Introduction
Glioblastoma (GBM) affects an estimated 18,500 patients annually, and there are approximately 15,000 deaths per year from GBM in the US. The incidence of GBM is increasing by approximately 1.2% per year, particularly in the elderly population. GBM represents about 17% of all brain tumors and 52% of primary brain tumors. The risk of GBM increases with age – with the greatest prevalence occurring in patients in their 60’s or 70’s, although it is not uncommon among younger patients.

GBM is a highly invasive and diffuse tumor, commonly occurring in white matter. There are no clear margins between tumor and brain tissue, which makes full surgical resection impossible. 90% of patients develop tumor recurrence at or near the surgical site.

Clinical management for newly diagnosed patients with GBM is determined through a combination of hybrid imaging and histologic, molecular, and clinical information. The molecular pathways involved in the pathogenesis of GBM are well-characterized, but have not led to molecular-targeted therapies. In general, treatment for GBM involves maximum safe surgical resection or biopsy followed by adjuvant radiation and chemotherapy.

Focused ultrasound (FUS) is an early stage, therapeutic technology that offers a possible adjuvant or alternative to current treatment strategies. It has the potential to improve quality of life, longevity, and healthcare-related costs by improving treatment efficacy, eliminating invasive procedures and reducing side effects. FUS uses concentrated energy to treat tissue deep in the body accurately, precisely, and noninvasively. Combining imaging techniques (magnetic resonance or ultrasound) with FUS permits target tissue identification with sub-millimeter accuracy, real-time treatment guidance and monitoring, and confirmation of treatment efficacy.

There are four primary areas in which FUS could potentially play a role in the treatment of GBM:

1. Tumor ablation: thermal ablation, histotripsy, or non-thermal microbubble-enhanced destruction/ablation
2) Drug delivery: focal delivery of chemotherapy agents to the brain – enhanced by FUS-induced opening of blood-brain barrier (BBB) – and reducing systemic toxicity of these agents

3) Adjunct to immunotherapy: focal delivery of immunotherapy agents and/or stimulating the immune response

4) Treatment adjuvant: radiosensitization, activation of ultrasound sensitive agents (e.g. heat sensitive liposomes, sonodynamic agents)

The application of FUS for the treatment of GBM (via thermal ablation) is currently in the early clinical development stage (Phase I) and trials are enrolling patients. FUS is FDA-approved for three indications: pain from bone metastases, uterine fibroids, and ablation of prostate tissue; although there are many more indications approved outside the US.

On November 9-10, 2015, the Focused Ultrasound Foundation (FUS Foundation), held a workshop on the use of focused ultrasound in the treatment of GBM. The meeting included presentations on the state of the field, discussion of relevant treatment mechanisms, and development of roadmaps for future preclinical and clinical research. The workshop was also intended to foster collaboration by bringing together a multidisciplinary group of thought leaders including neuro-oncologists, neurosurgeons, neuroscientists, physicists, biomedical engineers, and representatives from FDA, NIH, and industry.

**Background**

Current treatment options for GBM are limited. Untreated patients with GBM have a life expectancy of only a few months. Surgical resection increases this number to 3 months, and the addition of radiotherapy plus chemotherapy (temozolomide) improves overall survival to a median of 12 months. Surgery followed by concurrent radiation and temozolomide (TMZ) treatment remains the current standard of care for patients with GBM. TMZ is a well-tolerated oral alkylating chemotherapy with mild non-cumulative myelotoxicity. MGMT (06-methylguanine-DNA-methyltransferase) methylation status is a predictor for patients most likely to benefit from TMZ treatment. Epigenetic silencing of the MGMT DNA-repair gene by promoter methylation compromises DNA repair and increases overall survival in GBM patients who receive alkylating agents like TMZ. Several factors make drug development and treatment for GBM difficult, such as the difficulty of delivering drugs to the brain and the highly invasive and inhomogeneous nature of the tumors. It is challenging to deliver the desired therapeutic dose that is effective yet also has minimal systemic effects.

