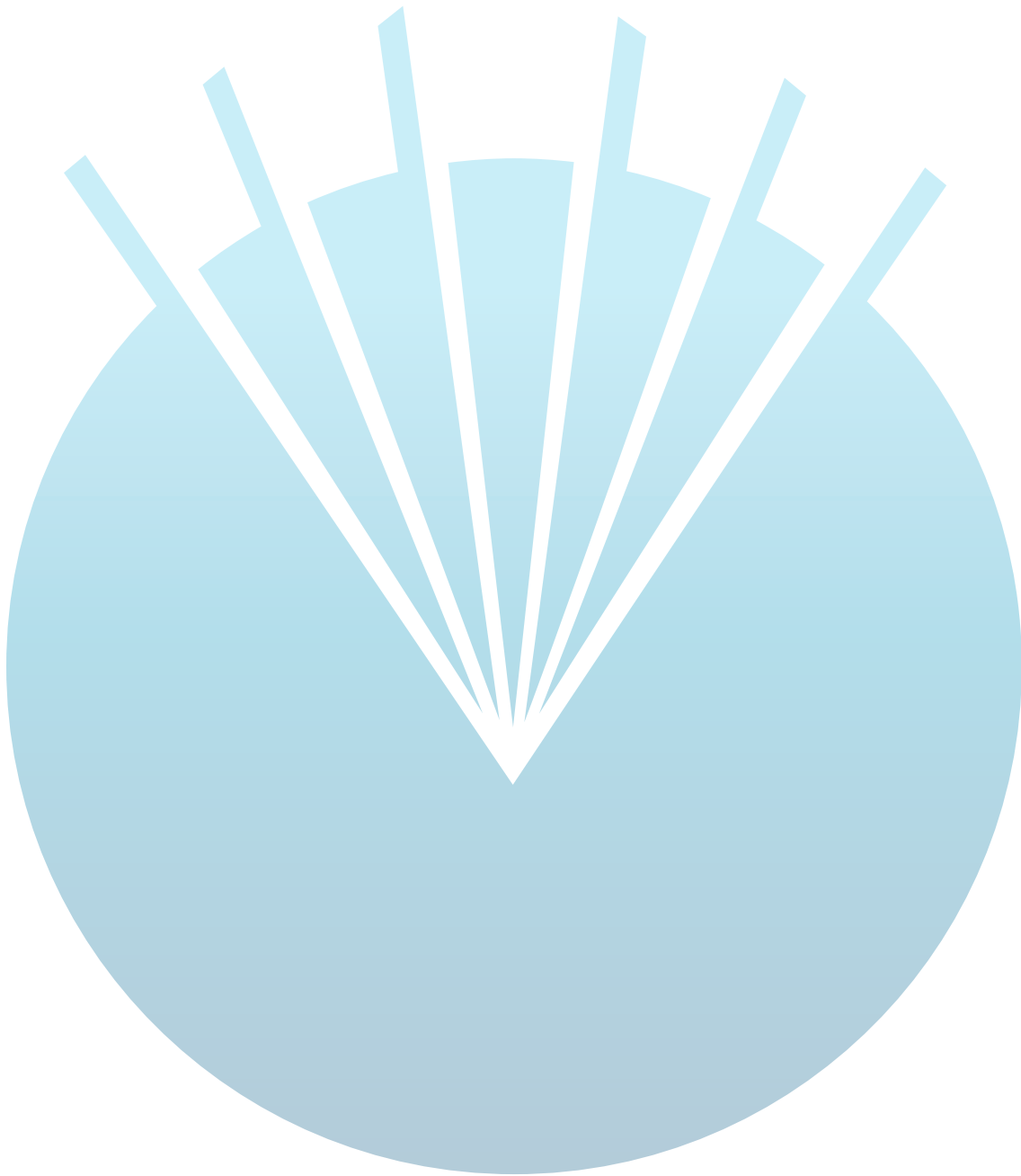
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