

Clinical Protocol

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Approvals:

Site	Sponsor
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This study is to develop preliminary data for regulatory clearance of this system for blood-brain barrier disruption in the treatment of brain tumours.

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BACKGROUND AND RATIONALE

1.1 Malignant Brain Tumours and the Blood-Brain Barrier

Malignant brain tumours portend a terrible prognosis regardless of whether they are of primary or metastatic origin (1-8). Glioblastoma is the most aggressive glial tumour, accounting for more than half of gliomas and 17% of all primary brain and CNS tumours, with an annual incidence of 5 cases per 100,000 (10). Far from the most common human malignancy, glioblastoma deserves attention on the basis of its universally poor prognosis. Treatment is hindered by the diffusely infiltrating nature of the tumour and in fact, glioblastoma may be regarded as a whole brain disease (11). There is no cure and until recently treatment consisted of surgical resection followed by radiation therapy, yielding a median survival between 9 and 12 months (5-8). Between 17-34% of patients were alive at 1 year and 3-5% at two years (5-8). The introduction of the oral alkylating agent temozolomide resulted in a modest improvement; a large randomized trial by the European Organisation for Research and Treatment of Cancer Brain Tumour and Radiation Oncology Groups and the National Cancer Institute of Canada Clinical Trials Group (EORTC and NCIC) showed that the addition of temozolomide to surgical resection and radiation resulted in an increase in median survival to 14.6 months and two year survival to 27.2% (3,4).

The World Health Organization grades brain tumours, including astrocytomas, along a spectrum of malignancy. Grade I astrocytomas (e.g. pilocytic astrocytomas) are well circumscribed and relatively benign; Grade II possess histologic evidence of atypical cells (e.g. diffuse astrocytoma); Grade III show evidence of cellular proliferation and mitosis (e.g. anaplastic astrocytoma) and Grade IV lesions are histologically and clinically aggressive, demonstrating necrosis and vascular proliferation (e.g. GBM). Treatment of Grade II and higher astrocytomas is centered on safe surgical resection with the addition of post-operative adjuvant therapy in the form of radiation and chemotherapy. There now exists good evidence that upfront surgery followed by chemoradiation can effectively prolong overall survival in patients with low grade gliomas (e.g. Grade II astrocytomas or oligodendrogliomas) (NEJM 2016), and high grade gliomas (Stupp, NEJM 2005). This rationale for concomitant chemotherapy with surgery provides the rationale for the current study which aims to investigate the safety of chemotherapy delivery to tumour.

The burden of metastatic disease is not as well characterized but it is estimated that metastatic tumours outnumber primary tumours ten-fold, occurring in 20-40% of cancer patients (9). These are most commonly due to lung, breast and melanoma carcinomas (9). The median duration from presentation with a single brain metastasis to death was 5 months while only 3 months for patients with multiple lesions in a large retrospective study (1). A similarly-sized study found the two year survival to be only 8.1% (2).

One of the difficulties with the treatment of brain tumours is that most chemotherapeutic agents generally do not penetrate into the brain. Unlike elsewhere in the body, the endothelial cells within the brain are interconnected by tight junctions forming what is known as the blood-brain barrier (BBB). The BBB is the major barrier to a number of chemotherapeutic agents used in the treatment of brain tumours, as it permits only small, lipophilic molecules to pass into the interstitial fluid space of the brain to any appreciable extent, unless a specific transport system

exists for the molecule of interest (13). Given that most chemotherapy agents do not fulfill the latter requirements, but demonstrate potent *in vitro* anti-tumour activity, increasing the permeability of the BBB to such agents may permit the utilization of many existing chemotherapeutics in the treatment of brain tumours, at more tolerable systemic doses.

The idea of trying to circumvent the BBB is not new. Pre-clinical studies have demonstrated an increase in the accumulation of a number of therapeutic molecules within tumours in the brain following osmotic or biochemical opening of the BBB (14). Furthermore, local interstitial therapies have been developed, including drug-eluting wafers positioned in the resection cavity and convection-enhanced delivery of a number of targeted toxins and chemotherapeutic agents (15-17). These studies have had mixed results, and all share a significant degree of invasiveness and heterogeneous drug delivery.

1.2 MR-guided Focused Ultrasound Blood-Brain Barrier Disruption

Focused ultrasound has proven to be an attractive modality for non-invasive thermal ablation of soft tissue, but additionally a number of pre-clinical studies have demonstrated the ability to non-invasively, focally and temporarily disrupt the BBB (18-23). Ultrasound has been known to cause disruption of the BBB for more than 50 years, at acoustic powers less than those required for thermal lesions (24,25), but it was uncertain whether this could be safely achieved through the skull until more recently. A number of groups have been able to demonstrate reversible and focal increases in the permeability of the cerebral vasculature in large mammals, including non-human primates, without permanent damage to the brain (unpublished data from the Hynynen lab, 26, 27).

The current treatment algorithm for BBB disruption begins with the acquisition of MR images of the brain and CT scan of the skull. The physician then reviews the MR images on the system workstation, identifies a target volume in the MR images, and delineates the treatment contours on the images. The system then calculates any corrections that are required as a result of propagation through the skull. During the treatment, an array of ultrasound transducers generates a point of focused ultrasound pressure waves, called a sonication (see Appendix B for Glossary of Terms). With the initiation of the sonication, an intravenous ultrasound contrast agent is delivered, consisting of a solution of protein or lipid shell encapsulated perfluorocarbon gas microbubbles. As the microbubbles pass through the vasculature within the sonicated volume, the microbubbles oscillate in size with the pressure waves, causing mechanical disruption of the BBB lasting for several hours. Post-BBB opening gadolinium-enhanced MR images provide a quantitative assessment of the target area to confirm the location and size of the focal BBB opening. The sonication process is repeated at multiple adjacent points to cover the entire prescribed treatment volume.

1.3 Pre-clinical studies of BBB Disruption using Focused Ultrasound

The first observation of BBB disruption as a result of ultrasound treatment of the brain was published in the 1950's (24,25). Over the past 50+ years the technology has undergone significant refinement and, with the advent of MRI and ultrasound contrast agents, the technique is now at a stage where safe and reproducible results can be achieved. Hynynen *et al* showed in 2005 that burst ultrasound could be used to create focal regions of BBB disruption in rabbits. Side effects were minimal; at acoustic powers sufficient to cause BBB disruption there was no

evidence of necrosis, but in some cases there were small areas of erythrocyte extravasation (19). The technique seemed to promote both trans- and para-endothelial movement of molecules of interest from the blood into the brain. A study by the same group in 2007 demonstrated similar effects could be achieved with the ultrasound contrast agent DEFINITY® (20). Subsequent studies using both metastatic and glioma tumour models in rats have demonstrated that BBB disruption using focused ultrasound and ultrasound contrast agents can be used to increase the accumulation of targeted antibodies and chemotherapeutic agents in tumour cells (18,21,22,23).

The promising results seen in rodent models led to more recent studies of BBB disruption in larger mammals. Xie *et al* showed in 2008 that an unfocused ultrasound transducer could be used through the temporal bone in combination with ultrasound contrast agents to temporarily disrupt the BBB in pigs (26). The first results of successful BBB disruption in non-human primates were presented at the 19th Annual Meeting and Exhibition of the International Society for Magnetic Resonance in Medicine by Nathan McDonald's group at Harvard University (27). Subsequently, Columbia University published their experience with BBB disruption in the primate brain, and did not report any significant adverse events (28). Most recently, the Harvard group has published their final results of BBB disruption in the rhesus macaque using the same clinical ultrasound device we intend to use in the present study. They found that repeated BBB disruption in the visual cortex, performed over several weeks, did not show any obvious functional or histological injury in a primate model, once again confirming that BBB disruption can be performed reversibly and safely without detectable adverse effects (29). Unpublished data from our lab has demonstrated that in a swine model, BBB disruption can be safely accomplished using the ExAblate Transcranial (220 kHz) system in conjunction with intravenous DEFINITY® ultrasound contrast.

1.4 Chemotherapy in Brain Tumours

There are several types of chemotherapy that have been used to treat patients with malignant brain tumours. For example, doxorubicin is an anthracycline antibiotic that is used in chemotherapy of a broad range of blood and solid tumours. Its anti-tumoural activity is via inhibition of topoisomerase II, blocking DNA and RNA synthesis (30). Doxorubicin does not cross the BBB to any appreciable extent, and as a result it has previously demonstrated little effectiveness in treating CNS malignancies when administered systemically (31,32). Further dose escalation is limited by cardiac toxicity. However, doxorubicin has been shown to be toxic to glial cell tumor lines *in vitro* (33-35). More recently, it has also been shown to be effective against malignant glial tumours *in vitro* when administered locally, not just in animal models, but also in human patients via an Ommaya reservoir (30,35-39). In rodents the concentration of doxorubicin can be significantly increased in the brain following intravenous administration of a liposomal formulation and BBB disruption with focused ultrasound, suggesting that doxorubicin is a promising chemotherapeutic agent to pair with the ultrasound treatment (21). Further supportive survival data from the same group has recently been published and demonstrated a significant survival advantage in a rat glioblastoma model when using doxorubicin in combination with ultrasound-mediated BBB disruption (40).

Our initial experience in this study demonstrated that chemotherapy (doxorubicin) can be safely delivered across the BBB in a patient with high grade glioma using MRgFUS (unpublished). To align our protocol with established clinical practice, a switch to deliver temozolomide through MRgFUS is now proposed. Temozolomide is an oral alkylating agent and is indicated for human

use of grade III and IV brain tumours (eg: anaplastic astrocytoma and glioblastoma). Use of TMZ is standard of care post-operatively as part of a prescribed program of adjuvant treatment for these tumours. We are now proposing to couple MRgFUS with an oral dose of TMZ in order to enhance delivery of TMZ to the entire tumour pre-operatively.

1.5 MRgFUS Through Intact Skull: ExAblate Transcranial System

Non-invasive focused ultrasound has special appeal for use in the brain, where it is desirable to treat tumours without damage to the surrounding brain tissue and structures. Ultrasound energy has long been known to propagate through intact skull and interact with deeper targets of interest. For example, in paediatric patients transcranial ultrasound has been used to detect midline shift of the brain (41). In adults, blood velocity in the carotid arteries can be monitored through the intact temporal bone using the Doppler Effect (42). The ability of focused ultrasound to produce focal thermal lesions deep in the brain has been shown in several studies as early as the 1950's; animal studies and early clinical studies provided encouraging results, showing well-defined tissue effects at the focal zone (43-45). Fry showed that a low frequency (around 0.5 MHz) beam could be focused through the skull (47,48). However, ultrasound is strongly attenuated by bone (49), and it had been thought that therapeutic ultrasound could not be delivered through an intact skull for two reasons:

- i. The variable thickness of the skull affects the wave propagation so much that it defocuses the beam and the focal spot is lost (50).
- ii. High temperatures are generated in the bone, due to energy absorption, and could damage the scalp, bone and adjacent brain tissue.

In many previous focused ultrasound treatments of the brain, the skull has been removed in order to obviate interactions with the ultrasound beam (44,51). However, this made the procedure invasive, with added risks and increased costs. The perceived necessity of cranial vault removal has prevented MRgFUS from being widely adapted for use in the brain, despite benefits compared to other non-invasive techniques. Focused ultrasound uses non-ionizing radiation, therefore treatments may be repeated without restrictions on cumulative dose, and tissue response is immediate, with the ability to guide and control the treatment in real time with MR imaging and thermometry (52). The device used in this study, the ExAblate transcranial (see below), addresses the above concerns regarding transcranial focused ultrasound by combining a large phased array, active water-cooling, acoustic aberration correction algorithm, and CT data analysis.

Large Phased Array Transducer

The system utilizes a large phased array transducer that is composed of hundreds of transducer elements. It has been shown that large hemispherical phased arrays can deliver adequate energy through the human skull without excessive temperature elevation on the skull surface (53, 54).