For patients with recurrent GBM there is no standard treatment protocol. There is a great deal of heterogeneity in recurrent GBM, making it difficult to predict which patients will respond to treatment. Management goals include: preserving neurological function, extending survival, and minimizing toxicities. The current gold standard as a clinical trial endpoint is 6 months progression free survival.
Current Developments in Immunotherapy for Glioblastoma

Historically the brain was considered immune-privileged, and there was an assumption that immunotherapy would not be effective in GBM. However, recent research has demonstrated that the brain has a lymphatic system, which has increased interest in the potential for immunotherapy to treat cancer in the CNS.\(^\text{12,13}\)

David Reardon from the Dana-Farber Cancer Institute described the current state of immunotherapy treatments for GBM. The major hurdles for current clinical trials are delivery of the agent (most do not cross the BBB well, if at all), heterogeneity of the tumor and its immune signature, and the complexity of GBM, as well as de novo and acquired resistance to treatment. Since 2010, several immunotherapies have received FDA approval for oncology indications, but none of these are approved to treat GBM. The majority of approved immunotherapies are check-point based immunomodulatory molecules (check-point inhibitors).\(^\text{8}\) These include antibodies that bind cytotoxic T lymphocyte antigen – 4 (CTLA-4), programmed death receptor-1 (PD-1) and its ligand (PD-L1). These antibodies reduce the tumor’s ability to evade attacks by the immune system by blocking pro-tumorigenic signals at different points in the immune response cascade.\(^\text{9}\) Both Anti-CTLA-4 and Anti-PD-1(PD-L1) treatment increase the activity of anti-tumorigenic cytotoxic T cells. Several publications have shown a reduction in tumor size (solid tumors and melanoma) after treatment with immunotherapy, e.g. nivolumab and lambrolizumab.\(^\text{10,11}\)

Preclinical work in a syngeneic glioma (C57BL/6) mouse model (GL261-luc2, orthotopic implant) found that immune checkpoint blockade increased long-term tumor-free survival following single agent anti-PD-1, anti-PD-L1, or anti-CTLA-4 therapy in 50%, 20%, and 15% of treated animals, respectively.\(^\text{14}\) Combination therapy of anti-CTLA-4 plus anti-PD-1 resulted in complete tumor regression in 75% of the animals.\(^\text{14}\) Extensive analysis of the immune response showed that tumor-specific immune memory responses were generated. Inhibitory immune checkpoint blockade increased the infiltration of anti-tumorigenic activated cytotoxic T cells and natural killer cells while decreasing the presence of pro-tumorigenic suppressive immune cells in the tumor microenvironment.

There are ongoing studies of immunotherapy in patients with GBM, including preliminary work with a tumor-specific vaccine targeting epidermal growth factor receptor variant III (EGFRvIII) . EGFRvIII is a constitutively activated and immunogenic mutation widely expressed in GBM.\(^\text{15}\) A Phase II randomized study suggests that the EGFRvIII peptide vaccine (rindopepimut) improves overall survival.\(^\text{16}\) Patients with recurrent GBM were randomized to receive bevacizumab or bevacizumab with rindopepimut, and the combination treatment improved overall survival. However, most of the patients in the trial eventually experienced further tumor progression. These tumors did not express EGFRvIII and would therefore be resistant to further rindopepimut treatment.
Tumor heterogeneity and the aggressive nature of GBM affect the potential success of immunotherapy. GBM has a high frequency of mutations, producing an immune signature that is unique to each patient’s tumor.\textsuperscript{17,18} Multi-targeted immunotherapy approaches through the application of multi-valent vaccines, multiple checkpoint blockades, and other combination strategies will most likely be required to produce significant gains in patient survival. Neoantigen load, a measure of tumor heterogeneity, may predict immune reactivity and patient outcomes, as demonstrated with CTLA-4 blockade in melanoma, colorectal cancer, and non-small cell lung cancer (NSCLC).\textsuperscript{19-22} There are several multi-valent vaccines in clinical trials for GBM. For example, a Phase I study of a personalized neoantigen cancer vaccine (NeoVax) in GBM is currently underway. In this study, next generation sequencing (RNA-Seq) is being used to investigate the intra-tumoral heterogeneity of the tumor for each patient.\textsuperscript{23}

Several early phase clinical trials are underway to investigate immunotherapy for the treatment of GBM. There are three main strategies: vaccine, immune checkpoint blockade, and modified T cell therapies. However, GBM tumors have a multilayered strategy of resistance and escape both through systemic immunosuppression as well as through the tumor microenvironment. Ultimately, combination approaches including one or two immunotherapies, with or without chemotherapy and/or other modalities (e.g. radiation) may be more promising strategies. Early phase clinical trials of such combinations are already underway in patients with GBM. Novel therapies such as FUS may also play a role in such combination approaches, though this research is very early stage.

\textit{Laser Thermal Ablation to Treat the Tumor and Radionecrosis}

Veronica Chiang from Yale University discussed lessons learned from laser interstitial thermal therapy (LITT) for the treatment of GBM. The LITT procedure is generally well tolerated and minimally invasive; patients go home the day following surgery. Given that most cases currently being referred for LITT are at the time of tumor recurrence, best operative results from use of the technology have been achieved using unusual surgical trajectories. Achieving these approaches has required cooperation between multiple companies to create compatible technology. One limitation of LITT is the inability to predict the distance and direction of heat penetration. While heat penetration tends to be faster through the lesion if it is comprised of more radionecrosis than tumor, there are other, as of yet undetermined, factors that make heat penetration different from case to case. Lesion visualization during treatment also remains restricted by bony proximity. Determining early outcome following LITT can also be challenging given the lack of definitive radiographic markers of treatment success until 3 to 6 months after LITT.

\textbf{State of the Field – Clinical Experience for Treating Glioblastoma with FUS}

Several brief presentations by workshop participants provided an overview of the current state of the clinical experience of FUS treatment for GBM.
Alexandra Golby from Brigham and Women’s Hospital reported on the initial experience with MRI-guided FUS for ablation in patients with GBM. The technology has been developed over several decades, and a hemispherical phased-array transducer allowed transcranial FUS application in clinical trials. A proof-of-concept trial with 3 patients showed that it was possible to non-invasively focus ultrasound beams on a target in the brain and to visualize the heating with magnetic resonance temperature imaging (MRTI). One patient had MRTI artifacts (due to blood products in the tissue) that caused a loss of MRI signal. After these results, a version of the ExAblate Neuro that operated at 220 kHz was manufactured and tested in one patient. High temperatures were produced along with thermal necrosis. However, sonication resulted in a larger than expected focal spot. In addition, damage in non-targeted structures was observed and liquefaction of the sonicated tumor was indicated. Several days later this patient developed an intraventricular hemorrhage and died. It is unknown whether this hemorrhage was the result of cavitation or other FUS-related effects. Since this trial, technological solutions have made heat estimates much more accurate. The trial demonstrated that FUS could penetrate an intact skull. However, it also demonstrated a critical role for thermometry and the risk of hemorrhage, which are important to consider in future use of FUS for thermal ablation.

In the past several years since this initial trial, the manufacturer InSightec has made many improvements to the MRgFUS technology and the current ExAblate Neuro has now been used to treat more than 200 patients with a range of neurological disorders through precise thermal ablation. No severe adverse events have been observed and the device has received regulatory approvals for treatment of movement disorders (essential tremor, Parkinson’s tremor) and neuropathic pain in several countries. FDA approval for the treatment of essential tremor is expected in 2016.

Javier Fandino from Kantonsspital Aarau (Switzerland) described an ongoing Phase I clinical trial of the safety and efficacy of thermal ablation using the ExAblate Neuro for the treatment of GBM. Only 4 patients have been treated, and tumor ablation has only been shown in one patient. In one patient, an implanted catheter interfered with sonication and resulted in the inability to adequately heat the tumor target. In another patient, an imaging artifact caused off-focus heating. In the case where ablation was observed, a 63-year-old patient presenting with a centrally located recurrent GBM underwent MR-guided FUS, and 25 high-power sonications were delivered. Partial ablation of the tumor was achieved with no observed neurological deficits or other adverse events for the patient.

Todd Mainprize from Sunnybrook Health Sciences Centre (Toronto) described the clinical experience with FUS-induced BBB opening using the ExAblate Neuro. The first patient was treated on November 5, 2015. On day one the patient was infused with liposomal doxorubicin, followed by sonication in conjunction with microbubbles (Definity, 0.3 mL (4 µl/kg)). The total
time for the FUS procedure was 2.5 hours. Sonications were performed in a 3x3 grid, with 3 mm spacing, and 300 ms of FUS was applied at each grid point. Increased gadolinium uptake was seen in the sonicated area, confirming BBB opening. On day 2, tissue samples were obtained and the tumor was surgically resected. Data will be available at a future date regarding blood levels of doxorubicin and histological analysis of the resected tissue. A current limitation of the procedure includes long delays between FUS sonications to allow for data processing, which necessitates additional microbubble and gadolinium injections. These additional doses can be costly or may face regulatory limitations. Future treatments should try to optimize this timing to enable the most efficient procedure.