Active Water-Cooling

The interface between the subject head and the transducer is filled with water, which provides the acoustic path. The system includes a cooling unit that circulates the water at a constant temperature so that the bone and scalp temperature remain within safety limits (See also Appendix C).

Acoustic Aberration Correction Algorithm

Acoustic aberration is created mostly by the variations in the bony structure of the skull. The degree of compensation necessary for each transducer element is based on predicting the aberration along the acoustic path from that element to the target and calculating the relative phase and amplitude correction necessary for that element. The result of this compensation is that the acoustic energy contribution from each element will arrive at the focal point in phase. This technique was first developed by measuring the phase distortions caused by the skull at the focus using a hydrophone. Analysis of these results showed it was possible to calculate the corrections necessary to create a focus after passing through the intact skull, thus solving the defocusing problem. Others have used imaging arrays to show that phase and amplitude compensation can be used to re-focus the beam through the skull (55). In diagnostic ultrasound imaging of the brain, phased arrays can be used to reduce or even eliminate beam distortion caused by the skull (50, 56).

CT Data Analysis

The phase/amplitude correction algorithm, based on ray acoustics methods, relies on an input that provides the bone density profile along a line between each acoustic element and the target point. This information is extracted from a three dimensional CT image of the skull (57).

Ex vivo experiments with human skulls have shown that the geometry and density information derived from CT images is sufficient to correct the distortions in the ultrasound wave caused by the skull (58), and to produce a sharp sonication focus (59).

To prove the method, an (unpublished) experiment was conducted using CT-corrected sonications in rabbit brain *in vivo* to produce focal coagulation without overheating of the skull. Consequently, the basic feasibility of performing trans-skull ultrasound treatments was demonstrated. Furthermore, since the original FDA approval of the current study protocol, preliminary clinical data using the transcranial ExAblate system is now available. This data shows the feasibility of the procedure as well as the safety and efficacy in terms of the ability to ablate targeted brain tissue.

For more technical details on the Acoustic Correction Table (ACT) Prediction Algorithm design and validation see Appendix D. More validation studies, through lab and animal experiments, are described in Appendix E.

ExAblate Transcranial System

The ExAblate Transcranial system combines a focused ultrasound surgery (FUS) delivery system and a conventional diagnostic 1.5 T or 3T MRI scanner. The ExAblate transcranial system provides a therapy planning algorithm, real-time thermal dosimetry, and closed-loop therapy control. The latter is achieved by utilizing the unique interactive MRI scan control features of the GE SIGNA MRI system.

To deliver the ultrasound treatment to the patient the ExAblate transcranial system uses a dome-shaped transducer (currently consisting of a 1000-element phased array transducer) positioned above the subject's head. During the treatment, the head is immobilized and, as previously described, the water surrounding the head is cooled.

The treatment setup of the ExAblate transcranial system is no different from the two previously approved MRgFUS studies at Sunnybrook. The treatment begins with a series of standard diagnostic MR images to identify the location and shape of tumour to be treated. Sonications are planned to encompass the tumour volume with a surrounding margin. The therapeutic molecule of interest is then administered intravenously in the patient so that it is in the circulation for the ultrasound treatments. The ExAblate Transcranial system (220 kHz) is used to deliver a very low power sonication, at approximately 1% of the power used to create a thermal lesion, at the same time as a bolus injection of 20 µl/kg of Definity® ultrasound contrast is administered. The sonication consists of 100 ms bursts of ultrasound energy repeated once every 10 seconds, for a total treatment duration of 2 minutes. Following the sonication, T2-weighted and gadolinium contrast-enhanced T1-weighted sequences are performed to assess the degree of BBB opening. Real-time MR thermometry confirms during the treatment that there is no heating of tissues within the brain.

1.6 Clinical Experience with ExAblate

1.6.1 ExAblate Body System for the treatment of Uterine Fibroids

The ExAblate 2000 system received FDA approval for the treatment of Uterine Fibroids in October 2004 (PMA # P040003). Furthermore, this system gained both AMAR authorization (Israel Ministry of Health) and CE (European and others) approval for the indication of treating Uterine Fibroids. Subsequent studies lead to a software upgrade and an enhanced sonication protocol. A further upgrade to the system to allow the transducer arm 3-dimensional movement is currently under IDE investigation as IDE G100127

1.6.2 ExAblate New Software Validation (IDE #G050221)

This was an FDA-approved study to validate the new ExAblate application software as well as the use of the ExAblate system with 3T MR scanners for the treatment of UFs. This was only a safety study. A total of 40 subjects were treated under this protocol IDE. The PMA-S was approved on February 27, 2007 under P040003/S002.

1.6.3 Enhanced Sonication Protocol (IDE #G060017)

This was an FDA-approved study to validate the new Enhanced Sonication feature of the ExAblate system, a detachable cradle, and several other modifications to the ExAblate 2000 system. This was a safety study only. A total of 50 subjects were treated under this protocol IDE. Following completion of this study, a full PMA supplement was submitted to FDA for review and approval [PMA# P040003]. Approval was granted on 12/22/2009 under PMA Supp P040003/S006. The system is marketed under the trade name ExAblate 2000/2100 and is indicated for use in treating symptomatic uterine fibroids.

1.6.4 Enhanced Sonication Post Marketing Study-P040003/S007

InSightec is currently recruiting subjects for a post-market study using the FDA approved enhanced sonication feature to demonstrate the safety of the enhanced sonication feature within current treatment guidelines of 100% individual fibroid ablation within established serosal and sacral treatment margins; this study has completed its enrollment. Data analyses and final report are in progress, and due for submission to the FDA in March-2013.

1.6.5 Validation of ExAblate UF V2 – IDE G100127.

InSightec is currently recruiting centers and initiating IRB review for study conduct in order to gain approval for the ExAblate Model 2100 Type 1.1 (also refer to as ExAblate UF V2). This ExAblate system will be operated with a NEW Clinical Application SW utilizing the added 5th degree of freedom of the transducer (A/P movement) in its overall planning and treatment of the uterine fibroids. This study will enroll 106 subjects under IDE # G 100127.

1.6.6 ExAblate Body System for the Treatment of Breast Cancer

InSightec conducted FDA approved clinical trials under IDE # G990184 and G990201 to evaluate the safety and efficacy of the ExAblate system in the treatment of breast carcinomas [81-83]. Both of these studies are now closed. Currently, InSightec has an FDA conditional approval for a new breast cancer phase-2 study (IDE # G060023).

1.6.7 ExAblate Ablation of Breast Fibroadenoma

InSightec conducted a feasibility FDA approved clinical trials under IDE # G930140 to evaluate the safety and efficacy of the ExAblate system in the ablation of breast fibroadenoma [2]. Under this study, a total of 11 subjects were treated. The results of this study showed that 8 of the 11 subjects who had ablations were either partially (>50%) or completely (>90%) successful. No adverse effects were reported, except for one case of transient edema in the pectoralis muscle two days after therapy.

Following this feasibility study, InSightec initiated an FDA approved pivotal protocol to study ExAblate ablation of Breast Fibroadenoma (IDE # G010225). A total of 110 subjects were approved for this trial, and only 27 subjects were treated before the study was closed for enrollment due to lack of subjects enrollment. No unanticipated adverse effects have been reported or detected by MRI. Clinically, acute pain and discomfort were tolerable, and no long-term complications occurred.

1.6.8 ExAblate Body System for the Treatment of Prostate Studies – Investigational Feasibility Studies

Prostate cancer is the second leading cause of cancer death in men in the United States. Most prostate cancers grow slowly with a high survival rate past 10 years if it is still confined to the prostate. The current treatment methods (surgical, external beam radiation therapy and male hormone suppression therapy) have significant side effects, such as impotence, incontinence, post-radiation colitis, etc. Because prostate cancer is usually slow growing, active surveillance at 6 month intervals using a prostate cancer marker (PSA – prostate-specific antigen) has been a primary treatment option. A focal treatment that could destroy the cancerous cells without harming the neurovascular bundle could provide a more palatable treatment with fewer side effects and extend life expectancy from prostate cancer causes.

Feasibility studies have been performed outside the United States to demonstrate the ability of the ExAblate to successfully target the prostate gland and ablate it (Total Gland Ablation – TGA). Additionally studies are now underway using focal therapy to ablate only cancerous foci within the prostate and leave the remainder of the gland intact. The difficulty here is in the methods available to identify the cancerous foci. These cancerous foci are generally not visible on MRI or CT, so careful, methodical biopsy mapping with multiple cores (minimum of 12 cores, commonly 16 cores for larger glands) must be performed in order to identify the portion of the gland with the cancer. In pilot studies with 14 subjects treated to date, the outcomes

generally have demonstrated a minimal degree of sexual and urinary side effects (except for transient obstructive urinary symptoms) unless the cancerous foci involve the neurovascular bundles and the conscious decision is made to include them in the treatment region-of-interest. Subjects that are eligible for participation are those with slow cancer growth being followed with active surveillance or with Gleason score of 6 (3+3) and no more than 2 cancerous foci in two or fewer adjacent sectors that would be amenable to ExAblate treatment. To date, feasibility studies are underway in Russia, India, Singapore, Italy, and will soon start in Canada. In the United States under FDA oversight, InSightec has received the full FDA approval under IDE # G100108) to initiate a feasibility study in the treatment of prostate cancer.

1.6.9 ExAblate Body System for the Palliative treatment of Metastatic Bone Tumors

First, InSightec performed FDA approved study for a feasibility study of ExAblate ablation of metastatic bone tumors under IDE # G050177. A total of 10 subjects were enrolled and treated at two (2) study sites. This study is now completed, and a final report was submitted to the FDA [84]. Most recently a PMA submission (PMA # P110039) with the pivotal study data was submitted to FDA on 5, DEC-2011 and approved as of October 2012.

1.6.9.1 Bone Feasibility Study IDE# G050177

The objective of this trial was to evaluate the safety and effectiveness of using ExAblate as a treatment for pain palliation in subjects with metastatic bone tumors. This study was designed as a prospective, one arm, non-randomized study. Ten subjects were enrolled at two sites. Nine subjects completed the study; one subject could not complete treatment due to limited device accessibility to the lesion.

Only 3 mild AEs were reported in the study with no device-related deaths, life-threatening injuries or permanent injuries, nor serious adverse events. There were 2 events of mild sonication-induced pain; both resolved the day that sonications ended. In addition, there was 1 event of a mild shivering reaction to conscious sedation lasting only a few minutes during the procedure with subsequent resolution. All of these events were anticipated side effects that were identified in the study protocol as possible treatment-related complications.

As noted above, effectiveness was measured by the level of pain relief (as measured by VAS), decrease in analgesics/opiate medication usage, and improved quality of life (as measured by SF-36). Prior to ExAblate treatment, the mean pain score was 5.6 ± 1.2 (N=10, score range: 4-7). A very rapid and sustained relief response was observed in subjects' pain relief. At 3 months, the mean score had dropped to 0.4 ± 0.6 (a 93% decrease from baseline). With respect to medication usage, all subjects maintained or decreased their medication usage.

Overall, these results demonstrate the safety and effectiveness of using ExAblate as a treatment for pain palliation in subjects with metastatic bone tumors.

1.6.9.2 Pivotal Bone Metastasis Study (IDE# G070022) – Brief Overview

InSightec received full approval for a phase-3 Pivotal study for the ExAblate treatment of bone metastases palliation (IDE # G070022). A total of 148 subjects were to be enrolled and treated at up to 20 sites. The pivotal clinical trial was a prospective, randomized (3:1), single-blind, sham-controlled, multicenter, two-arm study with sham-cross-over option. Consistent with prior correspondence between the company and FDA, InSightec has conducted an interim analysis of study data, providing for a statistical penalty addressing the early look at the data, in order to initiate PMA approval for this indication. This interim analysis has been performed under the

interim statistical analysis plan previously submitted to and approved by FDA (G070022/S54). The study objective is to evaluate the safety and effectiveness of an ExAblate thermal ablation treatment as compared to a sham treatment (where no energy is delivered) to reduce/relieve the pain of metastatic or multiple Myeloma bone tumors in subjects who are not suitable candidates for radiation therapy. FDA and Health Canada approval under PMA P110039 and License Number 91830, respectively.