Michael Canney from CarThera discussed a Phase I/IIa safety study of ultrasound for BBB opening in patients with recurrent GBM. Canney described the SonoCloud system, which is an ultrasound device – implanted through a small hole in the skull – that delivers planar energy directly to the brain and tumor. This strategy removes challenges imposed by the skull such as attenuation, aberration, and distortion. The implantation of the device can be done under local anesthesia in an outpatient setting. Preclinical studies in rabbits and nonhuman primates demonstrated safety.\textsuperscript{26,27} In July 2014, the Phase I safety trial started enrolling patients with recurrent GBM that were candidates for carboplatin chemotherapy. The primary endpoint of the trial was feasibility and tolerance. Secondary endpoints included BBB opening as evidenced by dynamic contrast enhanced MRI and clinical efficacy, as well as other factors. The BBB opening procedure takes about 6 minutes. There is no need for MRI monitoring during the procedure. Nine patients have been treated to date with no adverse events related to BBB opening. With this procedure, BBB opening results in greater delivery of chemotherapy into the brain. However, one limitation of the device is that ultrasound can penetrate a 1 cm diameter cylindrical volume deep into the brain but only in front of the device.

**FUS Mechanisms Relevant to the Treatment of Glioblastoma**

Workshop participants briefly presented on FUS mechanisms that have therapeutic potential for the treatment of GBM, including thermal ablation, non-thermal tissue destruction (histotripsy, microbubble-enhanced microvascular ablation, sonodynamic therapy), targeted drug delivery (BBB opening for treating GBM, nanoparticles), immunomodulation, and radiosensitization.

*Thermal Ablation*

Nathan McDannold from Brigham and Women’s hospital discussed FUS ablation with and without microbubbles. Preclinical studies in rabbits determined the threshold for FUS-induced thermal damage in the brain.\textsuperscript{28} The study used a simple phased array to create a spatially flat thermal region in the brain, and MR temperature imaging to determine the temperature at the center of the region. This was done for regions corresponding to tissue with and without
damage, and it provided more accuracy compared to other methods of estimating temperature in the brain. Lesions are not always immediately visible on MRI, and can increase in size over time. A low frequency (230 kHz) FUS study is currently being carried out in nonhuman primates. This system uses a continuous wave sonication that increases in intensity over time and uses acoustic feedback to control power levels.

Non-Thermal Tissue Destruction

McDannold also discussed non-thermal ablation using FUS plus a microbubble ultrasound contrast agent. In this method, the microbubbles isolate the effects of FUS to the vasculature.\textsuperscript{29,30} This technique enables non-thermal ablation with a low-intensity (< 1 MPa) burst sonication applied at a low duty cycle. This has been demonstrated in nonhuman primates (macaques) and produced well-defined lesions with no heating. Tissue damage appeared to be produced via ischemia, although there were some effects outside the focal region possibly caused by internal reflections or poor focusing. Non-thermal ablation can ablate deep brain structures without skull heating, although white matter tracts appear relatively resistant.

Charles Cain from the University of Michigan discussed transcranial histotripsy. Histotripsy is a mechanical fractionation of soft tissues by successive high intensity ultrasound pulses. This process occurs through non-thermal initiation and maintenance of dynamic ‘bubble-clouds’ that are a specific form of cavitation. This method is able to generate small lesions, which is ideal for transcranial procedures. Preclinical research in red blood cell phantoms indicates that monopolar pulses with a dominant negative phase could produce precise histotripsy-type lesions using an intrinsic threshold mechanism.\textsuperscript{31} Current work is being carried out \textit{in vivo} using histotripsy pulses.

Rich Price from the University of Virginia discussed the potential of microbubble-enhanced microvascular ablation in tumors. There are different modes of microbubble activation with FUS: stable cavitation (low peak-negative pressure) that opens the BBB for safe drug or gene delivery, and inertial cavitation (high peak-negative pressure) that opens the BBB and can cause occlusion of the tumor microcirculation. In a mouse model (Rag1\textsuperscript{-/-} bearing subcutaneous C6 gliomas), 1 MHz ultrasound was applied with varying duty cycles to the tumor every 5 seconds for 60 minutes in conjunction with intravenously injected microbubbles.\textsuperscript{32} This resulted in decreased blood flow to the targeted area. Tumor growth inhibition was dependent on ultrasound dose. Tumor samples from the treated area showed apoptosis and necrosis. Possible mechanisms of growth inhibition involve apoptosis/necrosis due to a lack of oxygen delivery, activation of innate immunity, and prolonged mild heating (activation of heat shock inhibitors, etc.). The limitations of this technique include the potential for hemorrhage and increased pressure in brain. In deep tissues, the approach may be complicated by “beam path” activation of microbubbles.
Zhiyuan Xu from the University of Virginia discussed sonodynamic therapy (SDT) in preclinical models. Photodynamic therapy in combination with 5-aminolevulinic acid (5-ALA) is FDA-approved for the treatment of several cancers (not GBM), but has limitations such as poor light penetration to deep-seated tissue, photosensitivity, invasiveness, and non-focal treatment. Photosensitizers can also be used as sonosensitizers. Sonodynamic therapy utilizes low-intensity ultrasound in conjunction with a sonosensitizer, which results in a low thermal rise and apoptosis secondary to reactive oxygen species. SDT has been investigated in vitro and in vivo. For example, verteporfin is a photosensitizer FDA-approved for use in ophthalmologic surgery, Verteporfin accumulates in abnormal blood vessels, and produces highly reactive short-lived singlet oxygen and other reactive oxygen radicals when activated by nonthermal red light, resulting in local damage to the endothelium and blockage of blood vessels. In Wistar rats with subcutaneous C6 glioma cells, ultrasound (1.1 MHz transducer, 175 mV) was used to increase tumor temperature to 42°C for 20 minutes; in the presence of verteporfin that accumulated in the tumor, this type of sonication resulted in decreased tumor size. Results of histology are pending.

**Targeted Drug Delivery**

Nathan McDannold presented on the potential of BBB opening for the treatment of GBM and metastatic disease. There are several barriers to drug delivery in brain tumors such as tumor recruitment of blood vessels from surrounding tissue, reduced permeability of brain metastases compared to those in other organs, and metastatic ‘seeds’ that are protected by the BBB. In an orthotopic rat glioma model, the concentration of liposomal doxorubicin increased in tumors after FUS plus microbubble treatment. Multiple BBB openings (3 times a week plus liposomal doxorubicin) in this model resulted in improved survival. In a rat model of intracranially metastatic Her2-positive breast cancer (BT747), 6 weekly BBB opening treatments in conjunction with trastuzumab enhanced delivery of drug to the tumor and resulted in decreased tumor volume in the treatment group. FUS-induced BBB/blood-tumor barrier (BTB) disruption can enable the delivery of therapeutic levels of chemotherapy in rodent tumor models. The therapeutic effect is variable, depending on tumor stage, treatment frequency and other factors. Safety studies show little or no histological or functional changes. However, current tumor models are not ideal and better models of GBM are needed, with improvements such as increased tumor infiltration in the brain and decreased immunogenicity.

Jung Soo Suk from Johns Hopkins University presented work on developing therapeutic nanoparticles for optimal drug and gene delivery to the brain. Due to electrostatic forces and tight junctions in the brain, it is difficult to get large or electrically charged nanoparticles across the BBB. Brain-penetrating nanoparticles containing DNA (DNA-BPN) and densely coated with low molecular weight polyethylene glycol (PEG) are capable of retaining their physiochemical properties in physiological environments and efficiently penetrating brain tissue, thereby providing widespread and high-level in vivo transgene expression in the brain. DNA-BPN provide a widespread p53 transgene expression and anti-cancer efficacy in an
orthotopic 9L rat brain tumor. A BPN particle containing paclitaxel demonstrated enhanced therapeutic effects (delayed tumor growth) in a rat glioma (F98) model compared to un-encapsulated paclitaxel.\textsuperscript{40} BPN in combination with FUS were able to cross the BBB and enter targeted brain regions with greater distribution than just the nanoparticles alone.\textsuperscript{41} Preliminary research has shown that FUS can also deliver BPN across the tumor vasculature beyond the level achieved by the EPR effect alone. Additionally, the combination of FUS and BPN can deliver gene therapies to specific anatomical brain regions.