1.7 ExAblate Transcranial Treatment of Brain

1.7.1 Feasibility Study for Brain Tumor IDE # G020182 – ExAblate Transcranial Low Frequency System

In 2002, the FDA approved an IDE for a feasibility clinical study for the ExAblate Transcranial system in the treatment of brain tumors[48]. The purpose of this study is to evaluate the safety of MRI-guided focused ultrasound thermal ablation of brain tumors performed through intact human skull using the ExAblate system. Specifically, the objectives of this non-randomized study are:

- a) To evaluate the safety of FUS delivered through intact human skull to the brain, during the treatment, and during the follow-up period of 3 months.
- b) To evaluate the effect of thermal ablation in the target tumor with contrast MR imaging to identify viable tumor, and non-viable thermally ablated tissue

This study was limited to subjects with a newly diagnosed glioma, recurrent glioma, or metastatic cancer to the brain for whom surgery was felt to be not indicated by a physician not associated with the study.

Per FDA order under IDE # G020182/S02, this study was approved for a total of 10 subjects. Also, per same FDA order, a report was requested after enrollment and treatment of the first 3 subjects in order to gain the FDA approval for continuation.

- During these 3 treatments, all safety subsystems and monitoring of the device provided the intended safety monitoring capabilities. The adverse events that were captured were Non-Significant, Anticipated, Treatment Side Effects and incidental to the treatment.

The treatment of the 4th subject was done with an upgraded system (approved under IDE G#020182/S04). The treatment day safety was no different than those previously reported. The skull/dura temperature change was in the range of previous treatments. Utilizing the burst sonication regime, the designated tumor was completely ablated. This was consistent with our overall plan to achieve the efficacy needed.

Despite an apparently uneventful treatment, this tumor subject died of an intracerebral hemorrhage five days after ExAblate. The Study Safety Committee determined the cause of the hemorrhage to be unknown but possibly multi-factorial. It was related to the propensity of glioblastomas to bleed, exacerbated by radiotherapy, medications and an underlying coagulopathy. The latter was suggested by the fact that this particular subject had a hemorrhage at the biopsy site long before ExAblate, skin bruising, and a peri-orbital hematoma that worsened dramatically at the time of his demise.

The neuropathologic findings raised the possibility that pre-existing changes in the vessels, such as mineralization and wall thickening, may have rendered those vessels more susceptible to damage by ultrasound at the doses or frequencies used. The Study Safety Committee

recommended protocol changes in the exclusion criteria (tumors with a known tendency to bleed, subjects with abnormal clotting studies or on drugs known to affect coagulation) and in clarification of the imaging criteria (target volume maximum size requirement < 2.5 cm diameter, or an 8 cc volume - the tumor volume may be larger, as long as true midline shift is < 5 mm and the subject is not clinically compromised; definition of midline shift > 5 mm – does not include tumor growth across midline). With these provisos, the Safety Committee recommended continuation of the study. The FDA approved the recommendation of the Safety Committee under IDE # G020182/S15. The full FDA approval to restart this study was covered under IDE#G020182/S19.

1.7.2 ExAblate Transcranial MRgFUS System and Thalamotomy Procedures

The InSightec ExAblate TcMRgFUS system has received CE Mark in Europe for thalamotomy treatment of neurological disorder such as neuropathic pain, essential tremor (“ET”), and Parkinson’s disease (“PD”) induced tremor. These studies were conducted under FDA IDE approvals (ET under IDE # G100169; enrolling for PD under IDE G # 120017), Health Canada (ET under Application # 166556), Swissmedic (NP001 study included Neuropathic pain, ET and tremor in PD subjects – Reference: 2011-MD-0001), and Korean FDA (ET under KFDA Application # 260) oversights. Overall, more than fifty (50) subjects received the ExAblate TcMRgFUS thalamotomy procedure.

1.7.3 Study for Neuropathic Pain Outside the United States

The original study was an investigator initiated and sponsored study (Study number BZ001) in the treatment of neuropathic pain that was conducted at the University Hospital Zurich (Zurich Switzerland) using the InSightec ExAblate Transcranial (650 KHz) system. The study was approved by and performed according to the guidelines of the ethics committee of the University and the State of Zurich. At the conclusion of this study, an InSightec sponsored study was conducted under Swissmedic (Reference #: 2011-MD-0001) and local IRB approvals. This study (NP001) included subjects with Neuropathic pain, Essential tremor, and those subjects with Parkinson’s disease induced tremor.

To date, more than thirty-five (35) subjects underwent selective central lateral thalamotomy (CLT) using the ExAblate Transcranial treatment. For all subjects, the treatment was well tolerated and did not result in any side effects or neurological deficits. The safety profile of these procedures was no different from those known to occur in any thalamotomy procedure such as DBS or RF ablation. To be specific, all the reported adverse events observed in these studies were all non-significant and anticipated events already identified as potential events in the study protocol. These were headaches, nausea, and vomiting. All resolved on the same day as treatment. The only significant event reported to date from these two (2) studies an event of neurological deficit (Subject BZ001-12), i.e. “dysmetria (dyscoordination) of the right hand, dysarthria, motor neglect and gait disorder”. This event, originally reported as part of IDE # G100169, was reported immediately following the last sonication. All symptoms improved significantly 1-hour post treatment. By 24 hours, 70-80% of the motor symptoms had abated and with time all dysmetric symptoms cleared except when the subject tried to write or speak (see Jeanmonod *et al* paper).

1.7.4 Study for Tremor in Parkinson Disease subjects - Outside the United States

As stated above, the Swissmedic NP001 included cohort of six subjects with Parkinson's disease induced tremor. Following the ExAblate treatment, the tremor suppression ranged from 20 to 50% at the 3-Months time point in 5 out of the 6 treated subjects.

1.7.5 Study for Essential Tremor - Outside the United States

In addition to the Swissmedic study that included cohort of 1 subject (study is still on-going) with Essential tremor, InSightec conducted separate studies for the treatment of Essential tremor in both Canada and in Korea. A total of 8 subjects were treated to date as follows: 3 subjects in Canada, and 5 subjects in Korea. Both of these study protocol were similar to the FDA feasibility study (IDE G#100169). Overall, the ExAblate treatment safety profile is no different from the FDA feasibility study (for more details, see Section-1.7.6) nor from the safety profile of typical thalamotomy device procedures such as RF ablation and DBS. In short, safety events such as Dizziness and vomiting were reported, and both resolved without sequelae.

1.7.6 ExAblate Transcranial System Feasibility Study for Essential Tremor IDE - G100169

InSightec received FDA approval for a feasibility study of ExAblate Transcranial System for unilateral thalamotomy in the treatment of Essential Tremor under IDE # G100169. Fifteen subjects were enrolled and treated at one site and the study report was recently completed. Both the safety and effectiveness profiles showed a very favorable outcome of the ExAblate Transcranial MRgFUS treatment of patients with medication-refractory movement disorders, namely ET. The clinical outcomes subscales as well as the quality of life measures showed a substantial improvement in subject disabilities and their quality of life.

The incidence (number of patients experiencing an event) and severity of all device and procedure related AEs are summarized in Table 1. This list of events includes the non-significant and the significant anticipated treatment side effects as well as the events incidental to the treatment.

Table 1 Overall Adverse Events and Severity: ET-001					
	Event	Mild	Moderate	Severe	Total # of Events
Non-Significant Anticipated Events	Ache – pin site	1	0	0	1
	Balance	7	0	0	7
	Chest Pain	1	0	0	1
	Neural Deficit - speech	2	0	0	2
	Neural Deficit – hand strength	1	0	0	1
	Emesis	2	0	0	2
	Fatigue	3	0	0	3
	Headache 1 Day	4	5	0	9
	Headache > 1 Day	1	2	0	3

Table 1 Overall Adverse Events and Severity: ET-001					
	Event	Mild	Moderate	Severe	Total # of Events
	Numbness -- appendage	1	0	0	1
	Nausea	2	4	0	6
	Paresthesia - appendage	3	0	0	3
	Paresthesia - face	4	1	0	5
	light headedness	7	1	0	8
	Twitch	1	0	0	1
	Warm Sensation	4	0	0	4
	Edema	1	0	0	1
	Ataxia		1	0	1
	Non-Significant Subtotal	45	14	0	59
Significant Treatment Anticipated Events	Balance	2	2	0	4
	Fatigue	1	0	0	1
	Infection – pin site	1	0	0	1
	Numbness – pin site	1	0	0	1
	Parasthesia - appendage	1	1	0	2
	Parasthesia - face	4	1	0	5
	Parasthesia - scalp	4	0	0	4
	Hypesthesia - scalp	1	0	0	1
	Burn – pin site	1	1	0	2
	Significant Subtotal	16	5	0	21
Incidental Events	Sinusitus	0	1	0	1
	Bronchitus	1	0	0	1
	Incidental Subtotal	1	1	0	2
	Total	62	20	0	82

The most significant results shown in Table 1 are that all these events are indeed anticipated events and were already identified as potential events in the study protocol. Furthermore, NONE of the adverse events reported to date were severe in nature. In fact, 76 % (62/82) were Mild events, and the remainder 24% (20/82) were Moderate. Most of these events resolved either the

same day (49% of the total events) or within 2 weeks of treatment (26% of the total events). Hence, 61 events (i.e. 74%) resolved within 2 weeks of the procedure.

Of all the events that did not resolve within the first 2 weeks post procedure, 14 out of 82 events (17%) resolved in more than 2 weeks (not counting the incidental sinusitis (n=1)), and 7 events (9%) were reported as ongoing.

1.7.7 Overall summary of ExAblate TcMRgFUS safety profile

The safety events reported in all these studies present a favorable safety profile for brain surgery using the ExAblate TcMRgFUS for ablative therapy. The majority of all events are mild and transient. They generally fall into the pattern of other techniques indicating that these are generally the result of stereotactic device use (e.g., headache) and thalamotomy in general, regardless of the mechanism of therapy, due to brain tissue insult or due to heating of tissue locally surrounding the lesion target. The overall effectiveness profile was also shown to be very favorable.

2.0 OBJECTIVES

The purpose of this study is to evaluate the safety of BBB disruption using transcranial MRI-guided focused ultrasound in conjunction with an intravenous ultrasound contrast agent to increase the accumulation of temozolomide in brain tumours and the adjacent brain using the ExAblate Transcranial system (220 kHz). Data will be collected to establish the basic safety of this type of treatment as the basis for later studies to evaluate its clinical efficacy.

2.1 Study Hypotheses

- i. Focused ultrasound under MRI-guidance can be safely delivered through an intact human skull in conjunction with timed intravenous ultrasound contrast agents to temporarily and focally disrupt the BBB, with a low risk of transient adverse effects, as evaluated during follow-up of up to 3 months.
- ii. A pre-defined target volume including the tumour rim and a surrounding margin can be made more permeable to intravenously injected MRI contrast agents and temozolomide, as shown on immediate post-treatment contrast-enhanced MRI and post-operative tissue-analysis, respectively.

2.2 Specific Aims

2.2.1 Safety

Safety will be evaluated by patient examination and MRI during the treatment, and by follow-up MRI and clinical visits. These are detailed in section 4.8. Data on the safety versus adverse effects of this treatment will be acquired during each visit through a combination of MRI evaluations, clinical assessments and neurological examinations.

2.2.2 Feasibility

The extent of BBB disruption will be determined by the degree of contrast enhancement seen on post-sonication contrast-enhanced MR images. Patients will undergo surgery within 24 hours of the ultrasound procedure and, following adequate specimen collection for pathology, the sonicated volume will be resected and collected for analysis.

3.0 STUDY DESIGN

This study is a prospective, single-arm, non-randomized, feasibility study to evaluate the safety of focal BBB disruption using the ExAblate Transcranial (220 kHz) system and Definity® ultrasound contrast in patients with malignant brain tumours. Patients will be approached after their selection by a team of neurosurgeons and oncologists who specialize in the management of brain tumours. The study will be described to them, and once informed consent is obtained, they will be enrolled in the trial. Key aspects of study design and methodology are described below.

4.0 METHODOLOGY

4.1 Patient Selection

Up to ten (10) subjects who meet the inclusion/exclusion criteria will be recruited at Sunnybrook Health Science Centre to undergo treatment.

4.2 Patient Enrolment

The attending physicians from either the neurosurgical service or the radiation oncology service will enrol the subjects. Subjects who appear eligible (case history, laboratory charts, latest MRI scan of brain tumours(s), physical and neurological status) will be asked if they are willing to participate in this study. Participation will be fully voluntary and rejection will not affect the availability of other treatment modalities that may be appropriate.

All patients' enrolment eligibility will be reviewed for confirmation in a multidisciplinary case conference involving neurosurgeons, radiation and medical oncologists based on the inclusion and exclusion criteria listed below.

The subjects willing to participate will complete a Health Care Proxy designating someone to make medical decisions, if necessary, and sign a consent form: (1) to approve gadolinium-enhanced MRI and non-enhanced high-density CT of the head for the final inclusion (see inclusion/exclusion criteria), (2) to approve the administration of Definity® ultrasound contrast, (3) to approve a single dose of temozolomide, and (4) to express willingness to undergo MRgFUS treatment once they meet all inclusion/exclusion criteria. The study staff will maintain a screening log of all subjects consented to be included in the study. In case inclusion is due to patient refusal to go through surgery, a patient advocate will be present in the consenting procedure to guarantee patients' rights are protected.

4.3 Inclusion Criteria

1. Men or women.
2. Age between 18 and 80 years, inclusive.
3. Able and willing to give informed consent.

4. Malignant brain tumour confirmed by biopsy or suspected based on imaging
5. Tumour is clearly defined on pre-therapy contrast enhanced MRI scans.
6. Size of the targeted portion of the tumour (i.e. prescribed ROT) is less than 2.5 cm in diameter (16 cm³). The non-targeted tumour tissue may exceed the targeted volume.
7. Karnofsky rating 70-100 (See Appendix A).
8. ASA score 1-3.
9. Able to communicate sensations during the ExAblate MRgFUS procedure.
10. Able to attend all study visits (i.e., life expectancy of at least 3 months).
11. At least 14 days passed since last brain surgery.
12. At least 6 months from last chemotherapy or brain radiation.

4.4 Exclusion Criteria

1. Tumour not visible on the pre-therapy imaging
2. Tumour presenting with the following imaging characteristics:
 - i. Following steroid treatment, brain edema and/or mass effect that causes midline shift or shift in wall of the third (3rd) ventricle of more than 10 mm
 - ii. Evidence of recent (less than 2 weeks) intracranial hemorrhage
 - iii. Containing calcifications in the focused ultrasound sonication beam path in the event system tools cannot tailor the treatment around these calcification spots
3. The sonication pathway to the tumour involves:
 - i. More than 30% of the skull area traversed by the sonication pathway is covered by scars, scalp disorders (e.g., eczema), or atrophy of the scalp
 - ii. Clips or other metallic implanted objects in the skull or the brain, except shunts
4. The subject presents with symptoms and signs of increased intracranial pressure (e.g., headache, nausea, vomiting, lethargy, and papilledema)
5. Cardiac disease or unstable hemodynamics including:
 - i. Documented myocardial infarction within six months of enrolment
 - ii. Unstable angina on medication
 - iii. Congestive heart failure
 - iv. Left ventricular ejection fraction below the lower limit of normal
 - v. History of a hemodynamically unstable cardiac arrhythmia
 - vi. Cardiac pacemaker
6. Severe hypertension (diastolic BP > 100 on medication)
7. Anti-coagulant therapy, or medications known to increase risk of hemorrhage, (e.g.:ASA, non-steroidal anti-inflammatory drugs (NSAIDs), statins)
8. History of a bleeding disorder, coagulopathy or with a history of spontaneous tumour hemorrhage
9. Abnormal level of platelets (< 100000), PT (>14) or PTT (>36), and INR > 1.3
10. Documented cerebral infarction within the past 12 months
11. TIA in the last 1 month
12. Cerebral or systemic vasculopathy
13. Immunosuppression (corticosteroids to prevent/treat brain edema are permitted)
14. Known sensitivity to gadolinium-DTPA
15. Contraindications to MRI such as non-MRI-compatible implanted devices
16. Large subjects not fitting comfortably into the MRI scanner (generally >250 lbs)
17. Difficulty lying supine and still for up to 4 hours in the MRI unit or claustrophobia
18. Untreated, uncontrolled sleep apnea

19. Positive pregnancy test (for pre-menopausal women)
20. Known life-threatening systemic disease
21. Severely impaired renal function with estimated glomerular filtration rate <30 mL/min/1.73m² and/or on dialysis
22. Right to left or bi-directional cardiac shunt
23. Allergy to eggs or egg products
24. Subjects with chronic pulmonary disorders.
25. Subjects with a history of drug allergies, asthma or hay fever, and multiple allergies, in particular subjects with a history of anaphylaxis
26. Subjects with a family or personal history of QT prolongation or taking concomitant medications known to cause QTc prolongation, or QT prolongation observed on screening ECG (QTc > 450 for men and >470 for women).
27. Subjects with evidence of Hepatitis B virus infection/carrier state.
28. Liver injury as indicated by liver function tests (AST >37 U/L;ALT >31 U/L)

4.5 Pre-Treatment Procedures

Subjects eligible according to the pre-screening data will be asked to sign the consent form. They will undergo a detailed examination (see Subject Examination Form) to review the inclusion/exclusion criteria:

1. Medical history;
2. Physical and neurological examination;
3. Examination by anesthesiologist;
4. Examination of the scalp;
5. Laboratory tests will include:
 - a. complete blood count (hematocrit, white cell count, platelet count);
 - b. blood type;
 - c. pro-thrombin time (PT); (coagulation profile)
 - d. partial thromboplastin time (PTT, aPTT);
 - e. serum chemistry 7 panel (Na, K, Cl, HCO₃, BUN, creatinine, glucose);
 - f. Liver function tests (albumin, bilirubin (direct and indirect), AST, ALP and ALT)
 - g. Screen for Hepatitis B Virus infection
6. Chest x-ray;
7. Electrocardiography (ECG);
8. Pregnancy test (for pre-menopausal women)
9. Baseline imaging required for this study:
 - i. CT Imaging: A high-resolution non-contrast CT scan will be performed for density skull definition and identification of intracranial calcification. The CT data is used by the system to adjust sonication parameters and compute phase correction values for achieving focus through the skull.
 - ii. MR Imaging: following the MRA imaging, the subject will remain in the supine headfirst treatment position. The images will be evaluated to identify the location and size of the brain tumour.

Note: The full MR and CT exams list requirements are listed in Appendix F.

In the pre-therapy treatment planning, the contrast-enhanced MR and CT images will be evaluated to verify that at least one of the tumours of the subject can be treated with the MRgFUS system. In addition, various parameters will be computed in preparation for the treatment (CT segmentation, registration markers specification, transducer location and orientation setup etc.) The MRgFUS treatment will be scheduled only if the subject successfully completes the aforementioned evaluations (1,2,3,4,).

The subject's head will be carefully shaved and examined for pre-existing scalp scars or any other scalp lesions. The subject's head will be placed in the immobilization unit (similar to frames used for stereotactic radiotherapy) for use during the actual MRgFUS treatment. The MRgFUS treatment will take place at least one day after the consent has been given. Corticosteroid therapy (e.g., Dexamethasone 4 mg PO q.i.d.) will be prescribed as medically necessary. The subject will be instructed to take nothing by mouth after midnight prior to the procedure in order to avoid the possible complications of emergent induction and intubation, should the need arise, for example in the case of intracranial haemorrhage.

4.6 Treatment Procedures

On the day of the treatment, at least the following clinical team members will be present:

Neurosurgeon and radiation oncologist trained in radiosurgery: they lead the planning and guidance of the treatment. They will also monitor the neurological performance and clinical status of the patient. They will be responsible of the overall management of patient. If patient is not able to respond to evaluation during the treatment, the procedure will stop, and the level of anesthesia monitored/modified until contact with the patient can be re-established and the neurological status can be evaluated.

Medical Oncologist or Oncology Nurse: monitor chemotherapy delivery and any side-effects for up to one hour.

Physicist: they are responsible for the mechanical running of the machine and for troubleshooting in the event of malfunction. They are also well-versed in the software and hardware components of the ExAblate system.

The overall treatment procedure will include the following steps:

1. The patient will be admitted through Same Day Surgery.
2. An intravenous line will be inserted for the delivery of fluids, any medications needed during the procedure, as well as the DEFINITY®.
3. Non-invasive monitoring of heart rate, blood pressure, systemic oxygen saturation, electrocardiogram, will be maintained throughout the procedure using standard MR-compatible monitoring devices. The neurosurgeon will administer appropriate medications for patient management if necessary. An MRI compatible ventilator will be available in the event of an emergency. The patient will be able to communicate during the entire course of the procedure.
4. A temozolomide capsule (150 mg/m² body surface area) will be orally taken by the patient under supervision of an oncology nurse. This will be administered 30-60 minutes prior to the ultrasound to allow assessment and treatment of any side-effects resulting

- from the Definity drug infusion. Prophylactic IV anti-emetics will be administered with the initiation of chemotherapy.
5. The patient's head will be carefully shaved and chemically depilated. Any scars or scalp lesions will be documented.
 6. TED stockings will be worn to prevent deep venous thrombosis in the lower limbs.
 7. The patient's head will be placed in the immobilization unit (similar to those used in stereotactic radiotherapy head fixation) for use during the actual ultrasound treatment.
 8. The subject will be positioned supine and headfirst on the MR/ExAblate Transcranial MRgFUS therapy table.
 9. The hemi-spherical dome containing the elements of the MRgFUS transducer will be positioned over the convexity of the subject's head in the treatment position. This will be done according to measurements taken from the pre-operative/imaging session(s).
 10. A rubber diaphragm will be attached to the patient's head and to the transducer to create the acoustic coupling system between the ultrasound transducer and the scalp.
 11. The immobilization system will be secured over the subject head to maintain a constant relationship between the target and the transducer.
 12. A baseline neurological assessment will be done before the treatment begins.
 13. A localizer scan (quick T1) and a non-contrast T2-FSE MR scan will be obtained to allow further refinement of the position the MRgFUS transducer focal point with respect to the targeted zone.
 14. The interface within the rubber diaphragm will then be filled with degassed water. This volume will be completely filled, taking care to avoid air bubbles between the face of the transducer and the scalp. Through active circulation and cooling, the water will be maintained at 18°C throughout the procedure to avoid undesired heating of the scalp and skull.
 15. A series of MR images will be acquired to identify the target area, and plan the actual treatment
 - i. T2 Weighted imaging exams along at least 2 axes (Axial and Coronal). Other MR imaging series may also be acquired
 - ii. Axial DTI imaging exam (mean diffusivity/ADC)
 16. The pre-therapy contrast enhanced MRI and CT image datasets will be registered to the T2 weighted MR images that were just acquired. This image fusion of pre-operative MR assists in the accurate delineation of the target area and determination of a safe sonication pathway
 - i. The fusion of the CT data is required for the computation of phase correction values to correct for skull aberration, and identification of intracranial calcifications
 - ii. Scars of the scalp will be designated to ensure the ultrasound beam avoids these specific areas
 17. The treatment volume and plan will be defined by the neurosurgeon, radiation oncologist and neuroradiologist. The ExAblate MRgFUS system will automatically calculate the phase and amplitude corrections necessary for the system to produce a focal spot at the desired location.
 18. A central point in the targeted volume will be sonicated at low power to confirm the targeting accuracy on the MR thermometry images. Focal point position and/or transducer location will be adjusted as necessary.
 19. Definity will be activated and prepared as per the manufacturer's directions for administration to the patient