\textit{Immunomodulation}

Or Cohen-Inbar from the University of Virginia presented on immunomodulation in response to FUS in preclinical models of GBM. There are many effective antitumor response strategies, including maintaining a local anti-tumorigenic cytokine microenvironment. Patients with GBM exhibit impaired antitumor immunity (modulated by T cells) and impaired systemic immunity (B cells). Early research from patients with GBM found that patients with a postoperative infection had longer survival, and this was attributed to a non-specific active immune response recruitment to the tumor milieu. Preclinical experiments demonstrated that FUS enhances immunogenicity through the induction of heat shock proteins (HSP-70).\textsuperscript{42,43} FUS also enriches tumor-infiltrating T-lymphocytes (TIL), populations with immunopotent cells such as CD8+ T cells, CD4+ T cells, and natural killer cells \textit{in vivo} in a variety of cancers, although this has not been shown in a model of GBM.\textsuperscript{44,45} Dendritic cell activity and proinflammatory cytokine secretion is enhanced by FUS \textit{in vivo}.\textsuperscript{46-48} In summary, FUS-induced immunomodulation can be harnessed in combination with current and developing immunotherapy approaches.

Rich Price discussed ongoing preclinical research on ultrasound activation of microbubbles to modulate the immune signature. A subcutaneous B16 tumor (melanoma) was implanted in C57BL/6 mouse (immunocompetent). Tumor growth inhibition and animal survival were enhanced by microbubble activation with a low duty cycle/high peak-negative pressure ultrasound treatment. Early results suggest activation of both the innate (macrophages, natural killer cells) and adaptive (CD4, CD8, and T\textsubscript{reg} cells) immune systems. Potentially, microbubble activation with ultrasound could transform the tumor environment to become sensitized to anti-PD-1 treatment. However, immune privilege in the brain may affect this response. Future studies will determine the immune response to microbubble activation in intracranial tumors.

\textit{Radiosensitization}

James Larner from the University of Virginia discussed using FUS for radiosensitization through the induction of hyperthermia. GBM is resistant to radiation, likely due to genetic mutations that alter multiple signaling pathways.\textsuperscript{49} The combination of heat and radiotherapy has potential synergistic therapeutic effects, but it is technically difficult to administer them both simultaneously.\textsuperscript{50} One of the challenges of hyperthermia is thermo-tolerance, which is resistance to subsequent heating via heat shock protein activation. To avoid thermo-tolerance, hyperthermia should be limited to one to two times per week. Hyperthermia sensitizes GBM
stem-like cells to the effects of radiation. The combination of hyperthermia and radiation is immunostimulatory, and the combination of heat and radiation has been validated in randomized controlled trials for the safe and effective treatment of breast cancer and GBM.

State of the Technology – ExAblate Neuro

Eyal Zadicario from InSightec provided a brief overview of the current state of the technology for the ExAblate Neuro systems. InSightec is currently looking at two possible modes of treating tumors, thermal lesioning and drug deliver by opening of the BBB. A platform is in development that will enable studies of both of these mechanisms. The existing ExAblate Neuro, used for thalamic lesioning, is limited in delivering a robust treatment for tumors. Treatment of tumor tissue is challenging: its location may be anywhere in the brain, it tends to be more vascular and made of nonhomogeneous tissue, and it may require a large treatment volume.

One solution to this is to use low frequency. Significant advances in technology have been made since earlier experiments, particularly safety protocols to prevent or avoid the effects of cavitation; namely, a real-time cavitation monitoring system has been developed. There is a low frequency 220 kHz system in development and 2-year *in vivo* study using this system is nearing completion.

The company plans to submit both preclinical and clinical safety data using this system to the FDA in 2016. This is expected to enable initiation of clinical research in 2016.

The first experience with BBB disruption shows great promise. The site at Sunnybrook has a one of a kind modified ExAblate Neuro that is being used in the first-in-human study. The company plans to collect the input from this study after 3-5 cases and develop a system configuration that can support a Phase I study using BBB opening. This is expected to support Real-time acoustic feedback which is necessary for safety monitoring, and this feature is currently in development. Clinical trials for BBB opening are in the planning stages and may include patients with either GBM or Alzheimer’s disease. Further clinical discussion needs to take place to define the Phase I and regulatory approach of these programs.