20. A 2 minute -3 W burst sonication will be initiated at the same time as a 4-20 μ l/kg bolus injection of Definity® ultrasound contrast. All sonications will be performed using the 230 kHz system. The ultrasound intensity will be determined by an iterative process described below.
21. Immediately following a sonication, a gadolinium-enhanced T1-weighted MR sequence will be performed to evaluate the degree of BBB disruption. If less than 20 % enhancement is seen, the acoustic power will be increased incrementally by 1 W and then step 19 repeated. This cycle is repeated until the 2min-10W sonication is reached. If no 20% enhancement and no sign of “inertial cavitation”, then power is increased in steps of 1W until inertial cavitation is detected.
22. In the event of discomfort or pain, the patient has the ability to abort the sonication at any time by activating the ‘Stop Sonication’ Button. Once this button is activated, the system will instantly stop the energy delivery. After addressing the patient concerns or discomfort, the procedure may continue without further delay.
23. The Neurosurgeon will also have access to a ‘Stop Sonication’ button in the event they need to deactivate for whatever reason(s).
24. All adverse events that may be cause of these potential activation(s) of the Stop Sonication Button will be captured on the CRFs.
25. The physician has the ability to terminate the sonication procedure at any time, and may include instances where:
 - i. The patient wishes to stop due to pain, severe discomfort, or any other reason,
 - ii. Development of focal neurological deficits,
 - iii. Occurrence of seizures,
 - iv. MRI signs of intracranial bleeding or significant brain swelling
 - v. Targeting difficulties, due to subject motion,
 - vi. Inability to observe the focal point during sonication,
 - vii. Inability to communicate with the patient
 - viii. Any other medically indicated reason.
26. After completion of the ExAblate MRgFUS treatment, a series of MR images will be acquired to assess the treatment effects:
 - i. T1-weighted imaging exam with and without contrast along at least 2 axes: Axial and Coronal
 - ii. Axial DTI imaging exam (mean diffusivity/ADC)
 - iii. Other MR imaging series may also be acquired.

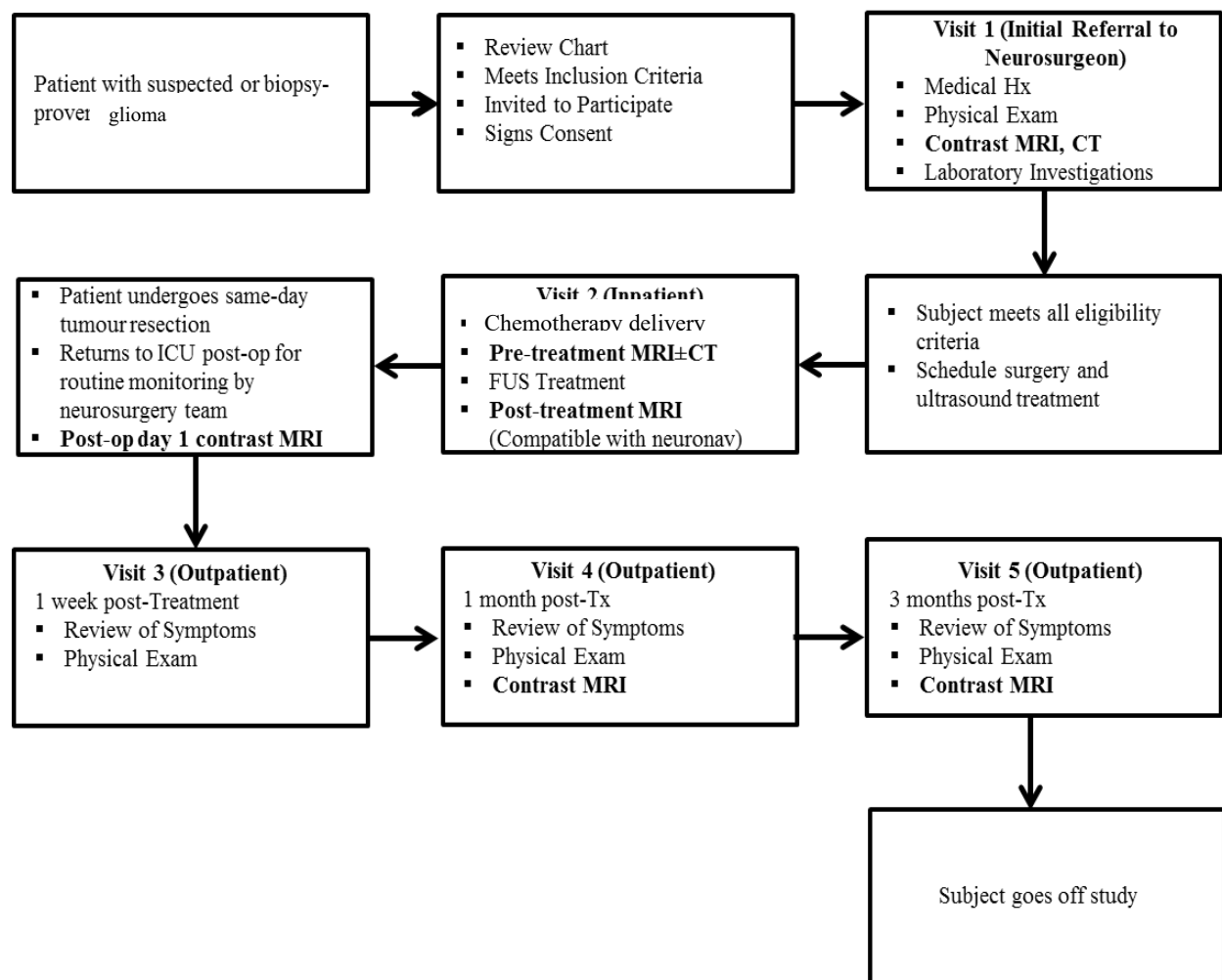
In the event of new neurological deficits or seizures, other imaging modalities (including CT) will be performed immediately in addition to neurological and physical examination.
27. The subject will be removed from the ExAblate table and monitored in S-wing for any signs of adverse events until such time as the care team is content to transport the patient to the operating room for tumour resection. Patients will undergo their pre-planned tumour resection the same day as the ultrasound treatment, but at most 24 hours after.
28. The location of the BBB disruption, as identified on the post-treatment MRI by the presence of increased contrast extravasation, will be registered on the intra-operative neuronavigation system to facilitate localization and resection/sampling of the treated tissue volume.
29. Study personnel will be available on the day of the surgery to collect the specimen. The specimen will be frozen in liquid nitrogen vapour, assigned a numeric identifier linked to the patient’s MRN through an encrypted master list, and stored in a secure -80°C freezer until chemotherapy quantification. A preparation of acidified alcohol (0.3 N HCl in 50%

EtOH) will be used to extract chemotherapy from homogenized harvested tissue samples for quantification. Samples will be destroyed following study completion.

30. The patient will then undergo the standard post-operative monitoring as per the neurosurgical team.

31. A contrast-enhanced MRI will be performed on post-operative day 1 and assessment of the patient's condition will be performed by study personnel.

Figure 4.1: Study Outline



4.7 Subject Discharge

The subject may be discharged when deemed appropriate by the neurosurgical team, generally 4-5 days postoperatively. This will require that the subject's neurological and physical status are stable, that there are no clinical symptoms of increased brain edema, and post-operative imaging

does not suggest increased brain swelling, hemorrhage, or any other complication. The observation period may be prolonged by the neurosurgeon for these or other medically indicated reasons (for full list of imaging requirements see Appendix F)

Corticosteroid treatment will be used as deemed necessary by treating neurosurgeon. Thereafter, the medication will need to be tapered as stipulated by clinical and medical requirements.

4.8 Follow-up Visits

The follow-up visits are scheduled at 1 week, 1 month, and 3 months after the treatment. During each visit, the subject is evaluated for safety by physical and neurological examination, and MR imaging with contrast (except at Week 1).

Figure 4.2: Follow-up allowance

<u>Follow- Up Visit</u>	<u>Window Allowance</u>
1 - Day	$\pm$ 0 Days
1 - Week	$\pm$ 3 Days
1 - Month	$\pm$ 10 Days
3 - Month	$\pm$ 2 Weeks

5.0 BIOSTATISTICAL ANALYSIS PLAN

For this study, the safety assessment will be descriptive with no statistical endpoints. The results will be examined and analyzed and used as a basis for determining the nature of future studies. The average concentration of treated versus control tumour chemotherapy levels will be compared using Student's t-test, as will pre-and post-treatment MRI contrast enhancement.

5.1 Safety

Because this is a feasibility study, identifying ExAblate device-related safety issues is the goal of the study. All subjects recruited to this study, by definition, have a serious neurological disease, which in and of itself present a significant risk of a deterioration of the subject's medical and mental status during the follow up period of the study.

Adverse events will be recorded and categorized according to severity, relationship to surgical procedure, drug and contrast media, and relationship to ExAblate device. Standard Code of Federal Regulation definitions for Serious Adverse Events (SAE's) and Unanticipated Adverse Device Effects (UADEs) will be used in assessment of AEs (See sections below for Adverse Events Analysis and Reporting.)

5.2 Efficacy

In this feasibility study, efficacy is not a primary endpoint. However, to evaluate the treatment effect, for each subject, the following data will be collected:

- i. Overall tumour volume and sonicated/unsonicated tumours volumes (by weight)
- ii. Change in contrast enhancement (intensity) following BBB disruption to pre-sonication images as a comparative ratio measured in percent as read from MR images and normalized to the contralateral hemisphere.
- iii. Sonicated versus non-sonicated tumour brain chemotherapy concentration

5.3 Subject Health Status

The results from the physical and neurological exams will be recorded in the CRFs and will be recorded in the CRFs.

5.4 Statistical Considerations

This is a feasibility study of ten (10) subjects. A statistical comparison will be made between ultrasound treated and control tumour and brain chemotherapy levels using Student's t-test. Similar comparisons will be made between pre- and post-treatment MRI contrast enhancement. Differences in control versus treated brain chemotherapy levels in pre-clinical studies have shown at least 3-4 time increases with ultrasound, so that we expect to be able to show a statistically meaningful difference with a small number of patients. Reported adverse events will be examined and analyzed and used as a basis for determining the nature and extent of future studies.

5.5 Subject Confidentiality

Subject confidentiality will be maintained throughout this study, including all publications. Data collected and entered into the CRFs are the property of the study sponsor. Representatives from the study sponsor or authorized sponsor representatives, the Institutional Review Board (IRB), Ethics Committee, Health Canada or the FDA may receive copies of the study records and may review medical records related to the study.

As previously stated, patient surgical specimens will be identified only by unique numerical identifier linked to their MRN through an encrypted master list. Specimens will be frozen and maintained in a secure -80°C freezer until such time as they are processed. Following tissue homogenization and analysis for chemotherapy content, the specimens will be destroyed.

6.0 SAFETY CONSIDERATIONS AND RISK

Worldwide, over 10,000 treatments have been performed to date with the MR guided FUS ExAblate body system. Although this proposed study will utilize MR guided FUS to disrupt the BBB, a different indication than previously utilized, the body and brain systems have demonstrated the safety of the technique and in this application the acoustic power used is much lower, although with the addition of ultrasound contrast agents. The potential risks described below will be explained to the subject in the informed consent process.

6.1 Risks Associated with MR Imaging

Risk of Magnetic Resonance Scanners

- MRI has no known deleterious biological effects in subjects with no contraindications. The incidence of claustrophobia during MRI examinations is approximately 10-15%,

although it is expected to be less frequent in the study population due to the use of sedation.