State of the Technology – CarThera SonoCloud

CarThera’s SonoCloud device, a 1 MHz ultrasound implant for performing BBB opening, is currently in a Phase I/IIa clinical trial in Paris for treating glioblastoma. The safety profile of the device has been excellent in this clinical trial, and efficacy of the BBB opening has been demonstrated for the highest ultrasound pressures of the escalating dose protocol. The efficacy profile of the device for glioblastoma patients, based on an increased drug uptake in the region where the BBB is opened, will soon be evaluated through a Phase IIb/III clinical trial. In addition
Overall Discussion and Evidence Gaps

Prioritize Treatment Mechanisms

There was a discussion on which treatment mechanisms seemed most promising: ablation (thermal or mechanical), immunomodulation, drug delivery via BBB opening, or radiosensitization.

Thermal ablation

- The FUS procedure is well controlled in normal brain tissue, but the methods in tumor tissue have not been refined.
- There was a discussion on the specific population of patients that could benefit from thermal ablation. The current surgical standard of care is limited and may not be able to reach deep metastases. Deep metastases would be an ideal target prior to surgery (surgical adjunct).
  - It is difficult to accrue patients in current FUS clinical trials because few patients meet the inclusion and exclusion criteria (e.g. tumors need to be central in the brain and not too large); additionally the laser therapy technique is competing for the same population of patients.
  - FUS could potentially be used in patients with residual tumor after surgery.
- A comment was made that surgery works well for 98% of patient; perhaps the interest for FUS is to investigate techniques that are not possible with surgery. For example, it is difficult to reach the thalamus and brainstem with surgical techniques.
- There was a comment that thermal ablation cannot be used in place of surgical debulking because a randomized comparative trial would not be possible to determine efficacy.

Mechanical ablation

- Histotripsy has a great deal of potential, and would take less time than thermal ablation.
- However, participants agreed that ablation does not have a lot of potential for FUS in patients with GBM. It is better suited for treating well-defined brain metastases.

Immunomodulation

- Biomarkers to measure the effectiveness of immunotherapies for treatment of GBM need to be developed.
- There was a discussion on the use of FUS to enhance the efficacy of immunotherapy in the tumor microenvironment. Immunotherapy can cause systemic toxicities; and local
delivery to the tumor (which could be enhanced by FUS) has the potential to lessen these effects. There are no immunostimulatory agents FDA-approved for the treatment of GBM, but there are several agents in late-phase clinical trials.

- The optimal brain target for immunotherapy was also discussed. Options include the main mass of the tumor, the border zone around the tumor, and the 2 cm edge around the tumor where there are satellite lesions. Stimulating the normal tissue in the brain to launch an immune response to the tumor has great potential, but these activated immune cells need to target the tumor.
  - Immunotherapy in combination with mechanical ablation was also discussed. A series of small lesions in a tumor would retain the vascularity of the tumor and provide access for infiltrating immune cells.
- PD-L1 is upregulated after radiotherapy, and the same response would be expected with FUS; therefore, checkpoint inhibitors seem like good therapeutic options for FUS.
- Most GBM interventions stimulate an immune response, and understanding this response would be useful in designing future trials for immunomodulation.
  - Adhesion molecules could serve as potential biomarkers for an active inflammatory response. There are simple and defined methods to measure adhesion molecules in the periphery.
- Immunostimulatory research should be done in immunocompetent animal models. Common GBM cell lines such as C6 or 9L are immunogenic and would not make good models for this kind of research. Additionally, many models have a mutational load that is much higher than human patients. F98 tumors implanted in rats might make a good preclinical model.
  - Future work in an immunocompetent rat model is needed to compare the efficacy of the different FUS mechanisms (ablation, histotripsy, hyperthermia, pulsed FUS, BBB opening).
  - There was a suggestion that canine patients would also make a good model as they are larger in size and clinical procedures are very similar to human patients. There are certain dog breeds with a tendency to develop spontaneous GBM tumors. Although the geometry of the canine skull makes thermal ablation difficult, other methods could be performed.
- The question of next steps to investigate immunotherapy in combination with FUS was addressed by the participants.
  - The first step is a rodent proof-of-principle study. Preclinical research has already shown that PD-1 inhibitors have efficacy, and the next step would be to determine whether the combination with FUS has additional efficacy.
  - Therapeutic agents to investigate include PD-L1 inhibitors because PD-L1 is upregulated in the tumor microenvironment. Other possibilities include microbubbles coated with cytokines delivered directly to the tumor.
- Resources for the preclinical work was discussed
Rich Price’s group is currently working on a preclinical study using B16 (melanoma) cells in C57BL/6 mice (immunocompetent) with FUS to open the BBB and deliver PD-1 and PD-L1 inhibitors, and CD27 agonists. The data should be available next summer. There is also an option to use additional preclinical models.

Joe Frank from the National Institutes of Health mentioned that his group could also provide resources. Experiments could be performed with implanted human tumor cell lines in immune-incompetent animals to stimulate native natural killer T cells to the tumor. Patient-derived tumors for experimental studies should also be considered.

Next steps: the FUS Foundation will put together a working group for preclinical research on FUS in combination with immunotherapy

**Drug delivery via BBB opening**

Sunnybrook plans to initiate a Phase II/III trial to study drug delivery methods over multiple time points. This trial would use FUS to open the BBB, administer temozolomide, followed by surgery and the standard of care. The combination of FUS with radiation could also be considered for inclusion into the study design. The researchers need to determine optimal timing for drug delivery.

- The point was raised that other therapies like BCNU could be considered particularly for recurrent disease.
- The next Sunnybrook trial will be designed by Todd Mainprize in consultation with neuro-oncologists and other experts.

There are still technical gaps in knowledge regarding opening the BBB in humans. The volume of BBB opening that is safe has yet to be determined; Nathan McDannold is investigating this question.

- The volume and duration of the BBB opening will be measured in future patients in the Sunnybrook trial via gadolinium-enhanced MR scans immediately after FUS-BBB opening and just prior to surgery.
- In order to appeal to a large number of patients and to increase the ease of repeatability of its use with a given patient, FUS treatment may need to be done without a stereotactic headframe.

- A comment was made regarding clinical efficacy of increased drug delivery; even if drug delivery was increased by 20% it is unclear if this will lead to an increased clinical effect.
- Proof-of-principle is needed to demonstrate that drug delivery is increased after BBB opening. The next step would be to investigate this question using promising chemotherap/immunotherapy agents in the development pipeline, particularly those that cannot cross the BBB on their own.

**Radiosensitization**
• Some preclinical work on BBB opening with microbubbles for radiosensitization has been studied by Kullervo Hynynen’s group at Sunnybrook Health Sciences Centre.
• Nathan McDannold mentioned a planned clinical trial for hyperthermia plus doxorubicin treatment in patients with GBM, he will also consider adding radiation to this protocol.

Technology Gaps

The participants discussed high-priority needs from FUS technology. Topics included:

• Develop a FUS method that does not require a stereotactic headframe
• Develop an MR-compatible microbubble injector
• A hair-sparing technique would be useful since some patients will not want to shave their heads
• Develop efficient software; there is a sonication delay with the InSightec system of 45 seconds, however, this is not the intrinsic limitation of the hardware
• Improve the configuration of the InSightec system for optimization of BBB opening
• Automate the acoustic feedback safety feature of the InSightec system
• The ability to have transducer movement planning would also be useful

OUTCOMES AND NEXT STEPS

At the end of the discussion the consensus was that research on FUS for the treatment of GBM should move forward in two primary areas: BBB opening for drug delivery and immunomodulation. The FUS Foundation will organize working groups in these topic areas over the next year. The FUS Foundation will continue engagement with this community to move the research forward.

ABBREVIATIONS

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<th>Abbreviation</th>
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<tr>
<td>BBB</td>
<td>Blood brain barrier</td>
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<tr>
<td>BPN</td>
<td>Brain-penetrating nanoparticles</td>
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<td>FDA</td>
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<td>FUS</td>
<td>Focused ultrasound</td>
</tr>
<tr>
<td>GBM</td>
<td>Glioblastoma multiform</td>
</tr>
<tr>
<td>LITT</td>
<td>Laser interstitial thermal therapy</td>
</tr>
<tr>
<td>MRTI</td>
<td>Magnetic resonance temperature imaging</td>
</tr>
<tr>
<td>NK</td>
<td>Natural killer cell</td>
</tr>
<tr>
<td>NSCLC</td>
<td>Non-small cell lung cancer</td>
</tr>
<tr>
<td>PEG</td>
<td>Polyethylene glycol</td>
</tr>
<tr>
<td>TIL</td>
<td>Tumor-infiltrating T-lymphocytes</td>
</tr>
</tbody>
</table>
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REFERENCES