Risk of Intravenous (IV) Catheter:

- There is a potential risk from the IV catheter used during the procedure. Participants can expect a small amount of pain and/or bleeding/bruising at the IV site. There is a small risk of infection. This procedure will follow the ‘standard of care’ at the study site.

Risk of Urinary Catheter:

- There is a potential risk if a urinary catheter is used during the treatment. Participant may expect varying levels of Urinary Tract Infection due to the use of the urinary catheter. In a different study for the ExAblate treatment of uterine fibroid (Pivotal study under IDE G020001 that lead to PMA approval under PMA # P040003), the incidence of this risk was found not to exceed 3.7%. This procedure will follow the “standard of care” at the study site.

Risk of Stereotaxic Head Frame:

- In this study, the Integra Radionics frame will be used for all treatments. The main part of this frame is a metal based ring that serves as a base for the rest of the fixation components. This frame comes with non-metallic posts mounts. Into the non-metallic post mounts, accompanying nylon encapsulated screws with a metallic tip are fitted through the posts. Only the metal tip of the screw is in contact with the patient. The risk of any skin event at or in the vicinity of the metallic tips is mitigated by the fact that the screws outer shell are out of nylon, and therefore eliminates any electrical conductivity to the skin. This is further mitigated by the fact that these screws are mounted on the non-metallic posts that links them to the metallic ring. Hence, the Integra frame is actually manufactured and sold with built in mitigating steps aimed at reducing / eliminating skin event risks. When the full set up is completed, the metallic ring is NOT in contact with the patient.

Risk of Contrast Agent

- This MR contrast agent being used is Magnevist®. It is an intravenously injectable contrast medium for MRI. It is contraindicated in patients with severe chronic kidney disease (GFR<30mL/min) and acute renal injury.
- The rate of serious adverse reactions is estimated to be on the order of 0.00002. The most common adverse events include headache (4.8%), nausea (2.7%), localized injection site coldness (2.3%), and dizziness. Additional adverse reactions occur with an incidence of less than 1%.
- Precautions should be exercised for patients with a history of grand mal seizures, ischemic heart disease and severely impaired renal function. The very unlikely possibility of an allergic reaction, including anaphylactic or cardiovascular reactions, should be considered especially for patients with a known sensitivity to gadolinium or history of asthma. Patients with known sensitivity to gadolinium as well as those with severe cardiac disease will not be considered for enrolment in this study.

- Nephrogenic Systemic Fibrosis (NSF) or Nephrogenic Fibrosing Dermopathy (NFD), kidney disorders, may occur in patients with moderate to end-stage kidney disease after they have had an MRI scan with gadolinium-based contrast agent. NSF causes fibrosis of the skin and connective tissues throughout the body. Patients develop skin thickening that may prevent bending and extending joints, resulting in decreased mobility of joints. NSF usually starts in the lower extremities. Fibrosis can also develop in the diaphragm, muscles in the thigh and lower abdomen, and lung vessels. For this reason patients with renal impairment will not be considered.

6.2 Risk of Definity Ultrasound Contrast Agent

- The risk associated with Definity® may be found in greater detail in the product insert (66). While Definity is approved for use as an MR contrast agent within Canada, the use here is to increase the accumulation of chemotherapeutic agents across the blood brain barrier into tumor cells (off-label).
- *Contraindications:* Patients with known or suspected right-to-left, bi-directional or transient right-to-left cardiac shunts, intra-arterial injection, or patients with known hypersensitivity to perflutren.
- *Deaths and Serious Cardiopulmonary Reactions:* Serious reactions, including fatal cardiac or respiratory arrest, have occurred during or following perflutren-containing microsphere administration. The risk for these reactions may be increased among patients with pulmonary hypertension or unstable cardiopulmonary conditions (acute myocardial infarction, acute coronary artery syndromes, worsening or unstable congestive heart failure, serious ventricular arrhythmias or respiratory failure, including patients receiving mechanical ventilation). Monitoring of vital signs, electrocardiography, and cutaneous oxygen saturation during and for at least 30 minutes after DEFINITY® administration should be performed. In the absence of these underlying conditions, patients should be observed closely during and following DEFINITY® administration.
- There is a 1.1% rate of serious cardiopulmonary adverse events, and a 0.4% rate of death. The deaths occurred several days after administration and appeared to be related to the course of underlying disease. Other serious adverse events, which appeared within days of the drug administration (2-15 days), all appeared to be a progression of underlying cardiac and non-cardiac disease. However, a role for DEFINITY® in the initiation or course of these adverse events could not be ruled out. Other uncommon but serious reactions observed during or shortly following perflutren-containing microsphere administration included loss of consciousness, convulsions, symptomatic arrhythmias (atrial fibrillation, supraventricular tachycardia, ventricular tachycardia or fibrillation), hypotension, respiratory distress or cardiac ischemia. Always have cardiopulmonary resuscitation personnel and equipment readily available prior to DEFINITY® administration and monitor all patients for acute reactions.
- *Anaphylactoid Reactions:* Post-marketing reports of acute anaphylactoid reactions including shock, bronchospasm, upper airway swelling, loss of consciousness, urticaria and pruritus, have occurred in patients with no prior exposure to perflutren- containing

microsphere products. Monitor all patients for signs and symptoms of anaphylactoid reactions.

- *Systemic Embolization of DEFINITY® in Patients with Cardiac Shunts:* In patients with right-to-left, bi-directional, or transient right-to-left cardiac shunts phospholipid-encapsulated microspheres can bypass the pulmonary particle-filtering mechanisms and directly enter the arterial circulation resulting in microvascular occlusion and ischemia. In an animal study utilizing intra-arterial administration of activated DEFINITY®, microsphere trapping was seen in small arterioles <15 µm, especially at branch points and in capillaries at all doses tested, including doses directly applicable to those used in humans. An animal study utilizing intravenous administration did not result in arterial microvascular obstruction presumably because of filtering by the lungs. Do not administer DEFINITY® by intra-arterial injection.
- *QTc Prolongation:* ECG parameters for doses up to 10 µL/kg were monitored in 221 subjects at multiple time points from 1 hour to 72 hours after the first bolus injection. In the 221 subjects, QTc prolongations of >30 msec were noted in 64 (29%) subjects. Forty-six out of 64 subjects with QTc prolongations were further evaluated and 39% (18/46) showed associated cardiac rhythm changes.
- The most common adverse events were reported in the central and peripheral nervous system (3.1%), Body as a Whole (2.4%) and Gastrointestinal system (1.8%). The most common events were headache (2.3%), back and renal pain (1.2%), flushing (1.1%) and nausea (1.0%).

6.3 Risks of Temozolomide

Patients enrolled in this study will receive one dose of TMZ prior to sonication of their tumour. The risks below are from the product monograph of TMZ:

- The most common adverse reactions (≥10% incidence) are: alopecia, fatigue, nausea, vomiting, headache, constipation, anorexia, convulsions, rash, hemiparesis, diarrhea, asthenia, fever, dizziness, coordination abnormal, viral infection, amnesia, and insomnia.
- The most common Grade 3 to 4 hematologic laboratory abnormalities (≥10% incidence) that have developed during treatment with temozolomide are: lymphopenia, thrombocytopenia, neutropenia, and leukopenia.
- Allergic reactions have also been reported. [1]
[SEP]
- Myelosuppression — monitor Absolute Neutrophil Count (ANC) and platelet count prior to dosing and throughout treatment. Geriatric patients and women have a higher risk of developing myelosuppression.
- Cases of myelodysplastic syndrome and secondary malignancies, including myeloid leukemia, have been observed.

- Pneumocystis pneumonia (PCP) – PCP prophylaxis required for all patients receiving concomitant TEMODAR and radiotherapy for the 42-day regimen for the treatment of newly diagnosed glioblastoma multiforme. ^[SEP]All patients, particularly those receiving steroids, should be observed closely for the development of lymphopenia and PCP. ^[SEP]
- Hepatotoxicity – fatal and severe hepatotoxicity have been reported with full dose temozolomide. Perform liver function tests at baseline, midway through the first cycle, prior to each subsequent cycle, and approximately two to four weeks after the last dose of alkylating oral chemotherapies. ^[SEP]
- Fetal harm can occur when administered to a pregnant woman. Women should be advised to avoid becoming pregnant when receiving chemotherapy. Pregnant or breast feeding women will not be enrolled into the study.

6.4 Risks Associated with Blood-Brain Barrier Disruption with Focused Ultrasound and Definity®

- There is a potential risk of conscious sedation, which includes reaction to the drugs or over-sedation.
- There is a potential risk to the patient of deep venous thrombosis (DVT) from lying stationary for several hours. The risk to the patient from lying still for this treatment should be no greater than that of lying still for any other reason. Patients will be provided compression stockings to use during treatment. All patients will be screened for DVT prior and post-ablation procedure. This will further reduce the risk to patients by screening out all those with DVT risk prior to ablation.
- There is a risk that the patient may experience neck pain or discomfort from lying in the same position during the treatment.
- There is a potential risk of haemorrhage during MRgFUS treatment. At the end of the MRgFUS procedure a contrast-enhanced MR scan is performed to assess the blood flow within the treated area and adjacent tissue. This exam constitutes an independent treatment assessment tool that provides further information on blood flow and can serve as a final check of the overall tissue status and alert the physician to any significant oedema or haemorrhage.
- There is a risk of discomfort to the subject caused by heating of tissue. The energy intensity at the level of the skin is quite low, and there should be no local heating and thus no pain in the scalp. The rise in temperature in the skull should be kept below the pain threshold by active cooling of the water in contact with the scalp. Because the focal point of the beam will be > 2.5 cm from the dura and there are no pain receptors in the brain, there should be no pain associated with BBB disruption. The subject will be in constant verbal contact with the physician and appropriate action can be taken in the event that a subject does experience discomfort. Remedies could involve lowering power levels, increasing the time interval between consecutive treatment pulses, or increasing the level of sedation and/or analgesia. The subject also has the ability to abort the sonication at any time by activating a handheld cut off circuit (i.e. stop switch).

- There is the risk that the tissue along the path to the target (scalp, skull, dura, brain, etc) could become heated to the point where tissue-damage or a burn might occur. This heating could be caused by improper treatment targeting, irregularities on the skin surface (e.g.: scars), treatment of a volume of tissue too close to the skin or bone, energy absorption by the bone, or the conduction of sufficient heat to cause a burn at the surface. In the case of a 1° or 2° burn of the scalp, the skin should heal without a scar. In the case of a 3° burn, a scar, or loss of sensation in the area of the burn could result.
- Although skin burn is a risk associated with both the ExAblate transcranial and body systems, there have been no cases of skin burn in ExAblate transcranial treatments; approximately 60 patients have been treated with the ExAblate transcranial system to date. However, over the last decade, there have been four reports of skin burns in breast treatments and two reports of skin burns in uterine fibroid treatments. These patients were treated with the ExAblate body system. The circumstances of these events are not applicable to the ExAblate transcranial system. However, the heating in the energy pass zone is always monitored and an additional cooling time can be administered when elevated temperatures are detected.
- If non-superficial tissue along the path to the target were to become heated to the point that damage occurs, there is the possibility that it would result in significant neurological damage or even death. To reduce the possibility that this could occur, several measures will be taken:
 - i. First, the procedure is performed in the MR scanner. During the treatment MR images will be acquired. Using specific scanning sequences and a rapid post-processing program, changes in temperature can be detected, and a thermal map of the brain generated. This thermal map will reveal any potentially dangerous elevations in temperature.
 - ii. Second, conscious sedation will be used so that the study personnel can regularly assess cognitive and motor function throughout the procedure as well as general neurological function. This will help to reveal any indication that tissue damage may be occurring along the beam paths.
 - iii. Third, MR-compatible pulse oximeter, blood pressure cuff, and EKG monitor will be monitored throughout the procedure.
 - iv. This information will permit detection of tissue damage, edema, or bleeding, if brain or blood vessels along the beam paths are injured by heat.
 - v. Finally, the subject and the neurosurgeon will each have a stop switch that can instantaneously interrupt the energy delivery at any time. The subject is given a stop switch in case aberrant tissue heating causes any compromise to speech, word finding, or other communication difficulties. The subject will be instructed prior to the proceeding that they should use the stop switch any time they feel excessive pain, discomfort, disorientation or any other unusual sensation. The neurosurgeon has a stop switch so that if there is any sign of neurological change, the energy delivery can be immediately stopped and the subject carefully evaluated. Temporary interruption of energy delivery will in no way compromise the potential for therapeutic benefit to the subject. Following subject evaluation

treatment can resume without delay.

- There is a risk of imprecise targeting of the focal point, and BBB disruption, haemorrhage or tissue damage outside of the planned treatment volume. If this occurs it is possible that serious neurological deficit or even death could result. To limit the risk of this occurring, the treatment process includes a mandatory verification step that requires the operator to first check the alignment of the subject anatomy, the focal point of the transducer and the MR imaging system. This procedure, done while the subject is in position for treatment, uses a very low energy sonication to confirm of the alignment of the focal point and the targeted treatment point in all three axes. For each sonication delivered during treatment, the operator gets continuous feedback on the position of the intended treatment point superimposed on the thermal dosimetry image and can make corrections where required if there is an elevation in tissue temperature. At any point in the treatment process this low-power verification of the localization may be repeated to ensure proper system focusing.
- There is a risk associated with subject motion during a sonication or between sonications. This could cause a movement of the tissue relative to the planned treatment volume on the system, and in extreme cases could result in the treatment of a point outside the planned treatment volume. Also, because the skull functions as a defocusing lens, the phase correction map computed for the target spot will become ineffective if the subject moves. To prevent or minimize this risk, there are several precautions taken to prevent motion, and to detect it, if it occurs:
 - i. During subject positioning every effort will be made to make the subject comfortable and the subject will be educated as to the importance of maintaining their position during the treatment.
 - ii. The subject will be managed with conscious sedation. This will enable them remain still during the procedure.
 - iii. The subject will be placed in a head immobilization unit based on a stereotactic frame. This technology has been effective in preventing movement in stereotactic neurosurgery, and has been adapted and modified to the specifications of MR-guided FUS.
 - iv. One or more members of the medical team will be in the room throughout the sonication to monitor the subjects' medical status and comfort.
- Risk of cavitation - There is a risk of cavitation in the tissue at the focal point. Cavitation is the collapse of rapidly developed gas bubbles at the focal point due an extreme intensity of ultrasound excitation. This rapid collapse could cause high pressure, shock waves, and high temperatures. However, we believe that through proper system design and careful selection of system operation envelope, there is a very minimal risk that cavitation could occur during a treatment, even in the event of user error. We have developed an automated treatment planner that takes as its input the targeted ablation area depth in tissue, focal volume and tissue absorption, and based on pre-set safe operating limits selects sonication parameters that will keep the intensity of ultrasound excitation well below the intensity levels that could cause cavitation.

6.4.1 Neurological Risks of Blood-Brain Barrier Disruption with Focused Ultrasound and Definity®

Ultrasound treatments in the brain may lead to increased edema or hemorrhage. These mechanisms may cause transient or permanent local neurological deficits or symptoms and signs of increased intracranial pressure. These complications are unlikely, but if they occur they may require medical management with increased dexamethasone and/or mannitol. In severe cases, the bone flap from the scheduled craniotomy could be removed or the craniotomy expanded to relieve increased intracranial pressure. Permanent neurological deficits or death could possibly result. Throughout the treatment itself, a neurosurgeon and anesthesiologist will be present, and the subject will be kept in the neurosurgical ICU for 24 hours post-treatment for observation.

6.5 Anticipated Treatment Side Effects from MRgFUS

All adverse events will be reported in the Case Report Forms (CRFs) and analyzed for their relationship to the treatment device. Based on previous treatment experience, the following anticipated side effects have been identified as possible treatment related complications of MRgFUS treatment. These can be classified into Non-significant and Significant Anticipated Treatment Side Effects based on their medical severity, additional treatment required, and long-term consequences for the subject.

Non-significant Anticipated Treatment Side Effects of MRgFUS treatment are those that normally resolve without sequelae within 1-14 days of the treatment:

- minor pain from subject positioning or system interface (i.e. back pain, soreness in neck).
- transient fever: oral temperature $>100.4^{\circ}\text{F}/38^{\circ}\text{C}$, lasting less than 24 hours
- minor (1° or 2°) skin burns less than 2 cm in diameter
- bruising of the skin along the treatment path
- asymptomatic increase in edema surrounding the treatment area as shown on MRI
- headache
-

Significant Anticipated Treatment Side Effects of MRgFUS are those which may require medical treatment, may have sequelae, and for which time of resolution is not defined. The following side effects are thought to be improbable but their relative risk remains to be defined:

Scalp in the sonication pathway:

- skin burns ($>2^{\circ}$) with ulceration of the skin
- scar formation
- loss of sensation
- atrophy

Bone in the sonication pathway:

- bone necrosis
- subdural bleeding
- vein thrombosis
- seizures
- symptoms from disturbances of eloquent cortical areas (motor, sensory, auditory, visual, speech)

Other brain tissue

- necrosis of normal tissue due to incorrect targeting
- bleeding in the treatment volume

- cerebral infarction
- neurological deficits
- moderate or severe increase in cerebral edema as shown by MRI scans
- symptomatic increase of intracranial pressure
- death

Cerebral arteries

- bleeding
- coagulation thrombosis
- vasospasm
- death

The experience of Significant Anticipated Treatment Side Effects in MRgFUS treatment in the breast and uterus have been less than 5% as shown in prior clinical experience. In brain treatments there is less prior experience with the ExAblate transcranial device and the probability of an adverse event is unknown. It is the purpose of this study to gain experience that will allow us to determine the safety of BBB disruption with focused ultrasound in the treatment of brain tumours.

6.6 Adverse Reactions and Precautions

The subjects will be educated as to what to expect during the procedure and the importance of immediately communicating any problems, unusual symptoms, or discomfort, to the investigator during the treatment and throughout the follow-up period. Subjects will also be educated as to what sensations or perceptions could indicate that neurological damage may be starting to occur. They will be told to use their handheld stop switch if they feel anything unusual may be happening, so that they can be neurologically assessed. All adverse reactions occurring in this study will be recorded in the Case Report Forms. Each will be assessed for its probable cause (unrelated to the treatment, device related, procedure related, etc) as described below.

6.7 Criteria for Removal from the Study

Subjects can be exited from the study at any time if in the opinion of the principal investigator it is not in the best interest of the patient to carry on as planned. In Addition, patient may also chose to exit the study at any time, but will be strongly encouraged to participate in the follow-up visits, in order to protect their health.

6.8 Adverse Event Reporting

It is the responsibility of the investigator to document all treatment related and device related Adverse Events (AE's), which occur during the course of the study. At each visit, the investigator will evaluate AE's. AE's not previously documented in the study will be recorded on the Adverse Event Log within the subject's CRF. The nature of each event, date and time (when appropriate) of onset, outcome, frequency, maximum intensity, action taken, and attribution will be recorded. AEs already documented in the CRF (i.e., at a previous assessment) and designated as 'ongoing', should be reviewed at subsequent visits as necessary. If these have resolved, the documentation in the CRF should be completed including an end date for the event.

If an AE increases in frequency or severity during a study period, a new record of the event will be started.

ICH E2A definitions for Serious Adverse Events (SAEs) will be used for evaluation of adverse events.

Serious: - Any untoward medical occurrence that at any dose:

- results in death,
- is life-threatening,
- requires inpatient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity, or
- is a congenital anomaly/birth defect.

All AE's (related or unrelated) meeting the criteria for an SAE require notification of the sponsor and the reviewing IRB as soon as possible, with subsequent completion of additional paperwork provided by the sponsor fully documenting the course of the event, all treatments, and final outcome. Initial reporting of an SAE should be made to the sponsor no later than two (2) working days after the PI learns of the incident.

Standard Code of Federal Regulation (CFR) definitions for Unanticipated Adverse Device Effects (UADEs) will be used for evaluation of this type of adverse event.

UADE [§812.3(s)] means any serious adverse event on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

Any UADEs will be reported to the Sponsor and to the reviewing IRB as soon as possible. However, in no event must this report be made later than two (2) working days after the PI learns of the incident.

Other common non-study or non-device related, minor health complaints will not be collected as AE's (for example: colds, sprains, headaches). Patients who have a progression of their primary disease or symptoms that lead to an alternative treatment will not be reported as an AE.

6.9 Adverse Events Analysis

A Data Safety Monitoring Board will be used to review all AE's on the study. Their role is to evaluate all AE's that occur throughout the study and determine if they are in fact related to the ExAblate, or some other cause. We would closely monitor all treatments for any AE's, and consider the following questions for AEs:

- Was the adverse event serious? (life-threatening, caused a disability, required or prolonged hospitalization, caused death)
- Was the adverse event device-related?
- Was the adverse event unexpected?
- Is there an unreasonable risk in continuing the trial?

If in fact it is determined that an ExAblate-treated patient experienced an AE that met all of the above criteria, we would stop the trial pending further investigation. If in the opinion of the

DSMB, a modification of the study protocol was necessary to provide adequate protection to future study participants, the modification would be implemented prior to reinitiating the study. Any such amendment would be reported to the IRB and Health Canada as required by the applicable regulations.

All adverse events will be assessed for their relationship to the study device or procedure. Standard Code of Federal Regulation (CFR) definitions for SAEs and UADEs will be used in assessment of adverse events.

7.0 MONITORING PLAN

Clinical Monitoring for this study will be managed by InSightec. The Clinical Monitor is qualified by training and experience to oversee the conduct of this study. The Clinical Monitor's responsibilities include maintaining regular contact with the investigational site through telephone contact and on-site visits, to ensure that:

- The trial is conducted according to Health Canada and GCP requirements;
- The Investigational Plan is followed;
- Complete, timely, and accurate data are submitted;
- Problems with inconsistent or incomplete data are addressed;
- Complications and unanticipated adverse effects are reported to the Sponsor and the IRB;
- The site facilities will be monitored to stay adequate to meet the requirements of the study.
- The Clinical Monitor will initiate the study during an on-site visit and will continue to perform on-site monitoring visits as frequently as deemed necessary. The first monitoring visit will usually be made as soon as possible after enrollment has been initiated. At this visit and all monitoring visits, the Clinical Monitor will compare the data entered onto the CRFs with the hospital or clinical records (source documents). Source documentation must be available to substantiate proper informed consent procedures, adherence to protocol procedures, adequate reporting and follow-up of AEs, and device procedure information. Findings from the review of CRFs and source documents during a monitoring visit will be discussed with the PI. Completed paper or electronic CRFs will be reviewed prior to data closure at each visit. The dates of the monitoring visits will be recorded in a Log to be kept at the clinical site. During monitoring visits, the Sponsor expects that the study coordinator and the PI will be available, the source documentation will be available, and a suitable environment will be provided for review of study related documents.

Every effort to contact all subjects for study follow-up and to encourage visit compliance should be made. A log should be kept of dates of attempted contact and results. After 3 unsuccessful attempts at contact (e.g., by telephone or email) and sending 1 certified letter to solicit compliance a subject may be considered lost to follow-up.

7.1 Electronic Data Capture (EDC)

Electronic CRFs (eCRFs) will be generated to capture protocol-specific information during the conduct of this study. This electronic data capture of the eCRFs is based on the Oracle Software system, and is designed, run and hosted by Sponsor (Haifa, Israel).

7.2 Investigator Responsibilities

All Principle Investigators will be required to sign the Investigator Agreement. All investigators will undergo extensive training on the protocol and operation of the MRgFUS system, and provide documentation of their specialized training.

8.0 POTENTIAL BENEFITS

This technique is still being investigated, so it may offer potential benefits, though this cannot be confirmed. The malignant tissue will be directly exposed to temozolomide, a chemotherapeutic agent known to have efficacy against malignant brain tumours. The chemotherapy will be targeted around the tumour margins, where there are higher concentrations of active tumour growth occurs. Given that all or most of the treated region will be resected after treatment with ultrasound, any residual malignant cells are unlikely to receive a therapeutic dose. There is no guarantee that this procedure will reduce the size of the tumour or otherwise treat the underlying disorder. Other subjects may benefit from this procedure in the future, if further trials prove it to be a safe and effective therapy, as unresectable infiltrating cells in the region surrounding the macroscopic tumour may be exposed to therapeutic levels of chemotherapy after BBB disruption is performed.

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APPENDICES

Appendix A: Karnofsky Score and ASA Classification

Karnofsky Performance Score

SCORE	FUNCTION
100	Normal, no complaint or evidence of disease
90	Able to perform normal activity; minor signs and symptoms of disease
80	Able to perform normal activity with effort; some signs and symptoms of disease.
70	Cares for self, unable to perform normal activity or to do active work
60	Requires occasional assistance, but is able to care for most needs
50	Requires considerable assistance and frequent medical care
40	Requires special care and assistance; Disabled.
30	Hospitalization indicated, although death not imminent; Severely disabled
20	Hospitalization necessary; active supportive treatment required, Very sick.
10	Fatal processes progressing rapidly; Moribund
0	Dead

ASA Classification:

Class I - healthy subject.

Class II - mild systemic disease with no functional limitation.

Class III - severe systemic disease with definite functional limitation.

Class IV – severe systemic disease that is a constant threat to life.

Class V – Moribund subject unlikely to survive 24 hours with or without operation.

All subjects that are Class 3 or above are excluded from this study.

Appendix B: FUS Glossary of Terms

Magnetic Resonance guided High Intensity Focused Ultrasound Glossary

Ablated Volume: The volume of tissue necrosed by the direct application of thermal energy.

Acoustic Power: The power (in Watts) actually coming out of the transducer. Prior to each sonication, the system displays the planned acoustic power. During sonication, the system displays the acoustic power being delivered.

Calibration: A stage in treatment planning process in which MR images are taken to identify the location and orientation of the FUS transducer. The operator then places a graphic object on the transducer image. This mapping from the FUS coordinate system to the MR coordinates allow the user to make all further adjustments to the treatment plan in directly on the MR images.

Cavitation: Cavitation refers to the collapse of rapidly developed gas bubbles at the focal point due an extreme intensity of ultrasound excitation. This collapse has the potential to result in high pressure, a propagating shock waves, and high temperatures. This action can also contribute to direct mechanical destruction of tissue.

Critical Thermal Dose: The Sapareto and Dewey equation predicts a family of curves relating temperature and time to a threshold that results in a 100% cell death from thermal effects. To compare these different time/temperature doses, standard notation describes critical thermal dose as the equivalent number of 240 minutes at 43°C.

Cooling time: This treatment protocol variable determines the minimum time the system will wait from the end of one sonication until it allows the user to initiate a succeeding sonication.

Focus score: This presents a measure of the defocusing effect a variable thickness fat layer in the pass zone will have on the ultrasound wave. A high score predicts a minimal tissue aberration effect at the focal point. A low score identifies significant aberrations that can potentially defocus the focal point and reduce the thermal dose delivered at the focus.

Fiducial: A feature that allows the user to place markers on the images on top of anatomical landmarks. These selected landmarks are used throughout the treatment to help identify subject movement.

FUS Subject Table: The table that houses the phased array transducer, and on which the treatment is performed.

MRgFUS: MR guided High Intensity Focused Ultrasound.

Non-perfused Volume: (NPV) The volume of tissue that has been rendered non-viable by the FUS treatment. It is identified on the post-treatment T1 contrast MR images. The measurement can be done within minutes after the last sonication. Gadolinium MR contrast material is administered to the subject intravenously and a multi-slice scan acquired of the entire anatomy surrounding the treated lesion. Because of the vascular nature of the targeted tumour, prior to

treatment these tissues normally enhance on MR. However, in areas that have been thermally ablated the vascular structure has been destroyed. These areas without perfusion show up as very prominent dark holes in the middle of a bright region.

Pass Zone: A graphic overlay displayed to the operator that shows the ultrasound energy path from the front of the transducer to the target and beyond. It assists the user in planning a treatment to avoid passage of ultrasound energy through undesired regions (e.g., bone, bowel)

Pass Zone Thermometry (PZT): Prior to the first sonication, the system acquires a set of T1-weighted images in the axial and sagittal directions at center of the prescribed volume. Periodically during a treatment, the system automatically acquires a new set of MR images, and calculates a relative magnitude difference map. This map can be coded to identify fatty tissue temperature within the Pass Zone. These maps may be helpful to monitor the temperature in the Pass Zone during treatment, and aid the operator in setting the cooling duration between sonications.

Prescribed Volume or Region of Treatment (ROT): The prescribed volume drawn by the user on the MR planning images to indicate the tissue to be treated. In the case of a single layer treatment, the volume of this will equal the area drawn on a coronal image multiplied by the computed sonication spot length.

Proton Resonant Frequency (PRF) Shift MR: A method of measuring temperature changes in tissue with MRI using information on the phase of the spins. The temperature dependence of the PRF is primarily due to temperature-induced changes in hydrogen bonds in water. It is a specific physical property of non-fatty tissue that has been measured over a range of field strengths and is independent of the specific hardware or software of an individual brand of MR system.

Region of Treatment (ROT): See Prescribed Volume.

Sonicate: A command by the user that results in the delivery of a pulse of ultrasound energy for a specified time and intensity.

Sonation Grid Density: A treatment protocol variable that determines the degree of overlap of adjacent sonications in both the horizontal and vertical direction.

Sonation duration: A treatment protocol variable that determines the time in seconds of the acoustic power delivery by the system during a single sonication.

Spot Size: The size (length and diameter of an ellipsoid) of the focal point that will exceed the critical thermal dose. It is predicted by the treatment planner based on sonication parameters, and tissue depth.

Tissue Aberration Correction (TAC): This is a system feature that can be used counter the defocusing effect of variable thickness of fatty tissue across the Pass Zone. Based on user identification of the layers of fat in the Pass Zone, the system calculates a corrected ultrasound phase pattern that will result in a refocusing of the ultrasound beam after passing through the fatty layer.

Treated Volume or Thermal Dose Volume: The summed total volume of tissue from all sonications that have exceeded the critical thermal dose, as measured by thermal MRI.

Treatment Protocol: This is a named set of parameters stored in the system that describe all of the user selectable variables (i.e. acoustic power in watts, cooling duration in seconds, sonication duration in seconds, sonication grid density, spot length, tissue aberration correction state, etc) for a particular type of FUS treatment (i.e. breast, uterine fibroid, brain)

Treatment Plan: Based on the Prescribed Volume and the Treatment Protocol, selected by the user the FUS system computes the location, intensity and number of sonications necessary to ablate the Prescribed Volume.

Verification: This is a 2-stage process:

Geometric Verification: Sonications performed using a low energy pulse resulting in a low temperature rise in the tissue. The focal point of this sonication is visible on the MRI thermal sequence and is used to verify the location of the sonication in 3 dimensions in-vivo relative to the planned treatment point. Any errors in the alignment of the system or variations in the location of the focal spot can be corrected by the user in this step.

Dosimetry Verification: Sonications performed at the therapeutic dose. This is monitored by the MRI thermal imaging sequence to allow the user to compare the actual thermal dose with that predicted by the treatment planning software and adjust energy up or down if required.

Appendix C: Imaging Exams Requirements

For this study, imaging exams are needed as follows:

For the overall evaluation of the patient at baseline: CT, MRA, MRI imaging

On day of treatment: pre and post BBB disruption imaging

During the course of the follow up period.

To allow consistency and comparison with baseline imaging, the following exams are suggested for each visit:

Visit	Imaging Required
Pre-Treatment	CT[2] MRA [3] MRI Sequences Protocol [1]
Treatment Day	MRI Sequences Protocol [1], [4]
24 hours Post Tx	MRI Sequences Protocol [1] MRA and CT ONLY if Needed
1 Week	MRI and CT ONLY if Needed; Protocol [1]
1 Month	MRI Sequences Protocol [1] MRA and CT ONLY if Needed
3 Months	MRI Sequences Protocol [1] MRA and CT ONLY if Needed

[1] MRI Sequences Protocol

1.1 Precontrast

T1-Weighted Sagittal / Axial / Coronal pre-contrast (consider using the fat sat and/or magnetization transfer techniques)

T2 Axial / Coronal

Axial Gradient echo/susceptibility weighted imaging/SWAN (ONLY in the event a hemorrhage is suspected or simply just to check for its potential)

Axial DTI (for white matter invasion and mean diffusivity/ADC)

2.2 Post Contrast MR Imaging Exam:

Perfusion imaging

Perform dynamic contrast enhanced MRI

T1-Weighted sagittal / Axial / Coronal [same as pre-contrast 1.1.a above]

[2] CT Imaging Protocol

For the purpose of this study, the CT Exam should be an Axial scan with bone filter, an image resolution of 512x512, and image thickness of 1mm with zero (0) spacing.

Note: The site MUST also provide the sponsor with the “raw” CT data.

[3] MR Angiography Imaging Protocol

This MR angiography imaging is performed to evaluate and ensure that the targeted volume is at least 5-mm from any major vascular structure. This MRA exam will be performed using the following suggested parameters:

3 DTOF MRA Sequence with Flow Comp, & Mag Transfer

Slice Interpolation set to: ZIP2, and parallel imaging

Acquisition parameters:

20 flip, min TR, min full TE, 25 bandwidth

22 FOV, .8 phase FOV, 1.2 mm slices, 36 slices per slab, 8 overlap

384x192, 1 NEX

Superior SAT, and Ramped RF I > S

[4] Neuronavigation Compatible MRI Sequence

PATIENT POSITION		IMAGING PARAMETERS	
Patient Entry	Head First	Gradient Mode	ZOOM
Patient Position	Supine	Imaging Mode	3D
Coil Configuration	8HRBRAIN	Pulse Sequence	SPGR
Plane	AXIAL	Imaging Options	Fast
Series Description	Ax 3D T1 FSPGR w Gad	Acceleration Factor	1.00
SCAN TIMING		SCAN RANGE	
Flip Angle	20	FOV	22.0
TE	In Phase	Slice Thickness	1.5
Number of Echoes	1	Location per Slab	120
Receiver Bandwidth	41.67	Overlap Locations	0
IMAGE ENHANCE		ACQ TIMING	
Filter Choice	None	Freq	270
		Phase	270
		Freq DIR	A/P
		NEX	1.00
		Phase FOV	0.80
		Auto Shim	Auto
		Phase Correction	No
GATING/TRIGGER		USER CVS	
Auto Trigger Type	Off	User CV23	100.00
FMRI		MULTI-PHASE	
PSD Trigger	Internal	# of Acquisition	0
Slice Order	Interleaved	Seperate Series	0
View Order	Bottom/Up	Mask Phase	0
# of Repetitions REST	0	Mask Pause	0
# of Repetitions ACTIVE	0		
SAT		ASSET	
Tag Type	None	Slice Acceleration Factor	1.00
		Phase Acceleration Factor	1.00
TRICKS		CONTRAST	
Pause On/Off	On	Contrast Yes/No	Yes
Auto Subtract	0		
Auto SCIC	2		
MULTI-STATION			
Number of Stations	0		
Station Number	0		

Appendix D: Attached Paper Reprints

Martin E, et al. Focused Ultrasound for Non-invasive Neurosurgery. *Annals of Neurology* 2009; 66(6):858–861.

McDannold N, Arvanitis CD, Vykhodtseva N, Livingstone MS. (2012). Temporary disruption of the blood-brain barrier by use of ultrasound and microbubbles: safety and efficacy evaluation in rhesus macaques. *Cancer Res* 2012;72:3652-3663.